Ex vivo reversal of effects of rivaroxaban evaluated using thromboelastometry and thrombin generation assay

B. Schenk[1],*, P. Würtinger[2], W. Streif[3], W. Sturm[4], D. Fries[1] and M. Bachler[1]

[1]Department of General and Surgical Intensive Care Medicine, [2]Central Institute for Medical and Chemical Laboratory Diagnostics, [3]Department of Paediatrics, and [4]Department of Internal Medicine, Medical University of Innsbruck, Anichstrasse 35, Innsbruck 6020, Austria

*Corresponding author. E-mail: bettina.schenk@i-med.ac.at

Abstract

Background: In major bleeding events, the new direct oral anticoagulants pose a great challenge for physicians. The aim of the study was to test for *ex vivo* reversal of the direct oral anticoagulant rivaroxaban with various non-specific reversal agents: prothrombin complex concentrate (PCC), activated prothrombin complex concentrate (aPCC), recombinant activated factor VII (rFVIIa), and fibrinogen concentrate (FI).

Methods: Blood was obtained from healthy volunteers and from patients treated with rivaroxaban. Blood samples from healthy volunteers were spiked with rivaroxaban to test the correlation between rivaroxaban concentration and coagulation tests. Patient blood samples were spiked with various concentrations of the above-mentioned agents and analysed using thromboelastometry and thrombin generation.

Results: When added *in vitro*, rivaroxaban was significantly ($P<0.05$) correlated with ROTEM® thromboelastometry EXTEM (extrinsic coagulation pathway) clotting time (CT), time to maximal velocity (MaxV−t), and with all measured thrombin generation parameters. *In vivo*, CT, MaxV−t, lag time, and peak thrombin generation (C_{max}) were significantly correlated with rivaroxaban concentrations. Regarding reversal of rivaroxaban, all tested agents significantly ($P<0.05$) reduced EXTEM CT, but to different extents: rFVIIa by 68%, aPCC by 47%, PCC by 17%, and FI by 9%. Only rFVIIa reversed EXTEM CT to baseline values. Both PCC (+102%) and aPCC (+232%) altered overall thrombin generation (area under the curve) and increased C_{max} (+461% for PCC, +87.5% for aPCC).

Conclusions: Thromboelastometry and thrombin generation assays do not favour the same reversal agents for rivaroxaban anticoagulation. Controlled clinical trials are urgently needed to establish doses and clinical efficacy of potential reversal agents.

Clinical trial registration: EudracCT trial no. 213-00474-30.

Key words: blood, anticoagulants; complications, haemorrhage; thromboelastography

Rivaroxaban (Xarelto®; Bayer, Germany) is used as thrombosis prophylaxis and therapy instead of anticoagulants such as unfractionated heparin, low molecular weight heparin, or vitamin K antagonists, because it is considered to have a wider therapeutic range and a more predictable dose–response relationship.[1–3]

According to the manufacturer, rivaroxaban does not require routine drug monitoring.[4–6]

In the European Union, Canada, and the USA, rivaroxaban is approved for the prevention of stroke and systemic embolism in patients undergoing hip- or knee-replacement surgery,

Editor's key points

- There is limited information on the best treatment of bleeding in patients taking newer direct oral anticoagulants.
- The effects of various procoagulant factors on laboratory coagulation parameters were assessed on blood treated *ex vivo* with rivaroxaban or blood from rivaroxaban-treated patients.
- Reversal of rivaroxaban anticoagulant effects *ex vivo* was possible, but varied between reversal agents and the coagulation assay, indicating that clinical trials are needed to validate reversibility *in vivo*.

prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation, treatment and prevention of recurrence of deep vein thrombosis and pulmonary embolism, and prevention of atherothrombotic events after acute coronary syndrome.[7] Dose reduction (15 mg once daily) is recommended or can be considered in patients with advanced renal impairment (creatinine clearance 30–49 ml min^{-1}).[8]

Although bleeding and bleeding complications in trauma and surgery are the most common complications and side-effects,[9] no evidence-based reversal strategy is available for a bleeding patient taking rivaroxaban.[10][11] A specific antidote is in development, but not yet commercially available.[12] With regard to off-label use of coagulation factor concentrates, it is still uncertain whether prothrombin complex concentrates (PCC),[13][14] recombinant activated factor VII (rFVIIa),[15] activated PCC (aPCC),[16] or fibrinogen concentrate (FI)[17][18] should be used in the management of rivaroxaban-related bleeding.

The aim of this study was to assess the effective *ex vivo* reversal of the effects of the new direct oral anticoagulant rivaroxaban on coagulation assays. We studied the following non-specific reversal agents: PCC (Beriplex® P/N; CSL Behring, Marburg, Germany), aPCC (factor VIII inhibitor bypassing activity, FEIBA; Baxter AG, Vienna, Austria), rFVIIa (recombinant activated factor VII, NovoSeven®; Novo Nordisk, Bagsværd, Denmark) and FI (human fibrinogen concentrate, FGTW; LFB Biomedicaments, Lille, France).

Methods

Ethics committee approval

This study was approved by the Human Subjects Review Board of the Medical University of Innsbruck, Austria (reference: UN4984_LEK) and by the national competent authority (*Bundesamt für Sicherheit im Gesundheitswesen*, BASG, Vienna, Austria; reference: LCM-717978) and registered with EudraCT (reference: 213-00474-30). Written informed consent was obtained from all study participants before study-related procedures were performed. The study was performed in compliance with the Declaration of Helsinki guidelines regarding ethical principles for medical research involving human subjects and followed Good Clinical Practice as defined by the International Conference on Harmonization (ICH-GCP).

Study population

Healthy volunteers

Blood samples were obtained from healthy volunteers aged 18–85 yr. Exclusion criteria were pregnancy, concomitant medication

with influence on anticoagulant or platelet activity, or presence of an inherited or acquired bleeding disorder.

