Role of Coagulation Factor Concentrates for Reversing Dabigatran-related Anticoagulation

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THE novel oral anticoagulation agents (NOACs) include dabigatran, a direct thrombin inhibitor, and rivaroxaban, apixaban, and edoxaban, direct Factor Xa inhibitors, indicated for either stroke prevention with nonvalvular atrial fibrillation or thromboprophylaxis.[1,2] The NOACs are administered orally, have predictable onsets within 2 to 4 h, and an offset more rapid than warfarin. Although monitoring is not required, dabigatran's effects can be assessed using standard coagulation assays such as thrombin times and partial thromboplastin times (PTTs), whereas Xa inhibitors require more specialized antifactor-Xa assays. However, managing any anticoagulated critically ill patient is problematic, because all anticoagulation agents can cause bleeding, and patients were anticoagulated for a specific prothrombotic issue. Despite concerns about the NOACs, warfarin and other commonly used anticoagulants including low-molecular-weight heparin are not completely reversible with standard therapies. For acute warfarin reversal, despite the extensive use of plasma, four-component prothrombin complex concentrates (PCCs) are recommended in recent guidelines, and now available in the United States.[3,4] Unlike warfarin, NOACs' anticoagulation effects normalize in approximately 24 to 48 h after discontinuing the drug with normal renal function. However, after trauma, emergency surgery, or major bleeding, acute therapeutic considerations are needed. Current information to help clinicians make decisions about managing bleeding in patients receiving NOACs is based on indirect information derived from multiple sources including *in vitro* data using human blood, animal models, and from volunteers anticoagulated with NOACs.[2]

In this month's Anesthesiology, van Ryn *et al.*[5] evaluated different coagulation factor concentrates (CFCs) to reverse bleeding induced by dabigatran etexilate using a rat tail bleeding model. CFCs available in most countries were compared including three-factor and four-factor PCCs, an activated PCC (factor VIII inhibitor bypassing activity [FEIBA]), and recombinant factor VIIa.[4] They used multiple coagulation tests to evaluate anticoagulation and reversal including PTT, thrombin times, ecarin clotting time, and prothrombin time. They also evaluated thrombin generation using a sensitive fluorometric assay with human plasma spiked with dabigatran at therapeutic and supratherapeutic values. Dabigatran increased bleeding times in the animal model approximately 2.7-fold with supratherapeutic dabigatran levels, and all the CFCs studied significantly reversed this prolonged bleeding time, usually returning to baseline within 5 min. However, the recombinant factor VIIa dose required was 500 μg/kg, a dose approximately five- to six-fold higher than that used for hemophilia with inhibitors, lower doses were ineffective in the model. The standard coagulation tests were prolonged three- to eight-fold over baseline dosing, and despite reducing bleeding, there were minimal changes in coagulation tests, and the assays were not predictive of bleeding reversal. In human blood samples, restoration of thrombin generation was dependent on the dabigatran concentration. Dabigatran reversal occurred at concentrations seen with therapeutic levels, but not at supratherapeutic levels. In summary, in the animal model, CFCs reversed dabigatran-induced bleeding, but routine coagulation assays did not predict this reversal, but there was variability based on the level of anticoagulation.[5]

Novel to clinicians in the United States is the application of PCCs as a therapeutic approach to bleeding. In the current study, higher doses of one four-component PCC (Beriplex/KCENTRA; CSL Behring, Marburg, Germany/King of

"… [coagulation factor concentrates] shortened bleeding time in the presence of dabigatran, but standard tests including [partial thromboplastin time] and prothrombin time did not predict this effect."

Image: M. R. England.

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Prussia, PA) produced complete hemostasis with formation of stable wound clots that did not reopen. This finding is consistent with different dosing strategies for PCC reversal of warfarin. The variability of CFCs on laboratory tests despite their beneficial effects on bleeding times may be related to heparin in certain CFCs, and the thrombin times and PTT are sensitive to heparin. As the authors note, the PTT is generally insensitive to CFCs, which predominantly affect the prothrombin time/international normalized ratio. In the current study, the lack of effect of dabigatran on anticoagulation reversal based on laboratory testing may also relate to the assays used. The lack of correlation between *in vivo* bleeding and coagulation assay response was also observed in a recent trial comparing four-factor PCC with plasma in patients treated with vitamin K antagonists with major bleeding.[6]

A novel test used in this study is the thrombin-generation assay that we have used in many studies evaluating anticoagulant and procoagulant effects of different agents. This assay is an important way to examine thrombin generation, a sensitive indicator of hemostatic activation, and critical to clot formation. In the current study, dabigatran decreased thrombin generation, whereas most of the CFCs increased thrombin generation except those containing heparin. Thrombin generation was increased in dabigatran-anticoagulated blood with several three- and four-factor PCCs and the activated PCC FEIBA. In plasma with therapeutic dabigatran levels, most CFCs increased thrombin generation but were not effective at supratherapeutic dabigatran concentrations. Of note was recombinant factor VIIa did not restore thrombin generation.

There are several important insights to consider from this study. This report is the first to compare all available CFCs on dabigatran-related bleeding. The "clinical end point" in this model was bleeding as measured by prolongation of bleeding times and noted that CFCs shortened bleeding time in the presence of dabigatran, but standard tests including PTT and prothrombin time did not predict this effect. This observation is consistent with the problems of using coagulation testing as predictors of bleeding and the lack of correlation between laboratory tests and clinical bleeding. As an example, in a factor XII–deficient patient, the baseline PTT is greatly prolonged, but patients have no clinically relevant bleeding issues. Also, coagulation tests can be corrected without correcting the underlying hemostatic defect as we have reported where recombinant factor VIIa corrects the international normalized ratio of warfarin-treated patients, but only PCCs actually restore thrombin generation.[7] One explanation for stopping bleeding without restoration of *in vitro* coagulation tests may be explained by prohemostatic effects at the site of vascular injury where tissue factor is expressed, thrombin generation can be intensified, and CFCs produce localized rather than systemic effects.

Another important aspect of the study by van Ryn is that it reports the potential for PCCs to mitigate the effects of dabigatran *in vivo*, even though these effects cannot be observed by standard coagulation testing. Also, this study gives clinicians some hope that PCCs might be helpful when treating dabigatran-related bleeding in patients as previous experiments provided minimal hope for benefit from PCCs, but it does not guarantee successful treatment of NOAC-related bleeding in humans by PCCs. Perhaps, the most interesting point that comes from this study is that exogenously added PCCs can enhance thrombin generation to the point where this "extra" thrombin has the potential to potentially "overcome" the anticoagulant effect of dabigatran. However, this effect is dependent on the NOAC concentration, and whether PCCs will stop bleeding in patients following overdosage, particularly when NOAC concentrations are high, remains to be determined.

What are the take-home messages for clinicians? As we described in our recent review in Anesthesiology, CFCs represent part of an important multimodal therapeutic plan in treating bleeding with NOACs, and initial measures should include hemodynamic and hemostatic resuscitation with volume and vasoactive support, identification of the bleeding source, and attempts at local hemostatic control.[2] For dabigatran, hemodialysis or hemoperfusion may be potential options but may not be feasible in emergencies or patients in shock.[8] As part of a multimodal approach, the off label use of PCCs including FEIBA represents a logical approach to treating bleeding when it occurs in dabigatran-treated patients. Overall, therapy of bleeding should be multimodal, with repletion and normalization of hemostasis with CFCs and transfusion factors.[2]

Despite concerns about NOACs and bleeding, a recent report compared the management and prognosis of major bleeding from randomized clinical trials of dabigatran or warfarin.[9] From 27,419 patients treated for 6 to 36 months, 1,034 patients had 1,121 major bleeds. The 30-day mortality after the first major bleed was 9.1% in the dabigatran group compared with 13.0% in the warfarin group, and dabigatran-treated patients required a shorter intensive care unit stay compared with that in warfarin-treated patients.[9] As I initially stated, managing any anticoagulated critically ill patient is problematic, because all anticoagulation agents can cause bleeding, and patients were anticoagulated for a specific prothrombotic issue. Despite the relative safety of NOACs compared with warfarin, additional clinical studies are needed to best determine the optimal therapy for bleeding when it occurs. Of note is a specific reversal agent currently under development for dabigatran, using an immunospecific Fab fragment (BI 655075).[10] This novel therapeutic approach is currently in early clinical testing and will soon enter into clinical trials.

Competing Interests

Dr. Levy serves on Steering Committees for Boehringer-Ingelheim (Ingelheim, Germany), CSL Behring (King of Prussia, Pennsylvania), J&J (Raritan, New Jersey), and Grifols (Research Triangle Park, North Carolina).

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Reversal of Dabigatran-induced Bleeding by Coagulation Factor Concentrates in a Rat-tail Bleeding Model and Lack of Effect on Assays of Coagulation

Joanne van Ryn, Ph.D., Johanna Schurer, Monika Kink-Eiband, Andreas Clemens, M.D.

ABSTRACT

Background: Dabigatran is a potent oral anticoagulant. Like any anticoagulant, there is an increased risk of bleeding associated with its use, and reversal may be needed in cases of severe bleeding.

