Reversal of apixaban induced alterations in haemostasis by different coagulation factor concentrates in patients after hip or knee replacement surgery

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Background. Apixaban is a direct oral anticoagulant (DOAC) with a specific inhibition of activated factor X (FXa). In case of bleeding or need of urgent surgery a direct antidote is not yet available. Off-label application of non-specific haemostatic agents, such as prothrombin complex concentrate (PCC) and recombinant FVIIa (rFVIIa), has been reported to reverse the effects of apixaban in *in vitro* and animal studies. The aim of this study is to measure the reversal potential of PCC and rFVIIa in patients with prophylactic apixaban concentrations.

Material and methods. Whole blood from patients under prophylactic therapy with apixaban was spiked with two doses of PCC or rFVIIa. Thromboelastometry (ROTEM®), prothrombin time (PT), and activated partial prothrombin time (aPTT) were performed.

Results. Prolongations in PT and aPTT were corrected by the different concentrates with variable efficacies (PCC<rFVIIa) for all time points after medication. Compared with baseline, the reversal effects ranged from partial correction (PCC) to overcorrection of the CT-ExTEM, PT and aPTT by rFVIIa.

Discussion. PCC partially reverses the effect of apixaban as measured by point-of-care coagulation testing and standard coagulation tests. Only rFVIIa reliably reverses apixaban anticoagulation.

Keywords: DOACs, apixaban, bleeding, reversal therapy.

Introduction

For a couple of years now, several direct acting oral anticoagulants (DOACs) have been available on the world market. These DOACs can be divided into the direct thrombin inhibitor dabigatran etexilate (DE) and the direct factor Xa inhibitors rivaroxaban, apixaban and edoxaban. Compared to vitamin k antagonists, the advantages of these new agents include their rapid onset and offset of action and a predictable anticoagulant effect so that routine coagulation monitoring is not required.

The DOACs have been shown to be as effective as enoxaparin in patients undergoing total knee replacement (TKR) or total hip replacement[1-3].

Apixaban has a good oral bioavailability (50%), is highly protein bound, shows only a few food/drug interactions, and reaches peak plasma concentration within 3-4 hours (h) after intake[4]. The ADVANCE-1-3[5-7] studies demonstrated that when compared with enoxaparin for efficacy, apixaban did not meet the pre-specified statistical criteria for non-inferiority in ADVANCE-1 and apixaban did meet the statistical criteria for superiority in ADVANCE 2 and 3. Apixaban use was associated with comparable bleeding rates compared with enoxaparin.

In case of bleeding in patients treated with oral factor Xa-inhibitors, a direct antidote (andexanet alfa, a recombinant FXa variant) is being studied in ANNEXA-4[8], a phase IIIb/IV study, but it is still awaiting approval and is not available on the market.

Previous studies investigated the effects of prothrombin complex concentrate (PCC) and recombinant FVIIa (rFVIIa) to reverse the anticoagulant effects of the Fxa-inhibitors, reporting differing effects[9-12]. These authors used considerably higher apixaban concentrations (<800 ng/mL) or higher doses of factor concentrates than recommended for clinical practice or investigated animal models only.

For bleeding in patients taking apixaban, the administration of PCC or rFVIIa should be considered[13]. The manufacturer also states that there is no clinical

evidence of reversing bleeding in individuals who have received apixaban.

Our *ex vivo* pilot study investigated two clinically highly relevant questions in the setting of apixaban-associated perioperative bleeding. First, can the anticoagulant effect of a prophylactic apixaban dosage be reliably measured by point-of-care (PoC) coagulation testing or standard coagulation tests? Second, which non-specific reversal agent is the most effective for reversal of prophylactic apixaban concentrations in case of need for surgery or serious bleeding?

Materials and methods

Study population and blood sampling

We recruited 15 patients with programmed elective knee or hip replacement surgery. Patients were eligible if they followed the Eliquis SmPC requirements for VTE prophylaxis after knee or hip replacement, i.e. 2.5 mg bid, first intake 12-24 h after surgery (so-called prophylactic dosage). Patients with pre-existing coagulopathies, on platelet inhibitors or non-steroidal anti-inflammatory drugs (NSAIDs) within ten days before study enrolment were excluded. After informed and written consent, blood samples were taken at the following time points: pre-operative on study enrolment day and on post-operative day (POD) 3 at 2, 4 and 8 h after apixaban intake. Blood samples were collected into 10 mL 0.106-M trisodium citrated tubes (S-Monovette®, Sarstedt, Nuembrecht, Germany), an EDTA tube for whole blood count, and a heparin tube for creatinine levels by venous puncture (BD Vacutainer® Safety-Lok™ Blood Collection Set 21Gx3/4"x12", Franklin Lakes, NJ, USA). Immediately after collection, each tube was gently inverted to achieve complete mixing. The study was approved by the ethics committee of Charité-Universitätsmedizin Berlin (EA1/024/14).