Patients

Blood samples were obtained from patients aged 18–90 yr receiving rivaroxaban (Xarelto®; Bayer, Germany). Exclusion criteria were pregnancy, non-compliance in taking medication, concomitant medication with an influence on rivaroxaban activity, or presence of haemophilia or an acquired or hereditary coagulation disorder.

Collection and preparation of blood samples

Blood samples were obtained by peripheral venipuncture 3 h after the patient took rivaroxaban (15 or 20 mg) in order to obtain peak plasma concentrations. Blood coagulation assays and determination of blood coagulation factor concentrations were performed using vacutainer tubes containing 1.106 mol l^{-1} trisodium citrate solution (Sarstedt, Nümbrecht, Germany). Coagulation analyses in whole blood were performed within 4 h after blood sampling. Plasma for thrombin generation assays was immediately frozen at −80°C (or −20°C for a maximum of 7 days) and thawed immediately before being analysed.

Reagents

Rivaroxaban was purchased from Bayer Pharma AG (Wuppertal, Germany) and diluted with 100% dimethyl sulphoxide (Fluka, Neu-Ulm, Germany), before being added in various concentrations (0, 100, 200, 300, 400, 500, 600, and 700 ng ml^{-1} final concentrations) to blood samples from healthy volunteers. Adding rivaroxaban solutions to whole blood resulted in dimethyl sulphoxide concentrations <5% and did not influence assays. To exclude diluting effects, PBS (Dulbecco's phosphate-buffered saline; Bio Whittaker®, Lonza, Belgium) was added to blood samples to produce equal volumes.

Reagents used for reversal of rivaroxaban effects were dissolved according to the instructions in product specification leaflets and added *ex vivo* to patient blood in concentrations corresponding to doses applied for clinical indications (lower doses) and maximal doses according to product specification leaflets (higher doses).

Spiking procedure

Blood samples from patients on rivaroxaban were spiked immediately after blood sampling to achieve the following final concentrations (assuming a body weight of 75 kg and blood volume of 5 litres): 0.3 or 1 U ml^{-1} PCC (corresponding to 1500 or 5000 U PCC); 1.5 or 2.25 U ml^{-1} aPCC (100 or 150 U kg^{-1} aPCC); 1.5 or 4.05 µg ml^{-1} rFVIIa (100 or 270 µg kg^{-1} rFVIIa); and 0.6 or 3 mg ml^{-1} FI (40 or 200 mg kg^{-1} FI).

Coagulation assays

Thromboelastometry

Within 4 h after blood collection, untreated baseline and spiked blood samples were analysed using thromboelastography (ROTEM®). ROTEM® parameters were determined using a ROTEM® gamma analyser (TEM Innovations GmbH, Munich, Germany). ROTEM® measurements were run at least until A30 values (clot firmness after 30 min) were reached, and all tests were performed according to manufacturers' instructions using the specific reagents provided by the manufacturer for EXTEM (extrinsically activated assay with tissue factor), INTEM

(intrinsically activated test using kaolin), and FIBTEM (extrinsically activated test with tissue factor and the platelet inhibitor cytochalasin D) measurements.

Thrombin generation assay
Thrombin generation measurements were performed using the Innovance ETP assay (Siemens, Marburg, Germany) on an automated coagulation analyser (BCS XP; Siemens). Coagulation was activated by adding phospholipids, human recombinant tissue factor, and calcium ions to platelet-poor plasma. The generated thrombin cleaves a chromogenic substrate (H-b-Ala-Gly-Arg-pNA), and the turnover of the substrate is recorded over time. The final concentration of substrate was 733 nM l^{-1} with CaCl 19 mM l^{-1}. The original curve was corrected for estimated α-macroglobulin-bound thrombin activity. From this curve, the following parameters can be obtained: total amount of generated thrombin in the reaction from initiation until return to baseline, also known as 'endogenous thrombin potential' (ETP), indicated as 'area under the curve' (AUC in mE, as a measure of the total endogenous generated thrombin); peak thrombin generation (C_{max}, in mE min^{-1}), which is the maximum of the first derivation of the ETP AUC; lag phase until initiation (t_{lag}); and time to peak thrombin activity (t_{max}).

Coagulation assays and rivaroxaban concentration
The PT and aPTT were determined on an automated coagulation analyser (aPTT, pathromtin® SL; and PT, thromborel®; S BCSxp; Siemens) for patients receiving rivaroxaban. Rivaroxaban concentrations were also measured on the BCSxp, using a chromogenic assay calibrated for rivaroxaban (BIOPHEN® DiXa-I; CoaChrom Diagnostica, Neuville-sur-Oise, France).

Statistical methods
The Wilcoxon signed-rank test was used to evaluate differences between baseline untreated blood samples and spiked samples from the same patient. Statistical analyses were performed using STATISTICA 10 software (StatSoft Europe GmbH, Hamburg, Germany). Pearson's correlation was used to evaluate correlations between rivaroxaban concentration and blood coagulation assays and coagulation factor activities. A value of $P \leq 0.05$ was considered statistically significant.

Results
Healthy volunteers
General
Thirteen healthy volunteers (six female, seven male) aged 22–56 (mean 36) yr were enrolled.

Influence of rivaroxaban on coagulation measurement
Thromboelastometry. Addition of rivaroxaban to blood samples from healthy volunteers prolonged EXTEM CT (clotting time) significantly in a dose-dependent manner (100–700 ng ml^{-1} rivaroxaban, $r=0.76$, $P<0.05$) from a mean (SD) of 68 (6) s at baseline to up to 425 (146) s at the highest dose of rivaroxaban tested (700 ng ml^{-1}; Fig. 1A).

The EXTEM MaxV–t (time to maximal velocity) was also significantly prolonged in a dose-dependent manner ($r=0.43$) from a mean (SD) of 197 (91) s at baseline to 416 (233) s at 700 ng ml^{-1} rivaroxaban (Fig. 1B).