Methods: In this study, six coagulation factor concentrates (CFCs) were tested for their ability to reduce bleeding induced by oral dabigatran etexilate (30 mg/kg) in a rat-tail bleeding model (n = 5 to 8 per group): three-factor (Profilnine [Grifols Biologicals Inc., Los Angeles, CA] and Bebulin [Baxter BioScience, Westlake Village, CA]) and four-factor prothrombin complex concentrates (Beriplex [CSL Behring, Marburg, Germany] and Octaplex [Octapharma AG, Lachen, Switzerland]), activated prothrombin complex concentrate (Factor Eight Inhibitor Bypassing Activity; Baxter AG, Vienna, Austria), and recombinant factor VIIa (NovoSeven; NovoNordisk, Bagsværd, Denmark). The effect of CFCs on prolongation of coagulation assays was measured. Thrombin generation after administration of each CFC was compared *in vitro* using human plasma (n = 5) spiked with dabigatran in concentrations corresponding to median peak (200 ng/ml) and supratherapeutic values (600 and 1,000 ng/ml).

Results: Dabigatran resulted in an approximately three-fold increase in bleeding time, consistent with supratherapeutic dabigatran plasma levels. Beriplex (35 and 50 IU/kg), Octaplex (40 IU/kg), Profilnine (50 IU/kg), Bebulin (60 IU/kg), Factor Eight Inhibitor Bypassing Activity (100 U/kg), and NovoSeven (500 μg/kg) significantly decreased this prolonged bleeding time over 30 min ($P < 0.001$). The coagulation assays were prolonged three- to eight-fold over baseline ($P = 0.01$). None of the CFCs produced a consistent change in these assays that was predictive of reduced bleeding. Thrombin generation reversal was dependent on the concentration of dabigatran and each CFC; normalization occurred at the lower concentration of dabigatran with most CFCs, but not at higher concentrations.

Conclusions: In this animal model, bleeding induced by high doses of dabigatran can be reduced by CFCs. However, routine coagulation assays do not predict this effect. **(Anesthesiology 2014; 120:1429-40)**

DABIGATRAN is a new oral anticoagulant used for stroke prevention in patients with nonvalvular atrial fibrillation. It is also effective for prophylaxis and treatment of venous thromboembolism. Like any anticoagulant, there is a risk of bleeding associated with its use. In particular, reversal of its anticoagulant effect may be needed in the event of severe bleeding or emergency surgery.[1]

Currently, there is no specific reversal approach available for dabigatran or any of the other novel oral anticoagulants although specific antidotes are in development.[2,3] In the case of non–life-threatening bleeding episodes, discontinuation of dabigatran and compression at the source of bleeding are usually sufficient.[1] However, in major or life-threatening bleeding where compression is not feasible or for emergency surgery, rapid reversal is needed. This has prompted investigation of the use of coagulation factor concentrates (CFCs) known to reverse hemostatic defects and enhance wound-localized thrombin generation.[4]

What We Already Know about This Topic

- Dabigatran is a potent anticoagulant that, as with all anticoagulants, is associated with an elevated risk of bleeding
- Whether the anticoagulant effect can be acutely reversed and if so, whether this can be assessed using coagulation assays is not known

What This Article Tells Us That Is New

- Administration of high-dose dabigatran etexilate to rats resulted in an increase in bleeding time which could be reduced by subsequent administration of coagulation factor concentrates
- Routine coagulation assays did not predict the effect of coagulation factor concentrates to reduce bleeding from dabigatran

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Prothrombin complex concentrates (PCCs) contain all the vitamin K–dependent coagulation factors and are therefore useful for the rapid reversal of coagulopathy and restoration of normal hemostasis in the setting of overanticoagulation induced by vitamin K antagonists.[5] Several nonactivated PCCs are approved for use, each effective in shortening the time to prothrombin time (PT)/international normalized ratio correction with a low risk of thrombotic adverse events.[5] These PCCs have variable composition and include four nonactivated vitamin K–dependent factors II, VII, IX, and X in similar ratios, together with anticoagulant proteins such as protein C and S; three-factor concentrates with relatively low amounts of factor VII (less than one third compared with factor IX)[4,6]; and an activated four-factor PCC containing factors II, IX, X, and protein C mainly in nonactivated forms and factor VII mainly in the activated form (table 1). In addition, recombinant activated factor VIIa (rFVIIa), an approved potent procoagulant and general hemostatic agent that can initiate hemostasis at sites of bleeding by direct activation of thrombin on the surface of platelets in the absence of tissue factor,[7] may have potential in reversing the anticoagulant effects.

The aim of this study was to compare six different CFCs for their ability to reduce bleeding induced by overdose of oral dabigatran in a rat-tail bleeding model. In addition, to determine the effect of different concentrations of dabigatran on anticoagulation, *in vitro* thrombin generation with increasing concentrations of each CFC was compared in human plasma spiked with dabigatran.

Materials and Methods

Reagents and Drugs

Dabigatran etexilate (Pradaxa®, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany) was prepared freshly as homogeneous 0.3% aqueous suspension in Natrosol® (Boehringer Ingelheim, Biberach, Germany) and hydroxyethylcellulose 0.5% in distilled water with the Covaris Ultrasonicator (Covaris Inc., Woburn, MS).

The six CFCs tested were NovoSeven® 90, 270, and 500 μg/kg (rFVIIa; NovoNordisk, Bagsværd, Denmark), Factor Eight Inhibitor Bypassing Activity NanoFiltered (FEIBA® NF; Baxter AG, Vienna, Austria) 100 U/kg, Beriplex® P/N 25, 35, and 50 IU/kg (CSL Behring, Marburg, Germany), Octaplex® 40 IU/kg (Octapharma AG, Lachen, Switzerland), Profilnine® SD 50 IU/kg (Grifols Biologicals Inc., Los Angeles, CA), and Bebulin® VH 60 IU/kg (Baxter BioScience, Westlake Village, CA). Dose selection was based on results in previous animal models[8] and current label recommendations.[9–14] The effects of different doses of NovoSeven and Beriplex on bleeding and systemic coagulation were also investigated.

Bleeding Model

All experimental procedures were approved by the Regional Governmental Animal Care and Use Office (Tübingen, Germany) and conducted in accordance with the German Animal Protection Act (Deutsches Tierschutzgesetz). A rat-tail incision bleeding model, as described by Gustafsson *et al.*,[15] was used with slight modifications using Hann Wistar rats (200 to 250 g body weight).

Table 1. Summary of Coagulation Factor Concentrates and the Composition According to the Label of Each Manufacturer

		Clotting Factors				Anticoagulant Proteins			
Product	Availability	FII	FVII	FIX	FX	Protein C	Protein S	AT	Heparin*
Three-factor PCCs									
Bebulin VH[10]	U.S.	24–38 IU/ml	<5 IU/ml	24–38 IU/ml	24–38 IU/ml	NA	NA	NA	<0.15 IU/IU FIX
Profilnine SD[11]	U.S.	NMT 150 U/100 FIX units	NMT 35 U/100 FIX units	100 U	100 U/100 FIX units	NA	NA	NA	None
Four-factor PCCs									
Beriplex P/N[12]	Europe, Canada, U.S. (as Kcentra)	20–48 IU/ml	10–25 IU/ml	20–31 IU/ml	22–60 IU/ml	15–45 IU/ml	12–38 IU/ml	0.2–1.5 IU/ml	0.4–2 IU/ml
Octaplex[13]	Europe, Canada	14–38 IU/ml	9–24 IU/ml	25 IU/ml	18–30 IU/ml	13–31 IU/ml	12–32 IU/ml	NA	5–12.5 IU/ml
rFVIIa									
NovoSeven[14]	None†	None	0.6 mg/ml	None	None	None	None	None	None
Activated PCC									
FEIBA NF[15]	U.S., Europe	1.3 U/U	0.9 U/U‡	1.4 U/U	1.1 U/U	1.1 U/U	NA	NA	None

Bebulin (Baxter BioScience, Westlake Village, CA); Beriplex (CSL Behring, Marburg, Germany); FEIBA (Baxter AG, Vienna, Austria); NovoSeven (NovoNordisk, Bagsværd, Denmark); Octaplex (Octapharma AG, Lachen, Switzerland); Profilnine (Grifols Biologicals Inc., Los Angeles, CA).

* Reported as anti-Xa levels in IU/ml. † Not approved in any country for this indication. ‡ Factor VII is mainly in the activated form.

AT = antithrombin; FEIBA = Factor Eight Inhibitor Bypassing Activity; FIX = factor IX; NA = information not available in product label; NMT = not more than; PCC = prothrombin complex concentrate; rFVIIa = recombinant activated factor VII; U.S. = United States.