Selecting concentration of haemostatic agents

We tested two different haemostatic agents (PCC and rFVIIa) which had been used in previous studies for reversal of apixaban-induced alterations of haemostasis[9-12]. Each agent was added to the patient's blood at two clinically recommended dosages.

Preparation of PCC solution

In our study we used Cofact® 500 IU (Sanquin, Amsterdam, the Netherlands) which is a heparin-free PCC. Cofact® 500IU contains factor IX (500 IU), factor II (140-350 IU), factor VII (70-200 IU), factor X (140-350 IU), protein C (111-390 IU) and protein S (10-80 IU).

We dissolved the lyophilised PCC with water for injection and achieved a stock solution of 70 IU/mL. To achieve the dosages of 25 IU/kg body weight (BW) and 50 IU/kg BW[10,14,15] to be administered for study purposes, we calculated that a patient with 75 kg BW and 70 mL blood volume *per* kg BW should receive 1,875 IU at a dose of 25 IU/kg (0.35 IU/mL) and 3,750 IU (0.7 IU/mL) at a dose of 50 IU/kg. Respectively, we added 25 µL (corresponding dose: 25 IU/kg) or 50 µL (corresponding dose: 50 IU/kg) of the stock solution to the 5 mL blood aliquots.

Preparation of rFVIIa solution

We used Novoseven® 1 mg (Novo Nordisk, Bagswaerd, Denmark) which contains 50 kIU activated recombinant factor VII. We reconstituted the rFVIIa powder with the provided sterile solution of L-histidine in water for injection and preserved a stock solution of 0.2 µg/µL. To achieve the previously tested dosages of 90 or 180 µg/kg, we spiked the blood samples with 1 or 2 µg rFVIIa per mL blood[16].

Study protocol

On the first post-operative day (POD), apixaban was commenced at a dose of 2.5 mg bid. Steady state conditions for apixaban[17] were assumed on the third POD. Whole blood samples were collected at 3 different times on the third POD (2, 4 and 8 h after apixaban intake). The samples were divided into 5 mL aliquots and spiked with the 2 different dosages of each haemostatic agent using 25 µL or 50 µL of the corresponding stock solutions.

After incubation for 5 min at room temperature, thromboelastometry was performed using the INTEM, EXTEM and NATEM assays. The remaining samples were centrifuged and immediately transferred to a 3 mL barcoded transfer tube and stored at −80 °C prior to shipment on dry ice to the laboratory for analysis.

Measurement methods/assays

Rotational thromboelastometry

Rotational thromboelastometry is an established viscoelastic *ex vivo* method to measure clot formation and strength[18]. The measurements were conducted simultaneously on 2 ROTEM® delta devices (courtesy of TEM International GmbH, Munich, Germany) according to the manufacturer's instructions, using the recommended test kits provided by the manufacturer. We chose the clotting time (CT) as the major measurement parameter; the CT is the time between start of measurement and the initiation of clot formation. All samples were analysed within 4 h after venipuncture with the activated assays INTEM and EXTEM (runtime 30 min at 37 °C).

Standard parameters of coagulation

Prothrombin time (PT) and activated partial prothrombin time (aPTT) were determined using a STA-R Evolution analyser with STA Neoplastin Plus (Roche/Stago [Grenzach-Wyhlen, Germany], reference

range >70% Quick is calculated approximately 16.5 seconds [s] from PT; 100% Quick is calculated approximately 13.2 s from PT) and STA APTT (Roche/Stago, reference range 26-40 s) as the reagents, respectively. Clotting times were measured in seconds. Fibrinogen was measured using method of Claus, fibrinogen STA (Roche/Stago, reference range 1.6-4.0 g/L), factor VII with STA factor VII (Roche/Stago, reference range 60-170%), and creatinine with the method of Jaffé on Cobas instrument (Roche [Grenzach-Wyhlen, Germany], reference range: male 0.7-1.2 mg/dL, female 0.5-0.9 mg/dL).

Measurement of apixaban anti-Xa activity

Anti-Xa activity was determined using a Coamatic® Heparin (Chromogenix, Maria Enzersdorf, Germany) assay run on a STA-R Evolution analyser. The results of the chromogenic anti-Xa assay were calibrated against an apixaban standard and subsequently reported in ng/mL; reportable range using a 1:10 dilution is from 10 to 2,000 ng/mL.