Thrombin generation. The t_{lag} was prolonged by rivaroxaban ($r=0.88$) from 31 (6) s at baseline to 96 (17) s at a rivaroxaban concentration of 700 ng ml^{-1} (Fig. 1c). The t_{max} was prolonged ($r=0.82$) from 56 (7) to 233 (98) s (Fig. 1D). The AUC decreased in a dose-dependent manner with increasing rivaroxaban concentration ($r=-0.46$) from 329 (43) to 250 (36) mE (Fig. 1E). The C_{max} was also reduced in a dose-dependent manner ($r=-0.81$) from 121 (22) to 48 (6) mE min^{-1} (Fig. 1F).

Patients
Twenty patients (eight female, 12 male) receiving rivaroxaban and aged 21–90 (mean 68) yr were enrolled. For subject characteristics refer to Supplementary data SI.

Rivaroxaban concentrations in plasma compared with coagulation parameters
Subjects treated with rivaroxaban had a mean (SD) rivaroxaban concentration in blood plasma of 225 (100) ng ml^{-1}. Rivaroxaban concentrations correlated best with a decrease in Quick value prothrombin time (PT), and significantly correlated with an increase in aPTT (Table 1).

ROTEM analysis. There was a significant, rivaroxaban-dependent prolongation and a clinically relevant increase in EXTEM CT and FIBTEM CT compared with normal values[19] in healthy subjects. The MaxV–t was prolonged in a dose-dependent manner in both EXTEM and FIBTEM. The INTEM CT was also prolonged in a dose-dependent manner by rivaroxaban and showed a clinically relevant increase compared with normal values.[19]

Thrombin generation. Rivaroxaban produced a clinically relevant, significant decrease in C_{max} compared with normal values (111–156 mE min^{-1}). There was also a clinically relevant prolongation of t_{lag} compared with normal values (19.6–25.6 s). The AUC ($P=0.26$) and t_{max} ($P=0.77$) were not significantly correlated with rivaroxaban concentration, whereas AUC was still in normal range and t_{max} was prolonged (compare also Table 4).

Significant correlations observed between coagulation tests are shown in Table 2.

Reversal of rivaroxaban-induced changes *ex vivo*
Thromboelastometry. All tested agents significantly ($P<0.05$) reduced prolonged EXTEM CT. Of the agents tested, rFVIIa 1.5 µg ml^{-1} reduced CT most, by 68%. Of all agents, rFVIIa was the only one to reach normal CT values.[19] Activated prothrombin complex concentrate (1.5 U ml^{-1}) reduced EXTEM CT by 47%. Fibrinogen concentrate reversed CT prolongation by 9% (0.6 mg ml^{-1} FI; compare also Fig. 2 and Table 3).

The INTEM CT was also prolonged, and the highest dose of rFVIIa (4.05 µg ml^{-1}) was able to reduce INTEM CT significantly by 21% from baseline down to the normal range.[19] Fibrinogen concentrate ($P=0.78$ for 0.6 mg ml^{-1}; $P=0.07$ for 3 mg ml^{-1}) and aPCC ($P=0.06$ for 0.3 U ml^{-1}; $P=0.49$ for 1 U ml^{-1}) did not significantly influence INTEM CT, but PCC significantly prolonged CT by 7% compared with baseline at the lower dose (0.3 U ml^{-1} PCC) and by 19% at the higher dose (1 U ml^{-1} PCC).

For FIBTEM CT, please refer to Supplementary data SII.