After fasting overnight, a single oral dose of dabigatran etexilate 30 mg/kg at a volume of 10 ml/kg body weight or treatment vehicle (same volume, control) was administered *via* gastric lavage. Dose selection was based on current experience, including previous findings in this model,[16] showing that peak plasma dabigatran concentration is achieved after approximately 45 min. Twenty-five minutes after oral treatment, the rats were anesthetized with sodium pentobarbital (Narcoren®; Merial GmbH, Hallbergmoos, Germany), 60 mg/kg intraperitoneally bolus followed by 20 $mg \cdot kg^{-1} \cdot h^{-1}$ infusion and placed on a 37°C heating pad to maintain internal body temperature during the experiment. Cannulas were placed in the left jugular vein for administration of the CFCs and the right carotid artery for blood sampling. The CFC or vehicle (n = 5 to 8 per group) was administered as an IV bolus 45 min after oral dabigatran etexilate.

Bleeding time was measured using a spring-loaded blade device (Surgicutt®; Loxo, Dossenheim, Germany), which was applied longitudinally on the surface of the tail, starting 2 to 3 cm from the tail root (9 cm from the tip), avoiding large vessels. Blood flow from the incision was blotted using filter paper held directly below, but not touching the wounds. The position of the filter paper was changed every 15 s until the filter paper no longer developed a red crescent (end of bleeding). Bleeding time was defined as the time from start to cessation of bleeding and was measured 5, 15, and 30 min after administration of the CFCs or vehicle (50, 60, and 75 min after administration of oral dabigatran etexilate).

Anticoagulant Activity, Ex Vivo

Arterial blood samples (2 ml, total volume) were taken at the same time as bleeding time measurements and collected in sodium citrate (final concentration of 0.313%). As a result of limitations due to blood volume and clotting of some blood samples, coagulation assays were not performed in all animals; however, there was a minimum sample size of five tests per assay in each treatment group. Assays of thrombin time (TT), ecarin clotting time (ECT), activated partial thromboplastin time (aPTT), and PT were performed using standard methods with a CL4 coagulation analyser (Behnk Elektronik GmbH & Co. KG, Norderstedt, Germany), as reported previously.[16]

Plasma concentrations of dabigatran in the rat model were determined by the clotting-based Hemoclot thrombin inhibitor assay (HYPHEN BioMed, Neuville sur-Oise, France).[17] This test uses a calibration curve to determine the concentration of dabigatran in plasma based on measurement by the diluted TT. To determine whether addition of CFCs could bias the assay result, increasing concentrations of dabigatran (50 to 1,000 ng/ml) were added to pooled human plasma obtained from healthy volunteers (after written informed consent) and incubated with CFCs for 5 min. Concentrations equivalent to plasma levels of the highest dose of each CFC were tested (NovoSeven at 100 nM, all others at 1 IU/ml). The diluted TT with and without addition of the CFCs was measured. Further details of the procedures and reagents are described in Supplemental Digital Content 1, http://links.lww.com/ALN/B45.

Thrombin-generation Assay, In Vitro

Measurement of thrombin generation in clotting human plasma was performed using the Calibrated Automated Thrombinography method with a Fluoroscan Ascent plate reader (Thermo Lab systems, Helsinki, Finland).[18] Human plasma was obtained from five healthy volunteers after written, informed consent.

Each CFC was tested over a concentration range that included plasma concentrations typically achieved by patients treated with recommended clinical therapeutic doses, based on Factor IX units specified by the manufacturers. For example, Beriplex is recommended at doses between 25 and 50 IU/kg, which would result in plasma levels of 0.35 to 0.7 IU/ml.[11] NovoSeven, although not recommended for reversal of anticoagulant-induced bleeding, has been used off-label at doses between 90 and 180 μg/kg corresponding to plasma concentrations of 2.6 μg/ml (52 nM). In each case, the CFC was added *in vitro* to platelet-poor plasma samples (1:100 dilution in plasma) obtained from healthy volunteers (n = 5) after addition of dabigatran (200, 600, and 1,000 ng/ml). Dabigatran 200 ng/ml corresponded to the approximate median peak concentration at steady state after administration of dabigatran etexilate 150 mg twice-daily,[19,20] whereas dabigatran 600 and 1,000 ng/ml were supratherapeutic concentrations that might be observed in patients with bleeding events.[21–23] Thrombin generation was initiated by addition of a platelet-poor plasma reagent containing 15 pmol/l of tissue factor and 4 pmol/l of phospholipids (Thrombinoscope BV, Maastricht, The Netherlands). Thrombin generation curves were calculated using thrombinoscope software (version 3.0.0.29; Thrombinoscope BV) to determine lag time (in min), peak concentration of thrombin, and endogenous thrombin potential (ETP, calculated as the area under the thrombin-time integral, nM × min, which reflects the total amount of thrombin generated as a function of time).

Statistical Analysis

Data from individual animals in each treatment group were combined and reported with descriptive statistics (mean ± SEM). Initially three to seven animals were studied in each treatment group. During the review process, additional experiments were added in each treatment group (n = 5 to 8 per group). Only those animals with a dabigatran plasma concentration of 200 ng/ml or greater before addition of each CFC were included in the analysis of bleeding time. This criterion was based on historical data showing that plasma concentrations of less than 200 ng/ml do not consistently prolong bleeding time, therefore, compromising the ability to show bleeding reversal with CFCs. The effects of the CFCs on bleeding time over a 30-min measurement period were compared between the treated groups and control (dabigatran alone) using a two-way ANOVA model, with two-tailed significance testing at 95% and a Bonferroni *post hoc* analysis. The ANOVA model

defined treatment as the between subjects factor and time as the repeated measures factor. No attempts were made to adjust the *P* values for a preliminary (interim) analysis.

Differences in initial dabigatran plasma levels between treatment groups were compared using an ANOVA and the Dunnett *post hoc* test for multiple comparisons. Coagulation assay results were calculated as the ratio of baseline measurements before administration of each CFC. Both the coagulation assays and *in vitro* ETP measurements were also tested using a two-way ANOVA. As previously mentioned, two-tailed significance testing at 95% was used with a Bonferroni *post hoc* analysis. The coagulation assay model defined treatment and time, whereas ETP model used dabigatran and each CFC as the two factors used to define any potential interaction. A *P* value of less than 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism (Version 5.01; GraphPad Software Inc., La Jolla, CA).

Results

A total of 72 animals were included in the study. Data from two animals were excluded from analysis, one each in the Beriplex 35 IU/kg and FEIBA groups, as initial dabigatran plasma levels were less than 200 ng/ml (159 and 180 ng/ml, respectively).

Effect of CFCs on Bleeding Time Prolonged by High Dose of Oral Dabigatran Etexilate

In the control group, mean bleeding time was 160.0 ± 12.0 s over the 30-min measurement period (fig. 1). Oral treatment with 30 mg/kg dabigatran etexilate resulted in a significant 2.3- to 2.7-fold prolongation of bleeding time over 30 min (ranging from 427.5 ± 43.1 s at 5 min to 371.3 ± 31.0 s at 30 min) (fig. 1), consistent with the supratherapeutic dabigatran plasma levels achieved (443 to 735 ng/ml). There was a dose-dependent reduction in bleeding time with increasing doses of Beriplex (25, 35, and 50 IU/kg IV) ($P < 0.001$); all doses significantly reducing bleeding time within 5 min of administration compared with dabigatran alone (fig. 1A); this was maintained for the two higher doses over 30 min but not for the lowest dose of Beriplex (25 IU/kg) when a new incision was made. Administration of NovoSeven also had a dose-dependent effect on dabigatran-induced bleeding ($P < 0.001$) (fig. 1B), the two lower doses had no significant effect on reducing bleeding time; however, the highest dose (500 μg/kg) reduced bleeding time to control levels over 30 min ($P < 0.001$ *vs.* dabigatran, nonsignificant *vs.* control). Octaplex and FEIBA (fig. 1C) as well as the three-factor coagulation