The inter-assay reproducibility is 6.8% relative standard deviation (mean 124.6 ng/mL) and 3.5% (mean 347.1 ng/mL).

Statistical analysis

Data are given as median with 95% confidence intervals in brackets. Initial statistical evaluation consisted of an exploratory data analysis of the primary and secondary end points, including structural analyses. Given the expected non-normal distribution of the observations (because of the limited numbers of samples), all statistical analyses were carried out as non-parametric tests (Wilcoxon's pairwise test). $p<0.05$ (two-tailed) was considered statistically significant. Statistical tests refer to comparisons against the baseline for each time point.

The p-values obtained are to be considered as exploratory since this study was designed as a pilot study. Therefore, no adjustment for multiple testing was made. Statistical analysis was carried out using IBM© SPSS© Statistics, v.20 and v. 23 (©IBM Corporation and its licensors 1989, 2010 and 2015) and SAS (v. 9.1, ©SAS software by SAS Institute, Inc., Cary, NC, USA).

Results

Demographic and baseline characteristics

Fifteen patients with programmed elective knee or hip replacement surgeries were enrolled onto the trial from May to October 2014. Five patients were excluded before the last blood sample: n=1 withdrawal of informed consent, n=2 adverse events (symptomatic anaemia, dizziness after blood draw), n=1 concomitant medication of acetylsalicylic acid, n=1 incorrect dosing regimen.

At study enrolment, whole blood count, and aPTT, PT and FVII activity were within the normal range for all patients. Two female patients showed slightly increased creatinine levels (1.11 and 0.98 mg/dL) and one patient a slightly increased fibrinogen level (4.26 g/L).

The knee or hip replacement surgery caused a decrease in red blood cell (RBC) count, platelet count, haemoglobin, haematocrit, and factor VII activity, and an increase in white blood cell (WBC) count, fibrinogen, aPTT and PT.

Effects of apixaban concentration

Peak plasma concentrations of apixaban were achieved 4 h post dosing. Compared with the pre-values at enrolment day, apixaban caused a statistically significant prolongation of CT-ExTEM, PT and aPTT. The lower apixaban plasma concentration 2 and 8 h post dose also altered all coagulation parameters, but to a lesser extent (Table I).

Effects of haemostatic agents

Prolongations in PT and aPTT were corrected by the different concentrates with variable efficacies (PCC<rFVIIa) for all times after medication. Compared with baseline, the reversal effects ranged from partial correction (PCC) to overcorrection of the CT-ExTEM, PT and aPTT by aPCC and rFVIIa (Tables I and II).

Effect of PCC

Prothrombin complex concentrate showed only partial effects. The CT-ExTEM did not decrease significantly after addition of PCC. The aPTT decreased significantly only 2 and 4 h after ingestion of apixaban. The PT showed significant decreases at all time points in both doses of PCC (Table I).

Effect of rFVIIa

Addition of either dose of rFVIIa decreased CT-ExTEM, aPTT and PT significantly at all time points. All values decreased below baseline results. The impact of rFVIIa on coagulation parameters was seen to be dose dependent (Table II).

Discussion

This study investigated the efficacy of PCC and rFVIIa to reverse the anticoagulant effects of prophylactic apixaban doses in patients undergoing total hip or knee replacement surgery measured *ex vivo* with thromboelastometry and standard coagulation tests.

Measurement of apixaban with a chromogenic apixaban-calibrated anti-Xa assay, thromboelastometry, aPTT and PT

The maximum measured plasma concentration of apixaban using a chromogenic calibrated anti-Xa assay was higher (median 92 ng/mL 4 h after intake) than expected compared to the pharmacokinetic study by Frost *et al.,* with maximum plasma concentration of 62

Table I - Effects of prothrombin complex concentrate (PCC) on thromboelastometry, PT and aPTT at 2, 4 and 8 hours (h) after apixaban intake.