Thrombin generation
Ex vivo administration of aPCC 2.25 U ml^{-1} significantly ($P<0.05$) increased the AUC by 232% from baseline. Adding aPCC 1.5 U ml^{-1} increased it by 154% ($P=0.028$). Also, PCC significantly increased AUC from baseline by 107%. Moreover, aPCC (2.25 U ml^{-1}) and
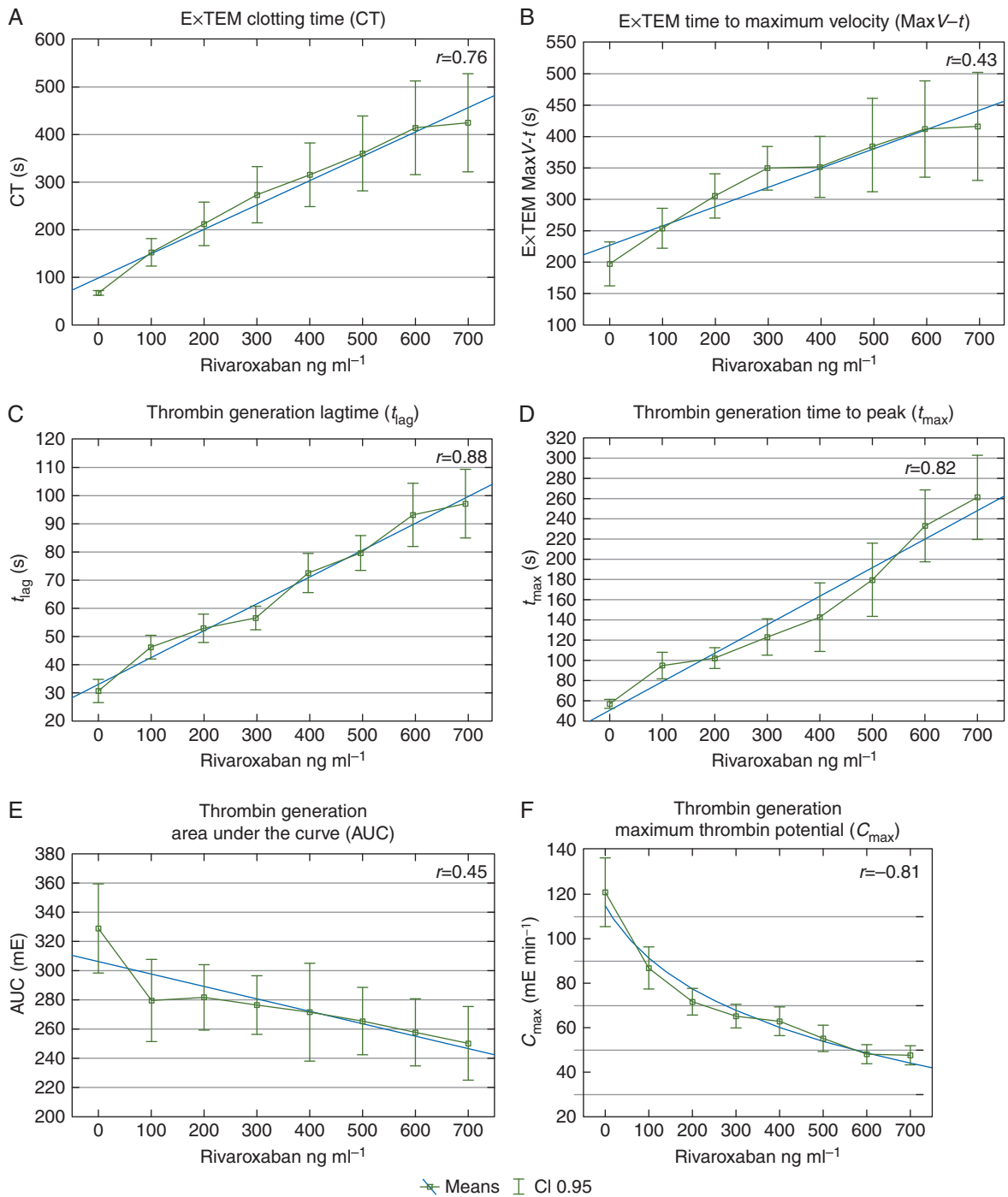
Fig 1 Correlation between various coagulation parameters and rivaroxaban plasma concentrations. Whole blood from healthy volunteers (n=13) was spiked with various doses of rivaroxaban (100–700 ng ml^{-1}). Significantly (P<0.05 for all) correlated parameters were as follows: (A) thromboelastometry EXTEM clotting time (CT, linear approximation for CT=47.7+51.1x); (B) thromboelastometry EXTEM time to maximal velocity (MaxV–t, linear approximation for MaxV–t=196.6+30.5x); (C) thrombin generation lag time (t_{lag}, linear approximation for t_{lag}=24.3+9.5x); (D) thrombin generation time to peak (t_{max}, linear approximation for t_{max}=25.7+27.2x); (E) thrombin generation area under the curve (AUC, linear approximation for AUC=314.5–8.5x); and (F) thrombin generation maximum of thrombin potential [C_{max}, logarithmic approximation for C_{max}=114.8–78.2×log10(x)]. X refers to the respective value on the x-axis. The strength of correlation was interpreted by evaluating the correlation coefficient, as follows: r 0.9–1.0 (–0.9 to –1.0)=very strong correlation; r 0.7–0.89 (–0.7 to –0.89)=strong correlation; r 0.5–0.69 (–0.5 to –0.69)=moderate correlation; and r 0.3–0.49 (–0.3 to –0.49)=weak correlation.

Table 1 Correlation between rivaroxaban concentrations and blood coagulation parameters. Values are the mean (SD) of all coagulation tests and correlation with rivaroxaban concentrations at baseline. The Pearson correlation was used to detect correlations between various blood coagulation tests and rivaroxaban concentrations at baseline. Tests were sorted according to the strength of correlation. aPTT, activated partial thromboplastin time; C_{max}, thrombin generation, peak thrombin generation; CT, clotting time; EXTEM, ROTEM, extrinsic coagulation pathway; FIBTEM, ROTEM, fibrinogen-dependent coagulation, thromboelastometry; INR, international normalized ratio; INTEM, ROTEM, intrinsic clotting time; MaxV−t, ROTEM, time from reaction start until the maximum of the first derivate of the curve is reached; PT, prothrombin time; Quick, Quick value; t_{lag}, thrombin generation, lag time until initiation. Correlation (r) with rivaroxaban concentration in patient blood. The strength of correlation was interpreted by evaluating the correlation coefficient: r 0.9–1.0 (−0.9 to −1.0)=very strong correlation; r 0.7–0.89 (−0.7 to −0.89)=strong correlation; r 0.5–0.69 (−0.5 to −0.69)=moderate correlation; and r 0.3–0.49 (−0.3 to −0.49)=weak correlation. Only significant correlations (P<0.05 for r) are shown

Test	Units	Mean (SD)	r	Reference range
Quick (PT)	%	63 (12)	−0.78	70–130
INR (PT)	–	1.34 (0.15)	0.75	0.8–1.2
CT (FIBTEM)	s	192 (83)	0.69	42–78
C_{max}	mE min^{-1}	88 (21)	−0.66	111–156
CT (EXTEM)	s	215 (89)	0.63	42–78
MaxV−t (FIBTEM)	s	221 (88)	0.62	–
aPTT	s	43 (12)	0.57	26–37
MaxV−t (EXTEM)	s	260 (101)	0.56	–
CT (INTEM)	s	245 (31)	0.54	134–218
t_{lag}	s	55 (16)	0.52	19.6–25.6

Table 2 Correlation between various blood coagulation assays. The Pearson correlation was used to detect the correlation between various blood coagulation tests. n.c., no significant correlation found; TG, thrombin generation; see Table 1 for definitions of the other abbreviations. Only significant correlations (P<0.05 for r) are shown

Assay	Quick (PT)	CT (FIBTEM)	C_{max} (TG)	CT (EXTEM)	MaxV−t (FIBTEM)	aPTT	MaxV−t (EXTEM)	CT (INTEM)
Quick (PT)	–	–	–	–	–	–	–	–
CT (FIBTEM)	−0.781	–	–	–	–	–	–	–
C_{max} (TG)	0.696	−0.682	–	–	–	–	–	–
CT (EXTEM)	−0.747	0.978	−0.596	–	–	–	–	–
MaxV−t (FIBTEM)	−0.795	0.972	−0.647	0.973	–	–	–	–
aPTT	−0.768	0.637	n.c.	0.616	0.609	–	–	–
MaxV−t (EXTEM)	−0.667	0.942	−0.579	0.972	0.944	0.514	–	–
CT (INTEM)	−0.740	0.786	n.c.	0.804	0.759	0.657	0.811	–
t_{lag} (TG)	−0.610	0.738	−0.587	0.741	0.684	n.c.	0.696	0.778

PCC (1 U ml^{-1}) increased C_{max} from baseline by 88 and 461%, respectively (Table 4).