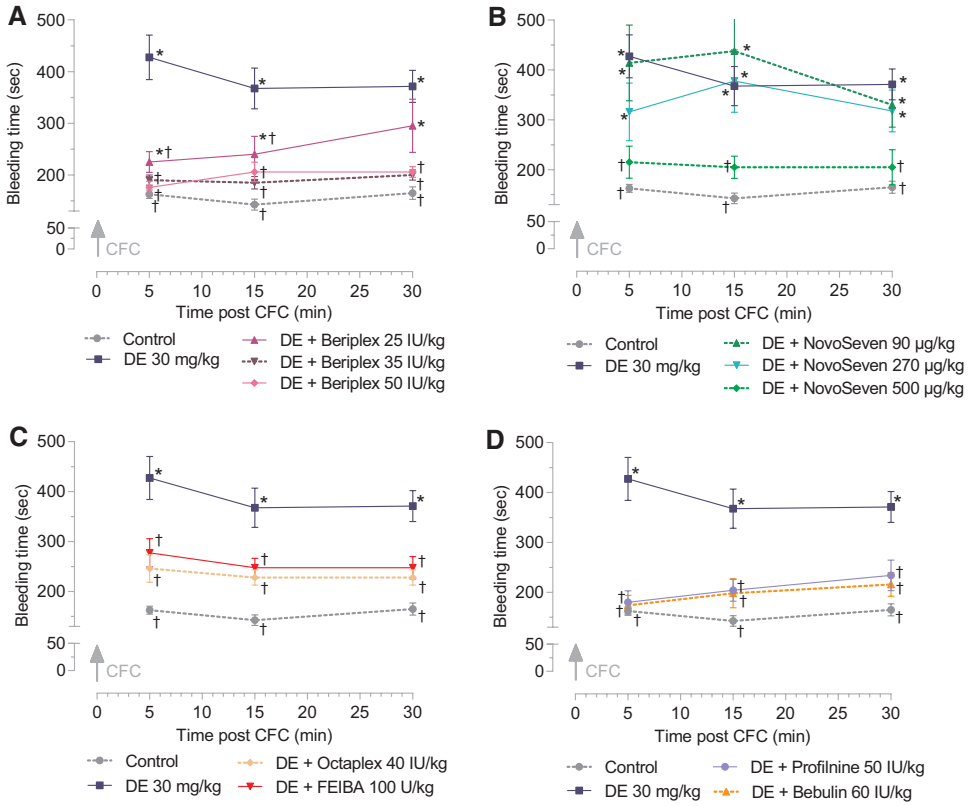

Fig. 1. Effect of dabigatran etexilate (DE) 30 mg/kg or control on bleeding time followed by (*A*) Beriplex, (*B*) NovoSeven, (*C*) Factor Eight Inhibitor Bypassing Activity (FEIBA) and Octaplex, and (*D*) Profilnine and Bebulin. Data represent mean ± SEM (n = 5–8 animals per group). *$P < 0.05$ *versus* control. †$P < 0.05$ *versus* dabigatran alone. The *vertical arrow* indicates the time of administration of each coagulation factor concentrate (CFC). Bebulin (Baxter BioScience, Westlake Village, CA); Beriplex (CSL Behring, Marburg, Germany); FEIBA (Baxter AG, Vienna, Austria); NovoSeven (NovoNordisk, Bagsværd, Denmark); Octaplex (Octapharma AG, Lachen, Switzerland); Profilnine (Grifols Biologicals Inc., Los Angeles, CA).

concentrates, Profilnine and Bebulin (fig. 1D), also significantly reduced the effect of dabigatran within 5 min and this was sustained over 30 min ($P < 0.001$).

Dabigatran Concentrations Achieved with or without CFC

Because diluted TT measurements are clotting based, it was important to establish that the presence of added CFCs in plasma did not influence diluted TT measurements of dabigatran *in vitro*. Addition of dabigatran to normal pooled plasma resulted in a linear dose-dependent increase in the diluted TT (fig. 2), as reported previously.[17] Addition of CFC to a second aliquot did not change the diluted TT assay and extrapolated dabigatran plasma concentration. Representative data for Beriplex, FEIBA, NovoSeven, and Bebulin are shown in figure 2. Similar results were observed for Profilnine and Octaplex (data not shown).

Dosing with 30 mg/kg dabigatran etexilate resulted in mean dabigatran plasma levels across the treatment groups between 443 and 735 ng/ml 45 min later (table 2). There was no significant difference in control plasma levels across the treatment groups ($P = 0.415$). Among rats treated with dabigatran followed by vehicle, mean plasma levels were 585 ± 50 ng/ml 45 min after dosing, representing the time

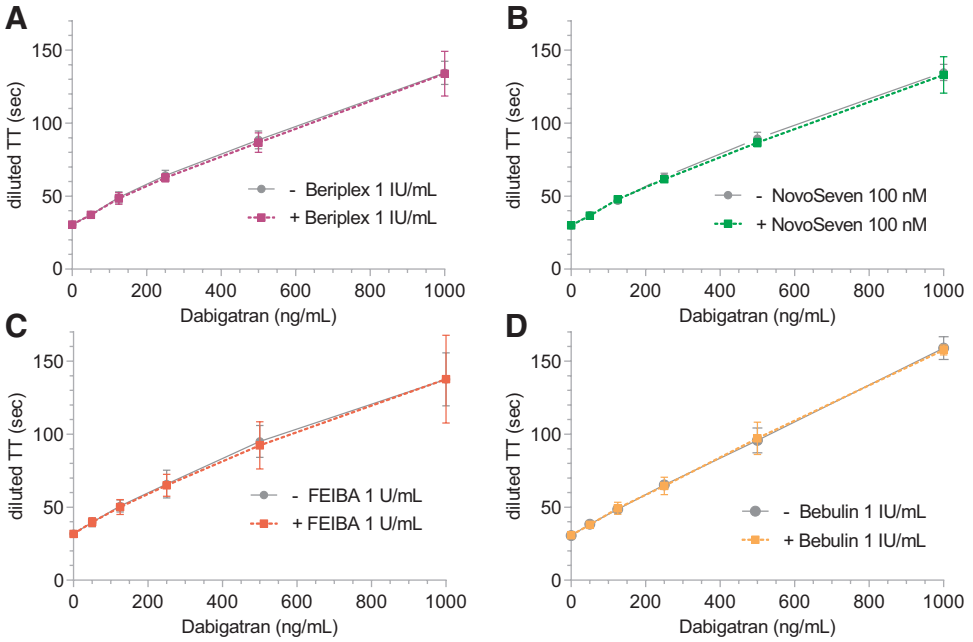

Fig. 2. Standard curves for dabigatran anticoagulation using the diluted thrombin time (TT) assay in the presence and absence of Beriplex (*A*), NovoSeven (B), Factor Eight Inhibitor Bypassing Activity (FEIBA) (*C*), and Bebulin (*D*). All coagulation factor concentrates (CFCs) were tested with a final concentration of 1 IU/ml, except NovoSeven (100 nM, equivalent to plasma levels of CFC achieved with approximately 500 μg/kg). Data shown as mean ± SD, n = 3 volunteers. Bebulin (Baxter BioScience, Westlake Village, CA); Beriplex (CSL Behring, Marburg, Germany); FEIBA (Baxter AG, Vienna, Austria); NovoSeven (NovoNordisk, Bagsværd, Denmark).

Table 2. Dabigatran Plasma Levels (ng/ml) Measured Using the Diluted Thrombin Time (Hemoclot) in Rats before and after Administration of CFCs

		Dabigatran Plasma Level (ng/ml) after Administration of CFC					
Time Point	Dabigatran + Vehicle (n = 7)	+ Beriplex 35 IU/kg (n = 5)	+ Octaplex 40 IU/kg (n = 5)	+ NovoSeven 270 μg/kg (n = 5)	+ FEIBA 100 U/kg (n = 5)	+ Profilnine 50 IU/kg (n = 5)	+ Bebulin 60 IU/kg (n = 5)
Predose*	585 ± 50 (100)	575 ± 123 (100)	735 ± 170 (100)	600 ± 109 (100)	472 ± 88 (100)	525 ± 42 (100)	443 ± 74 (100)
5 min after CFC	516 ± 67 (88)	474 ± 108 (82)	604 ± 149 (82)	498 ± 108 (83)	352 ± 70 (75)	410 ± 46 (78)	348 ± 51 (79)
15 min after CFC	408 ± 53 (70)	367 ± 90 (64)	458 ± 128 (62)	403 ± 90 (67)	278 ± 68 (59)	289 ± 45 (55)	216 ± 73 (49)
30 min after CFC	206 ± 57 (35)	295 ± 73 (51)	392 ± 105 (53)	305 ± 85 (51)	222 ± 53 (47)	172 ± 36 (33)	186 ± 62 (42)

Data shown as mean ± SEM; plasma level percentages relative to the predose baseline values are given in parentheses. CFCs in which multiple doses were tested (Beriplex and NovoSeven) showed similar plasma levels for all doses tested and therefore the results for a single dose are shown; n is the number of animals undergoing each treatment. Bebulin (Baxter BioScience, Westlake Village, CA); Beriplex (CSL Behring, Marburg, Germany); FEIBA (Baxter AG, Vienna, Austria); NovoSeven (NovoNordisk, Bagsværd, Denmark); Octaplex (Octapharma AG, Lachen, Switzerland); Profilnine (Grifols Biologicals Inc., Los Angeles, CA).

* Predose values are measured 45 min after oral dosing of dabigatran etexilate. Thus, dabigatran plasma levels at 5 min are 50 min postoral dabigatran etexilate.

CFC = coagulation factor concentrate; FEIBA = Factor Eight Inhibitor Bypassing Activity.

for peak dabigatran absorption, and levels were more than halved 30 min later (206 ± 57 ng/ml), reflecting the short half-life of dabigatran in rats. In the presence of CFCs, there was no difference in dabigatran plasma level clearance over the 30 min measurement period (P = 0.20). Thus, none of the CFCs significantly altered the clearance of dabigatran from plasma when measured as diluted TT compared with dabigatran etexilate alone.