	Enrolment day			**3.POD**		
	Pre-value	***2 h-apix***	***2 h-apix-PCC*** *25 IU/kg*	***p-value*** *(2 h-apix- PCC* vs *2 h-apix)*	***2 h-apix-PCC*** *50 IU/kg*	***p-value*** *(2 h-apix- PCC* vs *2 h-apix)*
Apixaban (ng/mL)	0 (0/0)	77 (41/95)	73 (45/100)		70 (39/95)	
CT-Extem (s)	63 (58/70)	72 (66/95)	79 (70/89)	0.705	79 (73/100)	0.232
aPTT (s)	35.1 (33.6/35.4)	40.5 (38.1/45.1)	40.6 (36.2/42.6)	0.037*	40.1 (35.9/41.8)	0.049*
PT (s)	13.3 (12.9/13.6)	14.6 (14.2/15)	13.3 (12.9/13.7)	0.002**	12.9 (12.5/13.4)	0.002**
	Enrollment day			**3.POD**		
	Pre-value	***4 h-apix***	***4 h-apix-PCC*** *25 IU/kg*	***p-value*** *(4 h-apix- PCC* vs *4 h-apix)*	***4 h-apix-PCC*** *50 IU/kg*	***p-value*** *(4 h-apix- PCC* vs *4 h-apix)*
Apixaban (ng/mL)	0 (0/0)	92 (62/104)	88 (57/103)		87 (56/105)	
CT-Extem (s)	63 (58/70)	80 (69/97)	75 (62/93)	0.232	78 (70/91.5)	0.828
aPTT (s)	35.1 (33.6/35.4)	42.8 (38/45)	39.9 (36.4/41.6)	0.002**	39.7 (35.3/41.4)	0.002**
PT (s)	13.3 (12.9/13.6)	14.7 (13.9/15.3)	13.5 (13.1/14.1)	0.002**	12.9 (12.6/13.6)	0.002**
	Enrollment day			**3.POD**		
	Pre-value	***8 h-apix***	***8 h-apixPCC*** *25 IU/kg*	***p-value*** *(8 h-apix- PCC* vs *8 h-apix)*	***8 h-apix-PCC*** *50 IU/kg*	***p-value*** *(8 h-apix- PCC* vs *8 h-apix)*
Apixaban (ng/mL)	0 (0/0)	59 (38/95)	57 (41/94)		59 (41/91)	
CT-Extem (s)	63 (58/70)	75 (66/84)	72 (65/86)	0.719	73 (66/92)	0.633
aPTT (s)	35.1 (33.6/35.4)	38.6 (35.8/41.8)	37.4 (34.6/40.1)	0.084	36.2 (34.5/39.6)	0.059
PT (s)	13.3 (12.9/13.6)	14.4 (13.6/15.1)	13.0 (12.5/13.6)	0.002**	12.7 (11.8/13.1)	0.002**

POD: post operative day; apix: apixaban; PCC: prothrombin complex concentrate (25 IU/kg=0.35 IU/mL; 50 IU/kg=0.7IU/mL); CT: clotting time; PT: prothrombin time; aPTT: activated partial prothrombin. Times are reported in seconds (s). Data for other parameters are median and 95% confidence interval in brackets. Pairwise Wilcoxon exact test (two-tailed): *p<0.05; **p<0.01.

ng/mL[17], but was equivalent to the 3 h post dose results of Yamahira *et al.*[16].

Apixaban prolonged CT significantly in CT-ExTEM at 2, 4 and 8 h after drug ingestion. In 2013, Martin *et al.* had reported similar findings for CT-ExTEM in a rabbit bleeding model for an apixaban concentration of 91 ng/mL[1-9]. This is consistent with the results of Dias *et al*. who measured a prolongation of R-time in thromboelastography, a value comparable to CT[19].

Conventional coagulation parameters such as aPTT and PT were only slightly prolonged by apixaban, but not beyond upper normal values. It was widely reported that both parameters are not suitable to measure reliably the presence or concentration of apixaban[20, 1], which is confirmed by our results.

Reversal with PCC and rFVIIa

Prothrombin complex concentrate is recommended by the manufacturer and by several guidelines as the first-line unspecific clotting factor concentrate for apixaban reversal[13]. Martin *et al*.[6] evaluated the ability of PCC and rFVIIa to reverse the effects of apixaban (concentration 60-113 ng/mL) in a rabbit model of hepatosplenic bleeding. Both PCC and rFVIIa were found to partially reverse bleeding time, but none of these factors decreased the hepatosplenic blood loss. It remains uncertain whether the doses of apixaban from the animal model can be compared to haemostasis in humans, because their dose-finding study was selected to increase the hepatosplenic blood loss, an event that is not to be expected for apixaban under normal circumstances. Nagalla *et al.*[22] report a recovery of peak thrombin by 25 IU/kg PCC in healthy volunteers taking therapeutic doses of apixaban. Case reports of PCC use for apixaban reversal describe differing efficacy[23,24] for apixaban reversal in clinical settings. An *in vitro* investigation by Escolar *et al*.[11] with peak levels of approximately 200 ng/mL showed an important reversal effect only for 270 µg/kg rFVIIa, supporting our findings in this and a previous study of our group investigating reversal of rivaroxaban[14]. Taking all published results into consideration, it appears that the potential of PCC

Table II - Effects of rFVIIa on thromboelastometry, prothrombin time (PT) and activated partial prothrombin (aPTT) at 2, 4 and 8 hours (h) after apixaban intake.