Fibrinogen concentrate (3 mg ml^{-1}) prolonged both t_{lag} (by 27%) and t_{max} (by 26%) and significantly reduced C_{max} (by 9%). At the lower concentration (0.6 mg ml^{-1}), FI significantly prolonged t_{lag} by 15%. Prothrombin complex concentrate (1 U ml^{-1}) also significantly prolonged t_{lag} by 33%. Activated prothrombin complex concentrate at 2.25 and 1.5 U ml^{-1} prolonged t_{max} from baseline by 96 and by 80%, respectively.

Discussion

Rivaroxaban-spiked whole blood led to significant dose-dependent anticoagulant effects on various coagulation assay parameters. The AUC and EXTEM MaxV−t showed moderate correlation. Blood obtained from patients undergoing rivaroxaban treatment showed a strong correlation between rivaroxaban concentrations and Quick (PT) and moderate correlations with other coagulation assay parameters. All tested reversal agents significantly reduced EXTEM CT, but only aPCC and PCC were able to improve ETP parameters.

The results of the first part of the study are consistent with those of previous studies.[20–22] Concerning in vivo analysis of rivaroxaban effects, our results are partly consistent with those of previous investigations;[23–25] Oswald and colleagues[26] also found a significant increase in ROTEM EXTEM CT and INTEM CT, as did another study conducted in 11 healthy male volunteers with significant prolongation of EXTEM and INTEM CT, whereas EXTEM was more strongly correlated with rivaroxaban concentrations than INTEM CTs.[27] Rathbun and colleagues[28] also observed only C_{max} and t_{lag} to be correlated with rivaroxaban, and Oswald and colleagues[26] also found AUC to be unchanged. In contrast, several other studies revealed impairment of AUC and t_{max}.[14 29 30]

Regarding ex vivo reversal, previous studies have reported that PCC and aPCC increased the AUC, whereas rFVIIa and aPCC were most effective in correcting thromboelastometry.[15 31] It was also shown that PCC improved AUC but not t_{lag} or t_{max}.[29] In an in vitro study by Perzborn and colleagues,[21] PCC was able to correct AUC and t_{lag} but not C_{max}; aPCC and rFVIIa corrected all parameters. In an in vivo study in healthy volunteers that attempted to reverse the effects of rivaroxaban using PCC, the AUC was also
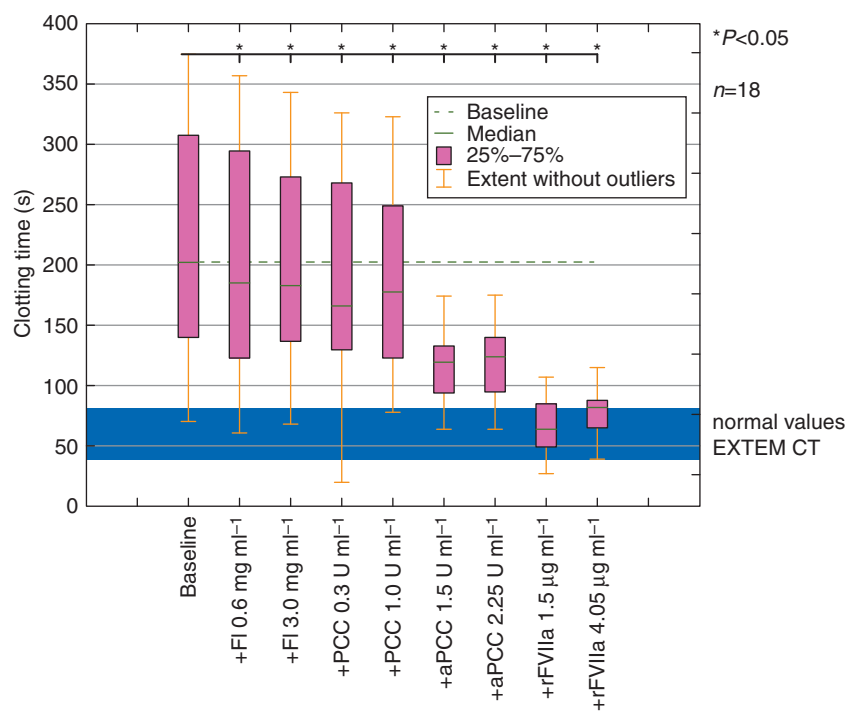
Fig 2 *Ex vivo* reversal of rivaroxaban-induced prolonged EXTEM clotting time (CT, in seconds) with potential non-specific reversal agents. Blood samples from patients on rivaroxaban (*n*=18, obtained 3 h after last medication intake) were defined as baseline values. Normal values (healthy persons without anticoagulation) of EXTEM CT are indicated (blue area). Blood samples were spiked with various non-specific reversal agents, as follows: fibrinogen concentrate (FI; FGTW, 0.6 or 3 mg ml^{-1}), prothrombin complex concentrate (PCC; Beriplex®, 0.3 or 1 U ml^{-1}), activated prothrombin complex concentrate (aPCC; FEIBA, 1.5 or 2.25 U ml^{-1}), and recombinant activated factor VII (rFVIIa; NovoSeven®, 1.5 or 4.05 µg ml^{-1}). Reversal from baseline was determined using Wilcoxon signed-rank test (*P<0.05).