Effect of CFCs on Coagulation Assays (High Dose of Oral Dabigatran Etexilate)

All coagulation assays were significantly prolonged (by approximately three- to eight-fold) after treatment with dabigatran etexilate (fig. 3). Across all treatment groups (n = 5 to 8 per group), dabigatran dosing led to a approximately three-fold mean increase in aPTT (from 19.0 ± 1.2 s with vehicle to 65.5 ± 5.7 s with dabigatran after 45 min) and

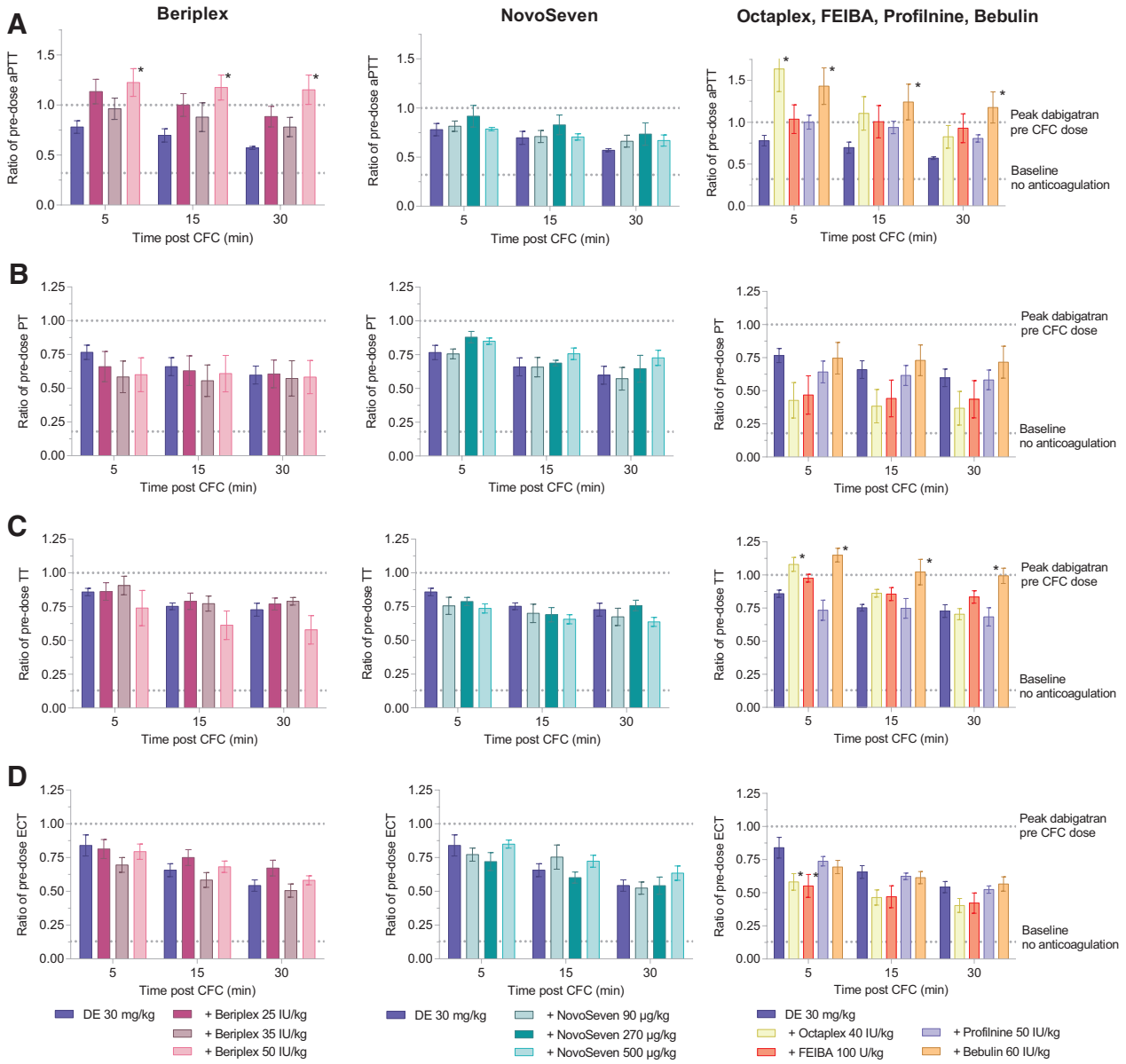

Fig. 3. Effects of coagulation factor concentrates (CFCs) on dabigatran anticoagulation as measured by (*A*) activated partial thromboplastin time (aPTT), (*B*) prothrombin time (PT), (*C*) thrombin time (TT), and (*D*) ecarin clotting time (ECT). Assay results at each time point for dabigatran alone and each CFC are reported as the ratio of the baseline value (time 0). The dabigatran coagulation level just before CFC administration (*i.e.*, peak dabigatran) is the *upper horizontal dotted line*. The *lower dotted line* shows control values after administration of vehicle. Values are shown as mean ± SEM (n = 5–8 animals per group). *P < 0.05 *versus* dabigatran alone at each time point. DE = dabigatran etexilate; FEIBA = Factor Eight Inhibitor Bypassing Activity. Bebulin (Baxter BioScience, Westlake Village, CA); Beriplex (CSL Behring, Marburg, Germany); FEIBA (Baxter AG, Vienna, Austria); NovoSeven (NovoNordisk, Bagsværd, Denmark); Octaplex (Octapharma AG, Lachen, Switzerland); Profilnine (Grifols Biologicals Inc., Los Angeles, CA).

PT (11.3 ± 0.3 to 43.3 ± 5.7 s); an approximately eight-fold increase in TT (21.1 ± 0.7 to 168.0 ± 7.0 s); and an approximately eight-fold increase in ECT (9.9 ± 0.4 to 79.0 ± 6.2 s) (see table, Supplemental Digital Content 2, http://links.lww.com/ALN/B46, which is a table showing coagulation assay results after administration of dabigatran etexilate and each CFC). Overall, there was an inconsistent effect of the CFCs on dabigatran-induced prolongation of clotting. In addition, none of the changes in these tests due to addition of CFCs predicted reduced bleeding time. The aPTT was not decreased by any of the coagulation concentrates (fig. 3A). Instead, some of the coagulation concentrates showed a trend for increase in aPTT, although this was only significant with Beriplex 50 IU/kg (all time points), Octaplex (after 5 min), and Bebulin (all time points). Data for Octaplex and FEIBA suggested reduced PT prolongation *versus* dabigatran alone although the changes were not statistically significant (fig. 3B). Octaplex and FEIBA significantly reduced ECT prolongation *versus* dabigatran alone at 5 min only (fig. 3C). The TT assay was significantly increased with Octaplex at 5 min and Bebulin at all time points (fig. 3D). NovoSeven had no effect on any of the coagulation assays, despite reducing the prolonged bleeding time at the higher 500 μg/kg dose.

Effect of CFCs on Thrombin Generation in Samples Supplemented with Dabigatran

The potential reversal of dabigatran anticoagulation was also tested at therapeutic (200 ng/ml) and supratherapeutic (600 and 1,000 ng/ml) plasma dabigatran concentrations *in vitro* in human plasma using a thrombin-generation assay. CFCs were added at concentrations that achieve plasma levels used in the management of bleeding in warfarin-treated patients.

Figure 4 shows representative thrombin generation-time curves for increasing concentrations of dabigatran and CFCs when given alone. Increasing dabigatran concentration was associated with prolongation of the lag time and a reduction in peak thrombin concentration with complete inhibition at the highest concentration of 1,000 ng/ml ($P < 0.001$; fig. 4). Differences were also seen in the thrombin generation curves for the different CFCs given in the absence of dabigatran (fig. 4, B–G). Beriplex, Bebulin, and Proflinine showed a similar effect on thrombin generation and increased peak thrombin generation in a concentration-dependent manner. NovoSeven was associated with a reduction in the lag time (fig. 4C), resulting in a slight shift of the curve to the left, but did not increase thrombin generation at these concentrations. Increasing concentrations of Octaplex (fig. 4D) resulted in a reduction in thrombin generation with increased lag time and a curve shift to the right. Increasing concentrations of FEIBA shortened the lag time compared with control (fig. 4E), consistent with the presence of activated FVII in the preparation, as well as increasing peak thrombin generation as seen with Beriplex, Bebulin, and Proflinine.

Dabigatran reduced the ETP in a concentration-dependent manner as compared with control plasma, with 600 and 1,000 ng/ml resulting in a significant reduction ($P < 0.001$) (fig. 5). When dabigatran was present at therapeutic peak concentrations (200 ng/ml), addition of CFCs (0.4 or 0.6 IU/ml) restored hemostasis to control levels or significantly increased the ETP *versus* control, as seen with all CFCs except Octaplex and NovoSeven ($P < 0.001$; fig. 5). FEIBA (0.8 U/ml, corresponding to a dose of 50 U/kg) resulted in a 2.1-fold increase in ETP ($P < 0.001$). At a dabigatran concentration of 600 ng/ml, higher concentrations of FEIBA, Bebulin, and Profilnine (0.6 and 0.8 IU/ml) normalized ETP as compared with control levels and significantly increased the ETP over 600 ng/ml dabigatran without CFCs ($P < 0.001$). At the highest dabigatran concentration (1,000 ng/ml), none of the factor concentrates normalized ETP, though weak but significant increases in ETP were seen at 1 IU/ml with Bebulin and Profilnine as compared with 1,000 ng/ml dabigatran without CFCs. NovoSeven and Octaplex had no significant effect on the concentration-dependent reductions in ETP by dabigatran.