	Enrolment day			**3.POD**		
	Pre-value	***2 h-apix***	***2 h-apix-rFVIIa*** *90 µg/kg*	***p-value*** *(2 h-apix-rFVIIa* vs *2 h-apix)*	***2 h-apix-rFVIIa*** *180µg/kg*	***p-value*** *(2 h-apix-rFVIIa* vs *2 h-apix)*
Apixaban (ng/mL)	0 (0/0)	77 (41/95)	71 (36/96)		74 (36/99)	
CT-Extem (s)	63 (58/70)	72 (66/95)	48 (44/50)	0.004**	48 (46/50)	0.004**
aPTT (s)	35.1 (33.6/35.4)	40.5 (38.1/45.1)	32.5 (31.2/35.4)	0.002**	31.0 (29.0/34.4)	0.002**
PT (s)	13.3 (12.9/13.6)	14.6 (14.2/15)	9.5 (9.0/9.9)	0.002**	9.0 (8.8/9.4)	0.002**
	Enrolment day			**3.POD**		
	Pre-value	***4 h-apix***	***4 h-apix-rFVIIa*** *90 µg/kg*	***p-value*** *(4 h-apix-rFVIIa* vs *4 h-apix)*	***4 h-apix-rFVIIa*** *180µg/kg*	***p-value*** *(4 h-apix-rFVIIa* vs *4 h-apix)*
Apixaban (ng/mL)	0 (0/0)	92 (62/104)	87 (60/105)		90 (64/109)	
CT-Extem (s)	63 (58/70)	80 (69/97)	47 (43/60)	0.01*	50 (48/57)	0.004**
aPTT (s)	35.1 (33.6/35.4)	42.8 (38/45)	33.2 (31.3/35.3)	0.002**	30.5 (29.5/33.2)	0.002**
PT (s)	13.3 (12.9/13.6)	14.7 (13.9/15.3)	9.6 (9.2/9.9)	0.002**	9.4 (8.9/9.7)	0.002**
	Enrolment day			**3.POD**		
	Pre-value	***8 h-apix***	***8 h-apix-rFVIIa*** *90 µg/kg*	***p-value*** *(8 h-apix-rFVIIa* vs *8 h-apix)*	***8 h-apix-rFVIIa*** *180µg/kg*	***p-value*** *(8 h-apix-rFVIIa* vs *8 h-apix)*
Apixaban (ng/mL)	0 (0/0)	59 (38/95)	61 (39/92)		58 (40/88)	
CT-Extem (s)	63 (58/70)	75 (66/84)	43 (40/47)	0.002**	49 (47/53)	0.002**
aPTT (s)	35.1 (33.6/35.4)	38.6 (35.8/41.8)	31.5 (29.8/34.6)	0.006**	30.1 (28.5/32.0)	0.004**
PT (s)	13.3 (12.9/13.6)	14.4 (13.6/15.1)	9.2 (8.8/9.7)	0.002**	9.0 (8.6/9.8)	0.002**

POD: post operative day; apix: apixaban; rFVIIa: recombinant activated factor VII; 90 µg/kg=1 µg/mL; 180 µg/mL=2 µg/mL; CT: clotting time; PT: prothrombin time; aPTT: activated partial prothrombin. Times are reported in seconds (s). Data for other parameters are median and 95% confidence interval in brackets. Pairwise Wilcoxon exact test (two-tailed): *p<0.05; **p<0.01.

and rFVIIa is uncertain, and the risk of thromboembolic events should not be underestimated, considering that after major surgery patients have a high risk for thromboembolic events.

Study limitations

This study is a pilot study and shows data for prophylactic concentrations only. To confirm these data, measurements in patients with therapeutic apixaban levels and larger study cohorts are needed. This would allow a more reliable conclusions to be drawn as to the appropriateness of thromboelastometry as a measurement approach and correlation with apixaban concentrations.

Conclusions

The specific antidote andexanet alfa is still undergoing clinical tests and is not yet available for clinical use in bleeding induced by oral factor Xa-inhibitors. Although its approval in Europe and North America is expected in the near future, it is unclear whether all emergency departments and healthcare providers will have access to this reversal agent. This study shows that PCC did not reverse completely the effect of apixaban as measured by PoC coagulation testing and standard coagulation tests. Only rFVIIa reversed apixaban anticoagulation reliably in our measurements.

Authorship contributions

MKK and KS planned and performed the study together with EL, KK, MS, EJ, CvH and KDW. MKK and KS analysed the data and wrote the manuscript. All Authors contributed to the final draft of the manuscript.

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