Table 3 Thromboelastometry clotting times. *Ex vivo* reversal of rivaroxaban-induced prolonged clotting times with potential reversal agents. Blood samples were spiked with various non-specific reversal agents, as follows: fibrinogen concentrate (FI; FGTW), prothrombin complex concentrate (PCC; Beriplex®), activated prothrombin complex concentrate (aPCC; FEIBA), and recombinant activated factor VII (rFVIIa; NovoSeven®). All values are means (sd). *P<0.05 compared with baseline values (Wilcoxon signed-rank test)

Treatment	EXTEM (n=18) 42–78 s	INTEM (n=16) 134–218 s
Baseline	215 (89)	247 (31)
+FI 3 mg ml^{-1}	197 (81)*	254 (33)
+FI 0.6 mg ml^{-1}	195 (89)*	243 (37)
+PCC 1 U ml^{-1}	193 (66)*	293 (60)*
+PCC 0.3 U ml^{-1}	179 (82)*	265 (34)*
+aPCC 2.25 U ml^{-1}	121 (29)*	239 (40)
+aPCC 1.5 U ml^{-1}	114 (29)*	233 (38)
+rFVIIa 4.05 µg ml^{-1}	78 (18)*	195 (30)*
+rFVIIa 1.5 µg ml^{-1}	68 (23)*	221 (42)*

significantly increased by PCC.[13] Fibrinogen concentrate seemed to have an impairing effect on thrombin generation in our study, but it was able to correct ETP in a rabbit model of rivaroxaban overdose.[17]

When comparing the first and the second part of this study, there is correlation with regard to ROTEM parameters but not thrombin generation parameters. This discrepancy could be caused by the high concentration range of rivaroxaban used in the first part of the study and the much narrower concentration range used *in vivo*. Moreover, platelet-poor plasma was used for thrombin generation assays, and patients concomitantly took other medication, which probably influenced the results.

The influence of rivaroxaban on PT, aPTT, and thromboelastometry assays differs widely depending on the reagents used[32 33] and must be evaluated for each particular reagent. Consequently, normalizing effects of rivaroxaban on the various assays can also be expected to differ from reagent to reagent.

As expected, t_{lag} was strongly correlated with ROTEM CT, which is in line with previous investigations; a study in 100 patients with thrombophilia or haemophilia revealed that both methods were comparable.[34] After *ex vivo* addition of potential reversal agents, this correlation was not evident in the present study, which might be attributable to different methods and reagents or to the combination of *in vivo* rivaroxaban treatment and *ex vivo* administration of haemostatic interventions. Consequently, the efficacy of reversal of rivaroxaban seems to be highly dependent on the methods and parameters used and on whether experiments are conducted *in vivo* or not.

We showed that EXTEM and FIBTEM CT were comparable to plasma PT, whereas INTEM CT was comparable to plasma aPTT. Only rFVIIa was able to reverse INTEM CT to baseline values, which might be triggered by the 'Josso loop', in which FVIIa,

Table 4 Thrombin generation assay. Blood samples were spiked with various non-specific reversal agents, as follows: fibrinogen concentrate (FI; FGTW), prothrombin complex concentrate (PCC; Beriplex®), activated prothrombin complex concentrate (aPCC; FEIBA), and recombinant activated factor VII (rFVIIa; NovoSeven®) and analysed with thrombin generation. AUC, area under the curve; C_{max}, peak thrombin generation; t_{lag}, lag time until initiation; t_{max}, time to peak thrombin activity. All values are means (SD). *$P<0.05$ compared with baseline values (Wilcoxon signed-rank test)

Treatment	t_{lag} (n=13) 19.6–25.6 s	t_{max} (n=13) 50.8–72.0 s	AUC (n=13) 312–441 mE	C_{max} (n=13) 111–156 mE min⁻¹
Baseline	55 (16)	114 (37)	332 (20)	88 (21)
+FI 3 mg ml⁻¹	70 (14)*	144 (40)*	325 (111)	80 (22)*
+FI 0.6 mg ml⁻¹	63 (16)*	131 (29)	339 (90)	83 (15)
+PCC 1 U ml⁻¹	73 (32)*	180 (106)	686 (297)*	494 (1011)*
+PCC 0.3 U ml⁻¹	71 (15)	175 (46)	475 (204)*	88 (29)
+aPCC 2.25 U ml⁻¹	77 (10)	223 (27)*	1102 (434)*	165 (60)*
+aPCC 1.5 U ml⁻¹	70 (8)	205 (27)*	842 (434)*	136 (60)
+rFVIIa 4.05 µg ml⁻¹	63 (12)	139 (52)	392 (206)	96 (27)
+rFVIIa 1.5 µg ml⁻¹	63 (22)	164 (73)	362 (151)	78 (20)

originally part of the extrinsic coagulation pathway, can activate factor IX or the intrinsic coagulation pathway.[35] Prolongation of INTEM CT by PCC can be explained by the composition of PCC; PCC also contains anticoagulants, such as protein C, protein S, heparin, and antithrombin. Nilsson and colleagues[36] found a significant increase in INTEM CT by recombinant human activated protein C. The heparin effect of PCC has been reported previously.[37]

Prolongation of the thrombin generation assay parameters t_{lag} and t_{max} by addition of potential reversal agents can be explained by the test and reagents used, because other studies showed a reduction in t_{lag} after rFVIIa and aPCC for rivaroxaban.[15 21] Moreover, the prothrombin content of PCC and aPCC is a major determinant of their potential to generate excessive thrombin, which clearly influences the test outcome of thrombin generation assays. This also depends on the type of assay and type of chromogenic substrate or tissue factor content used, as demonstrated in other studies.[30]

All potential reversal agents in this study were added ex vivo, and dose-dependent reversal effects of potential reversal agents cannot be detected in vitro, as already reported by Perzborn and colleagues.[21] This constitutes a major limitation of the present study, because the discrepancy between the in vitro and in vivo measurements and the ex vivo addition of potential reversal agents emphasizes the difficulty in demonstrating in vivo effects ex vivo. It also highlights the need for in vivo investigations and controlled clinical trials to evaluate the real potential of non-specific reversal agents.