Changes in peak thrombin generation after administration of the CFCs were similar to those observed with ETP (see figure, Supplemental Digital Content 3, http://links.lww.com/ALN/B47, which shows the effect of increasing doses of CFC on peak thrombin generation and lag time in human platelet-poor plasma spiked with dabigatran). In addition, none of the CFCs reversed the prolongation of the lag time by dabigatran (Supplemental Digital Content 3, http://links.lww.com/ALN/B47).

Discussion

This study demonstrates that clinically relevant doses of most CFCs can reduce bleeding time in rats prolonged by high-dose dabigatran; however, NovoSeven was only effective at a supratherapeutic dose. Dabigatran also prolonged coagulation tests to varying degrees in rats at these high doses and treatment with CFCs does not substantially affect these assays. In addition, dabigatran reduces thrombin generation (as measured by a decrease in ETP) *in vitro* in a concentration-dependent manner; with several three- and four-factor PCCs (Beriplex, Profilnine, and Bebulin) and the activated PCC FEIBA able to reverse these effects and restore thrombin generation. Higher CFC concentrations were required to show these effects as dabigatran concentrations increased. Octaplex and NovoSeven had no effect on the ETP.

There is a lack of randomized controlled trials testing potential reversal strategies with novel oral anticoagulants such as dabigatran, and most data come from *in vitro* studies or in animals and healthy human volunteers.[8,24–35] Although a specific, humanized antibody fragment targeting dabigatran is in development as an antidote to dabigatran, it is not yet commercially available.[3] In this study, we used the rat-tail bleeding time as it is a well-characterized experimental model that measures bleeding from small vessels.[36] All

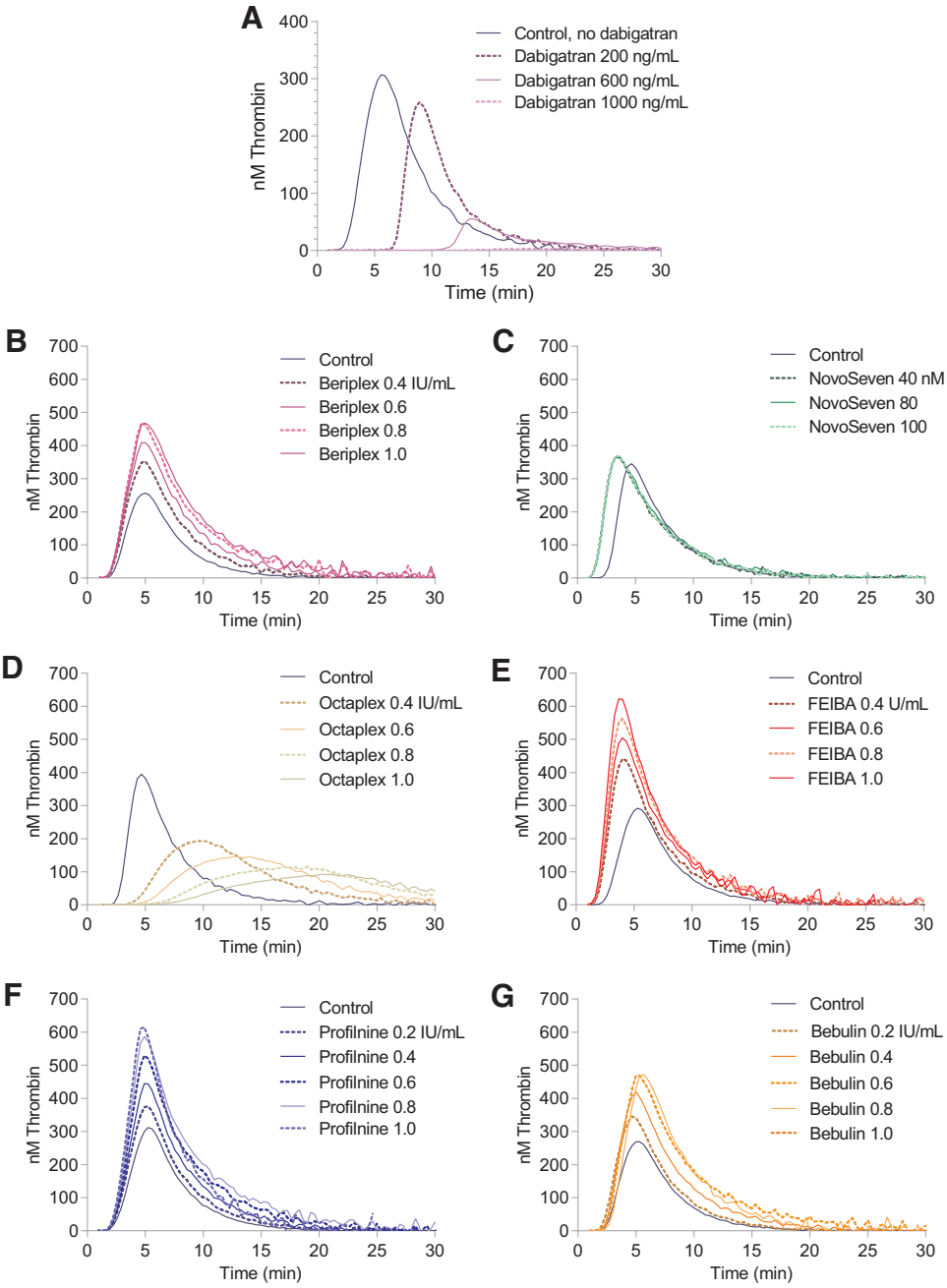

Fig. 4. Calibrated automated thrombogram curves showing the effect of increasing doses of dabigatran (*A*) and coagulation factor concentrates (*B–G*) on endogenous thrombin potential over 30 min in human platelet-poor plasma from healthy volunteers (n = 5). FEIBA = Factor Eight Inhibitor Bypassing Activity. Bebulin (Baxter BioScience, Westlake Village, CA); Beriplex (CSL Behring, Marburg, Germany); FEIBA (Baxter AG, Vienna, Austria); NovoSeven (NovoNordisk, Bagsværd, Denmark); Octaplex (Octapharma AG, Lachen, Switzerland); Profilnine (Grifols Biologicals Inc., Los Angeles, CA).

CFCs were effective in reducing bleeding time although the dose of factor concentrate required to achieve this seemed to be dependent on the initial concentration of dabigatran, as shown for the Beriplex and NovoSeven data. Subjective assessment also suggested that wound clots with lower Beriplex doses were more unstable with only partial hemostasis as the wounds reopened easily. In contrast, wounds in rats given higher doses of Beriplex had complete hemostasis with formation of stable wound clots that did not reopen.

The observed reduction in bleeding time after administration of CFCs was consistent with findings in other models. In a rabbit kidney injury model,[8] Beriplex 50 IU/kg reduced blood loss induced by dabigatran. In addition, in a murine model, Beriplex 100 IU/kg prevented intracerebral hematoma expansion.[25]

The effect of CFCs on prolongation of coagulation by dabigatran, as assessed by each assay, was quite variable. In general, most showed no effect with the addition of each CFC, despite