The potential risk for thromboembolic events has to be evaluated before using any potential reversal agent to reverse rivaroxaban-induced bleeding.[38] No controlled clinical studies in humans using reversal agents in bleeding situations are available. This illustrates a difficulty in evaluating potential reversal agents for the management of life-threatening bleeding in rivaroxaban-anticoagulated patients.

Our data indicate that great care must be taken when analysing data gained in vitro and translating such data to in vivo situations. It is not clear which reversal agent is best for the treatment of bleeding patients treated with rivaroxaban, and thrombin generation assay and ROTEM® do not favour the same agents. Coagulation assays cannot predict the bleeding tendency, and animal models might not be representative for human models. Therefore, controlled clinical trials are needed, even if specific antidotes are in development, because not all hospitals will be able to store these antidotes, and there is still little knowledge about the real efficacy of alternative reversal agents.

Authors' contributions

Study concept and design: W. Streif, D.F., M.B., B.S.
Application to ethics committee and authorities: B.S., D.F., M.B.
Patient recruitment: W. Sturm
Data acquisition: B.S., P.W., W. Streif, W. Sturm
Laboratory experiments: B.S., P.W., M.B.
Data analysis and interpretation: B.S., W. Sturm, D.F.
Writing of the first draft of the manuscript: B.S.
Responsible for data integrity and accuracy of data analysis: B.S.
Critical revision of the manuscript for important intellectual content: P.W., W. Streif, W. Sturm, D.F., M.B.

Supplementary material

Supplementary material is available at *British Journal of Anaesthesia* online.

Declaration of interest

D.F. has received study funding and honoraria for consultancy and board activity from Astra Zeneca, AOP orphan, Baxter, Baer, BBraun, Biotest, CSL Behring, Delta Select, Dae Behring, Edwards, Fresenius, Glaxo, Haemoscope, Hemogem, Lilly, LFB, Mitsubishi Pharma, NovoNordisk, Octapharm, Pfizer, and Tem-Innovation. All other authors declare that they have no conflicts of interest.