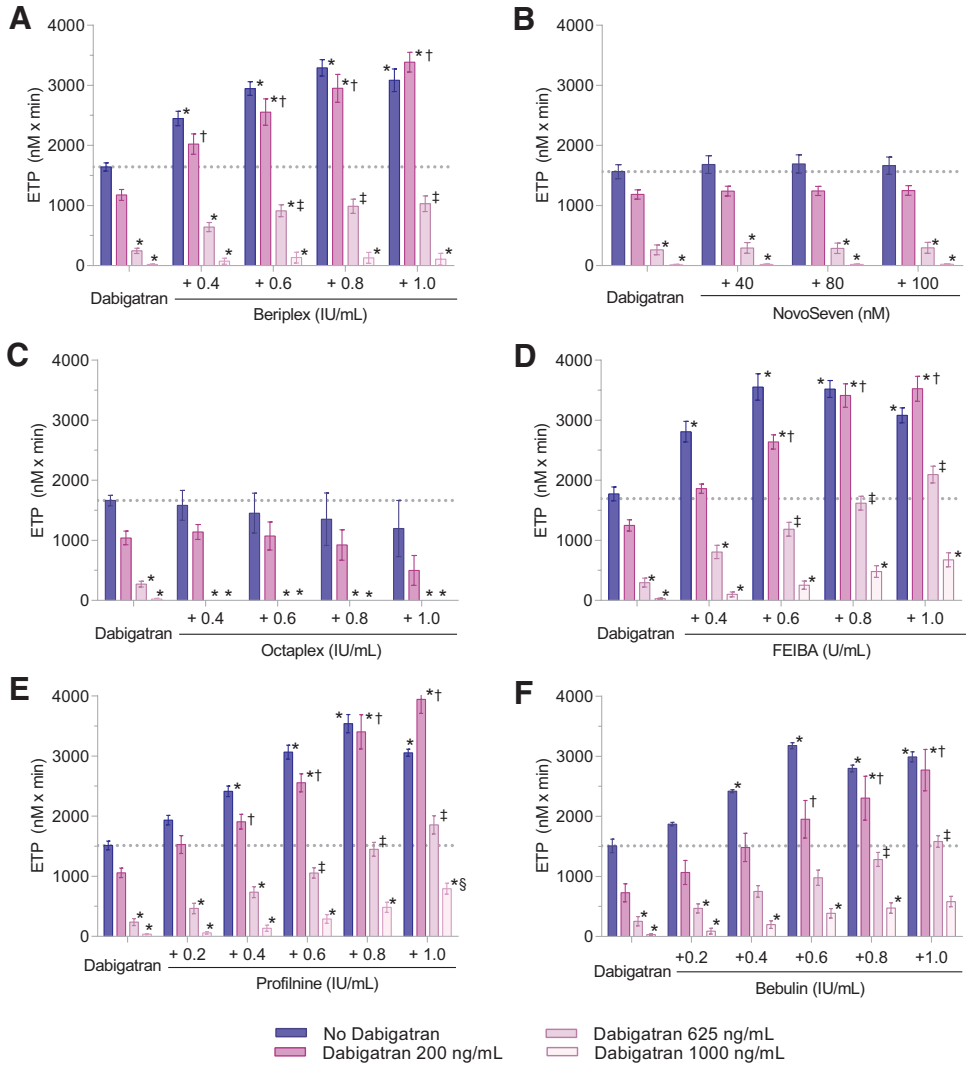

Fig. 5. The effect of increasing doses of coagulation factor concentrate (CFC) on human platelet-poor plasma anticoagulated with different concentrations of dabigatran; (*A*) Beriplex, (*B*) NovoSeven, (*C*) Octaplex, (*D*) Factor Eight Inhibitor Bypassing Activity (FEIBA), (*E*) Profilnine, and (*F*) Bebulin. Data represent mean ± SEM (n = 5 per group); endogenous thrombin potential (ETP; area under the thrombin-time integral, nM × min). **P* < 0.05 *versus* control (untreated plasma), *dotted line*. †*P* < 0.05 *versus* dabigatran 200 ng/ml, no CFC. ‡*P* < 0.05 *versus* dabigatran 600 ng/ml, no CFC. §*P* < 0.05 *versus* dabigatran 1,000 ng/ml, no CFC. Bebulin (Baxter BioScience, Westlake Village, CA); Beriplex (CSL Behring, Marburg, Germany); FEIBA (Baxter AG, Vienna, Austria); NovoSeven (NovoNordisk, Bagsværd, Denmark); Octaplex (Octapharma AG, Lachen, Switzerland); Profilnine (Grifols Biologicals Inc., Los Angeles, CA).

evidence that all factor concentrates had some effect on reducing bleeding time. Octaplex and FEIBA caused a significant reduction in the ECT at 5 min and a nonsignificant trend over the remaining 30 min. This was not apparent when animals were treated with Beriplex or other CFCs, despite all reducing the bleeding time. Thus, although the ECT is an effective assay to measure the presence of dabigatran in plasma, it is not useful for predicting a reduction of dabigatran-induced bleeding after administration of CFCs. Similarly, the diluted TT, which is a useful and sensitive measure for dabigatran in patients, was not influenced after addition of CFCs.

The assays that are sensitive to heparin, such as TT and aPTT, were prolonged in this study, notably at 5 min, with Octaplex or Bebulin. This finding may be attributable to heparin which is included in the preparations. As heparin is cleared more rapidly from the circulation, an increase would be expected early after administration, consistent with our finding. However, despite variable response with the different coagulation assays, it is clear that no assay was able to predict whether a CFC will be effective in reversing the prolongation of bleeding in this model. The lack of effect of the CFCs on coagulation assays prolonged by dabigatran is consistent with previous data.[8] Eerenberg *et al.*[24] reported that a single 50 IU/kg bolus of four-factor PCC did not reduce increases in aPTT, ECT, and TT associated with administration of dabigatran 150 mg twice-daily for 2 days in healthy volunteers.

The apparent discrepancy between the effects of each CFC on plasma coagulation assays and on bleeding time in these studies raises questions regarding the suitability of these assays for monitoring the potential of CFCs to reduce bleeding associated with dabigatran. Coagulation assays are only surrogate markers for increased bleeding tendency. In particular, the aPTT is generally insensitive to CFCs, which predominantly influence the extrinsic pathway as reflected by changes in the PT/international normalized ratio. Furthermore, the use of ECT or TT assays bypasses the additional prothrombin concentrates, because the stimulus for the assay activates thrombin or prothrombin. Thus, the lack of reversal effect with CFCs on dabigatran anticoagulation may relate to the assays used to test this, as shown in this study. This discrepancy was also observed in a recent trial comparing four-factor PCC with fresh-frozen plasma in patients with major bleeding treated with vitamin K antagonists.[37] Although the PCC was effective at reducing PT/international normalized ratio levels, an effect on hemostasis was limited to a subgroup of patients with visible or musculoskeletal bleeding.

Another test that is sensitive to CFCs, endogenous thrombin generation, was also tested in the presence of dabigatran using human plasma *in vitro*. Dabigatran induced a concentration-dependent decrease in the ETP, predominantly by prolonging the lag time, but at higher concentrations reduced peak thrombin concentrations. All CFCs except Octaplex and NovoSeven increased the ETP in the absence of dabigatran, highlighting the sensitivity of the assay for these agents. Octaplex showed features in the thrombin generation curve that were similar to those observed with dabigatran, which may possibly relate to the heparin component of this concentrate that may on occasion be higher than what is reported in the product leaflet.[38]

The dabigatran-induced reduction of the ETP was normalized with several three- and four-factor PCCs and the activated PCC FEIBA. However, the results were dependent on the starting concentration of dabigatran. Low CFC concentrations fully restored thrombin generation in human plasma spiked with 200 ng/ml of dabigatran, with higher concentrations required to normalize thrombin generation with 600 ng/ml dabigatran and no agents effective at dabigatran concentrations of 1,000 ng/ml. Administration of Octaplex and NovoSeven did not result in any change in the ETP for any of the dabigatran concentrations tested. Thus, even though a general trend of assay normalization to control levels is seen with the majority of these CFCs when measuring ETP and some dabigatran concentrations, Octaplex and NovoSeven had no effect on ETP even though bleeding time prolongation was reduced.

In the pursuit of an assay appropriate for predicting reduced blood loss, there seems to be a level of complexity that is not well understood.[34] Several factors may be relevant, including the assay used to measure reversal of anticoagulation, the initial concentration of anticoagulant, and the concentration of CFC. It is clear that large gaps remain in our understanding of the assays used to measure reversal of anticoagulation and thereby reduce bleeding with the new oral anticoagulant therapies.

Several case reports have reported the use of CFCs to reverse dabigatran-related bleeding but with varying degrees of success.[23,39–50] This may reflect the complexity of the clinical setting, with factors such as severity of blood loss, dose and timing of administration of the CFC in relation to the bleeding, and the plasma concentration of the anticoagulant. In one case report, "off-label" use of high-dose NovoSeven (3 × 30 μg/kg doses followed by 2 × 90 μg/kg doses) in combination with hemodialysis was effective in the management of postsurgical bleeding in a patient taking dabigatran 150 mg twice-daily.[40] However, in our study, only the highest dose of rFVIIa (500 μg/kg) reduced bleeding, which may in part be explained by the recognized differences between animals and humans with respect to the action of rFVIIa and tissue factor.[51,52] However, it may also be that lower doses of rFVIIa are not as effective at reversing dabigatran-induced bleeding compared with other CFCs. Apart from rFVIIa (where supratherapeutic doses were required), bleeding was reduced with CFC doses recommended for human therapeutic use.

There are a number of strengths to our study, including the use of a large number of CFCs, both three- and four-factor PCCs; oral administration of dabigatran; and multiple coagulation assays in conjunction with an assessment of bleeding. However, we also acknowledge a number of limitations. First, as coagulation factor levels were normal in this animal model, sufficient FVII was present to compensate for the missing FVII in the three-factor PCCs. Second, the range of doses for each of the CFCs may not be applicable to those used in clinical practice. Third, extrapolation of findings from this animal study to humans requires caution. Although bleeding time is a recognized surrogate for bleeding in humans, it has limitations.[53] Thus, it is uncertain how the observations in this rat model will be predictive of pathological bleeding in humans with high plasma levels of dabigatran. However, given the rarity of events involving major life-threatening bleeding, and that placebo-controlled randomized clinical trials are extremely challenging to perform, further *in vitro* or *ex vivo* studies evaluating different CFCs are likely to be undertaken.

Conclusions

In this animal model, bleeding induced by high doses of dabigatran was reduced by administration of CFCs. However, routine coagulation assays do not consistently predict this effect. This may be due to the fact that these agents preferentially act locally at the site of injury rather than systemically or the inadequacy of routine assays for detecting the reversal of the anticoagulant effects of dabigatran *in vivo*.