References

1. Haas S, Spannagl M, Schellong SM. Novel oral anticoagulants – key messages for the angiologist. *Vasa* 2012; **41**: 177–91
2. Eikelboom JW, Weitz JI. New anticoagulants. *Circulation* 2010; **121**: 1523–32
3. Varughese CJ, Halperin JL. Prevention of stroke in patients with atrial fibrillation: anticoagulant and antiplatelet options. *J Interv Card Electrophysiol* 2012; **35**: 19–27
4. Abraham ME, Marcy TR. Warfarin versus dabigatran: comparing the old with the new. *Consult Pharm* 2012; **27**: 121–4
5. Riva N, Lip GYH. A new era for anticoagulation in atrial fibrillation. Which anticoagulant should we choose for long-term prevention of thromboembolic complications in patients with atrial fibrillation? *Pol Arch Med Wewn* 2012; **122**: 45–52
6. Turagam MK, Velagapudi P, Leal MA, Kocheril AG. Aspirin in stroke prevention in nonvalvular atrial fibrillation and stable vascular disease: an era of new anticoagulants. *Expert Rev Cardiovasc Ther* 2012; **10**: 433–9
7. Levy JH, Spyropoulos AC, Samama CM, Douketis J. Direct oral anticoagulants: new drugs and new concepts. *JACC Cardiovasc Interv* 2014; **7**: 1333–51
8. Fox KA, Piccini JP, Wojdyla D, *et al*. Prevention of stroke and systemic embolism with rivaroxaban compared with warfarin in patients with non-valvular atrial fibrillation and moderate renal impairment. *Eur Heart J* 2011; **32**: 2387–94
9. McDonald CJ, Kalisch Ellett LM, Barratt JD, Caughey GE. A cross-country comparison of rivaroxaban spontaneous adverse event reports and concomitant medicine use with the potential to increase the risk of harm. *Drug Saf* 2014; **37**: 1029–35
10. Kaatz S, Kouides PA, Garcia DA, *et al*. Guidance on the emergent reversal of oral thrombin and factor Xa inhibitors. *Am J Hematol* 2012; **87** Suppl 1: S141–5
11. Ferrandis R, Castillo J, de Andrés J, *et al*. The perioperative management of new direct oral anticoagulants: a question without answers. *Thromb Haemost* 2013; **110**: 515–22
12. Siegal DM, Curnutte JT, Connolly SJ, *et al*. Andexanet alfa for the reversal of factor Xa inhibitor activity. *N Engl J Med* 2015; **373**: 2413–24
13. Eerenberg ES, Kamphuisen PW, Sijpkens MK, Meijers JC, Buller HR, Levi M. Reversal of rivaroxaban and dabigatran by prothrombin complex concentrate: a randomized, placebo-controlled, crossover study in healthy subjects. *Circulation* 2011; **124**: 1573–9
14. Levi M, Moore KT, Castillejos CF, *et al*. Comparison of three-factor and four-factor prothrombin complex concentrates regarding reversal of the anticoagulant effects of rivaroxaban in healthy volunteers. *J Thromb Haemost* 2014; **12**: 1428–36
15. Marlu R, Hodaj E, Paris A, Albaladejo P, Crackowski JL, Pernod G. Effect of non-specific reversal agents on anticoagulant activity of dabigatran and rivaroxaban. A randomised crossover ex vivo study in healthy volunteers. Thromb Haemost 2012; **108**: 217–24
16. Perzborn E, Gruber A, Tinel H, *et al*. Reversal of rivaroxaban anticoagulation by haemostatic agents in rats and primates. *Thromb Haemost* 2013; **110**: 162–72
17. Godier A, Miclot A, Le Bonniec B, Durand M, Lecompte T, Samama CM. Evaluation of prothrombin complex concentrate, recombinant activated factor VII and fibrinogen concentrate to reverse rivaroxaban in a rabbit model of bleeding and thrombosis. *Eur J Anaesthesiol* 2011; **28**: 87
18. Martin AC, Le Bonniec B, Fischer AM, *et al*. Evaluation of recombinant activated factor VII, prothrombin complex concentrate, and fibrinogen concentrate to reverse apixaban in a rabbit model of bleeding and thrombosis. *Int J Cardiol* 2013; **168**: 4228–33
19. Lang T, Bauters A, Braun SL, *et al*. Multi-centre investigation on reference ranges for ROTEM thromboelastometry. *Blood Coagul Fibrinolysis* 2005; **16**: 301–10
20. Körber MK, Langer E, Ziemer S, Perzborn E, Gericke C, Heymann C. Measurement and reversal of prophylactic and therapeutic peak levels of rivaroxaban: an in vitro study. *Clin Appl Thromb Hemost* 2014; **20**: 735–40
21. Perzborn E, Heitmeier S, Laux V, Buchmüller A. Reversal of rivaroxaban-induced anticoagulation with prothrombin complex concentrate, activated prothrombin complex concentrate and recombinant activated factor VII *in vitro*. *Thromb Res* 2014; **133**: 671–81
22. Gerotziafas GT, Elalamy I, Depasse F, Perzborn E, Samama MM. In vitro inhibition of thrombin generation, after tissue factor pathway activation, by the oral, direct factor Xa inhibitor rivaroxaban. *J Thromb Haemost* 2007; **5**: 886–8
23. Perzborn E, Strassburger J, Wilmen A, *et al*. In vitro and in vivo studies of the novel antithrombotic agent BAY 59-7939—an oral, direct factor Xa inhibitor. *J Thromb Haemost* 2005; **3**: 514–21
24. Asmis LM, Alberio L, Angelillo-Scherrer A, *et al*. Rivaroxaban: quantification by anti-FXa assay and influence on coagulation tests: a study in 9 Swiss laboratories. *Thromb Res* 2012; **129**: 492–8
25. Altman R, Gonzalez CD. Simple and rapid assay for effect of the new oral anticoagulant (NOAC) rivaroxaban: preliminary results support further tests with all NOACs. *Thromb J* 2014; **12**: 1477–9560
26. Oswald E, Velik-Salchner C, Innerhofer P, *et al*. Results of rotational thromboelastometry, coagulation activation markers and thrombin generation assays in orthopedic patients during thromboprophylaxis with rivaroxaban and enoxaparin: a prospective cohort study. *Blood Coagul Fibrinolysis* 2015; **26**: 136–44
27. Casutt M, Konrad C, Schuepfer G. Effect of rivaroxaban on blood coagulation using the viscoelastic coagulation test ROTEM™. *Anaesthesist* 2012; **61**: 948–53
28. Rathbun S, Tafur A, Grant R, Esmon N, Mauer K, Marlar RA. Comparison of methods to determine rivaroxaban anti-factor Xa activity. *Thromb Res* 2015; **135**: 394–7
29. Dinkelaar J, Molenaar PJ, Ninivaggi M, de Laat B, Brinkman HJ, Leyte A. In vitro assessment, using thrombin generation, of the applicability of prothrombin complex concentrate as an antidote for rivaroxaban. *J Thromb Haemost* 2013; **11**: 1111–8
30. Graff J, von Hentig N, Misselwitz F, *et al*. Effects of the oral, direct factor Xa inhibitor rivaroxaban on platelet-induced thrombin generation and prothrombinase activity. *J Clin Pharmacol* 2007; **47**: 1398–407
31. Escolar G, Arellano-Rodrigo E, Lopez-Vilchez I, *et al*. Reversal of rivaroxaban-induced alterations on hemostasis by different coagulation factor concentrates. *Circ J* 2015; **79**: 331–8
32. Hillarp A, Baghaei F, Fagerberg Blixter I, *et al*. Effects of the oral, direct factor Xa inhibitor rivaroxaban on commonly used coagulation assays. *J Thromb Haemost* 2011; **9**: 133–9
33. Halbmayer WM, Weigel G, Quehenberger P, *et al*. Interference of the new oral anticoagulant dabigatran with frequently used coagulation tests. *Clin Chem Lab Med* 2012; **50**: 1601–5
34. Siegemund A, Saile S, Siegemund T, Scholz U, Schobess R. Thrombin generation—comparison of ROTEM and technothrombin TGA. In: Scharrer I, Schramm W, eds. *37th Hemophilia Symposium*. Berlin, Heidelberg: Springer, 2008; 212–6

35. Josso F, Prou-Wartelle O. Interaction of tissue factor and factor VII at the earliest phase of coagulation. *Thromb Diath Haemorrh Suppl* 1965; **17**: 35–44

36. Nilsson CU, Hellkvist PD, Engström M. Effects of recombinant human activated protein C on the coagulation system: a study with rotational thromboelastometry. *Acta Anaesthesiol Scand* 2008; **52**: 1246–9

37. Scharbert G, Thaler U, Weilnböck C, Wetzel L, Kozek-Langenecker S. Heparin-induced effects of prothrombin complex concentrates in thromboelastometry. *Wien Klin Wochenschr* 2012; **124**: 320–5

38. Levi M, Levy JH, Andersen HF, Truloff D. Safety of recombinant activated factor VII in randomized clinical trials. *N Engl J Med* 2010; **363**: 1791–800

Handling editor: H. C. Hemmings