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Competing Interests

All authors are employees of Boehringer Ingelheim (Biberach, Germany), the manufacturer of dabigatran etexilate. The authors declare no other competing interests.

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CRITICAL CARE

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Prothrombin complex concentrates and a specific antidote to dabigatran are effective *ex-vivo* in reversing the effects of dabigatran in an anticoagulation/liver trauma experimental model

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Abstract

Introduction: New oral anticoagulants are effective alternatives to warfarin. However, no specific reversal agents are available for life-threatening bleeding or emergency surgery. Using a porcine model of trauma, this study assessed the ability of prothrombin complex concentrate (PCC), activated PCC (aPCC), recombinant FVIIa (rFVIIa) and a specific antidote to dabigatran (aDabi-Fab) to reverse the anticoagulant effects of dabigatran.

Methods: Dabigatran etexilate (DE) was given orally for 3 days (30 mg/kg bid) and intravenously on day 4 to achieve consistent, supratherapeutic concentrations of dabigatran. Blood samples were collected at baseline, after oral DE, after intravenous dabigatran, and 60 minutes post-injury. PCC (30 and 60 U/kg), aPCC (30 and 60 U/kg), rFVIIa (90 and 180 μg/kg) and antidote (60 and 120 mg/kg) were added to blood samples *ex-vivo*. Coagulation was assessed by thromboelastometry, global coagulation assays and diluted thrombin time.

Results: Plasma concentrations of dabigatran were 380 ± 106 ng/ml and 1423 ± 432 ng/ml after oral and intravenous administration, respectively, and all coagulation parameters were affected by dabigatran. Both PCCs and aDabi-Fab, but not rFVIIa, reversed the effects of dabigatran on thromboelastometry parameters and prothrombin time. In contrast, aPTT was only normalised by aDabi-Fab. Plasma concentration (activity) of dabigatran remained elevated after PCC and rFVIIa therapy, but was not measureable after aDabi-Fab.

Conclusion: In conclusion, PCC and aPCC were effective in reducing the anticoagulant effects of dabigatran under different conditions, while aDabi-Fab fully corrected all coagulation measures and decreased the plasma concentration of dabigatran below the limit of detection. No significant effects were observed with rFVIIa.

Introduction

Uncontrolled post-traumatic bleeding is the leading cause of potentially preventable death among trauma patients [1]. Early coagulation abnormalities are frequent in severely injured trauma patients and are associated with substantially increased mortality [2]. Patients' tendency towards perioperative and trauma-induced bleeding is further increased by anticoagulant medication. The direct oral anticoagulant (DOAC) dabigatran, a direct thrombin inhibitor, is administered orally as the prodrug dabigatran etexilate (DE) and is characterised by a rapid onset of action, few drug or food interactions, no requirement for routine coagulation monitoring and a short half-life of 12 to 14 hours [3].

Dabigatran is approved in many countries for the primary prevention of venous thromboembolic events in adult patients who have undergone elective total hip or total knee replacement surgery, and/or for the prevention of stroke and systemic embolism in adult patients with non-valvular atrial fibrillation [3,4]. Dabigatran directly inhibits both free and clot-bound thrombin, and this impedes the conversion of fibrinogen to fibrin, thus preventing thrombus development. Results from a large multicentre randomised controlled trial including 18,113 patients with atrial fibrillation (RE-LY) suggest a low overall risk of bleeding complications [5]. Because of this

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and the fact that clinical use of DOACs began relatively recently, experience in the management of bleeding complications associated with DOACs is limited. However, in the case of life-threatening bleeding following injury, immediate and prompt reversal of anticoagulation is required [6]. At the present time, no specific antidotes are licensed for reversal of the anticoagulant effects of dabigatran.

Experimental data show that dabigatran may be removed from the circulation by dialysis [7]. This approach is reported to be effective in patients with end-stage renal disease and several case reports have been published showing that dialysis is effective in reducing plasma concentrations of dabigatran [8-10]. However, this procedure may not be feasible in haemodynamically unstable patients with haemorrhagic shock. Prothrombin complex concentrate (PCC), activated PCC (aPCC) and recombinant activated factor VII (rFVIIa) have been proposed as candidates for reversing the anticoagulant effects of dabigatran. However, results from initial experimental trials are inconclusive and they do not account for different dabigatran concentrations or the combined effects of dabigatran and severe injury such as trauma [11-13]. Data from the few studies that have been performed in humans are also inconclusive. One study of PCC in healthy volunteers previously receiving dabigatran showed an increase in endogenous thrombin potential [14], whereas in another study PCC did not reverse the effect of dabigatran as measured by activated partial thromboplastin time (aPTT) [15]. A specific antibody fragment to dabigatran (aDabi-Fab) is in development, and in a rat model of anticoagulation it rapidly reversed the anticoagulant activity of dabigatran [16]. However, this antidote is not yet licensed for clinical use.

This porcine study was performed to evaluate the potential use of commonly available haemostatic agents (PCC, aPCC and rFVIIa), as well as the specific antidote aDabi-Fab, to reverse dabigatran-induced coagulopathy in an anticoagulation/trauma model. In addition, the study investigated the sensitivity of different coagulation tests, including thromboelastometry variables, for diagnosis and reversal of dabigatran/trauma-induced coagulopathy.

Materials and methods

Ethics and anaesthesia

All experiments were performed in accordance with German legislation governing animal studies following the *Principles of Laboratory Animal Care*. Ethical approval for these studies was obtained from the regional governmental animal care and use office (No. 84–02.04.2012.A197). Before surgery, pigs were housed in ventilated rooms and allowed to acclimatise to their surroundings for a minimum of seven days. Animals were fasted overnight before surgical procedures, with unrestricted access to water.

Prior to surgery, DE (Pradaxa, Boehringer Ingelheim, Biberach, Germany) was administered orally twice daily for 3 days (30 mg/kg *bid*). On the day of surgery, animals received an intramuscular injection of 4 mg/kg azaperone (Stresnil, Janssen, Neuss, Germany) and 0.1 mg/kg atropine (atropine sulphate, B Braun, Melsungen, Germany) as premedication. Anaesthesia was induced by intravenous injection of 3 mg/kg propofol (Disoprivan, Astra Zeneca, Wedel, Germany) followed by orotracheal intubation. The animals were ventilated with a tidal volume of 8 mL/kg and 16 to 22 breaths/minute (Cato, Draeger, Luebeck, Germany) to maintain end-tidal carbon dioxide between 36 and 42 mmHg. Anaesthesia was maintained with isoflurane (Forane, Abbott Laboratories Inc., Abbott Park, IL, USA) at an end-tidal concentration of 1% and a continuous infusion of fentanyl (Janssen, Neuss, Germany) at 3 to 4 μg/kg/h. Ringer's solution (Sterofundin, Braun, Germany) was infused at 4 mL/kg/h initially, increasing to 10 mL/kg/h after laparotomy until infliction of trauma. Throughout the experiment, body temperature was maintained at 36.5 to 37.0°C with a warming blanket.

Monitoring included electrocardiography (ECG), tail pulse oximetry, temperature, and arterial and central venous pressure measured by femoral catheters connected to a standard anaesthesia monitor (AS/3, Datex Ohmeda, Helsinki, Finland).

Surgical preparation and dabigatran infusion

Two 8.5-Fr catheters were surgically implanted in the right and left jugular veins for volume substitution and insertion of a pulmonary artery catheter. The right femoral artery was cannulated with an 18-G catheter to collect blood samples and to measure continuous arterial pressure. After line placement, a midline laparotomy with cystostomy was performed. Subsequently, dabigatran (active substance; Boehringer Ingelheim, Biberach, Germany) was infused at a rate of 0.77 mg/kg/h for 30 minutes and 0.52 mg/kg/h for 60 minutes to achieve consistent, supratherapeutic plasma concentrations of dabigatran.

A reproducible blunt liver injury was induced by clamping once through the parenchyma of the right middle liver lobe, using a custom-made instrument; the procedure has been described previously by our group [17]. Five minutes after injury and following haemorrhagic shock, all animals received a fluid bolus of 35 mL/kg of Ringer's solution followed by continuous infusion of 40 mL/kg/h until four hours post-injury. Sixty minutes after trauma, the abdomen was reopened and the blood was collected to determine the total blood loss post-injury.

Haemostatic agents

The following haemostatic agents were tested: PCC (Beriplex P/N 250 (US brand-name Kcentra), CSL Behring GmbH,

Marburg, Germany; lot 56560111C), aPCC (FEIBA, Baxter, Vienna, Austria; lot VNP5L003), and rFVIIa (NovoSeven, NovoNordisk, Denmark; lot LR04350). In addition, a specific antibody fragment to dabigatran being developed as an antidote (aDabi-Fab, Boehringer Ingelheim, Biberach, Germany; lot 6001325) was also tested [16]. PCC, aPCC and rFVIIa were reconstituted with sterile water according to the manufacturer's instructions immediately prior to administration. The aDabi-Fab was obtained in Tween 20 buffer (25 mM acetate, 220 mM sorbitol and 0.2% polysorbate 20) at a concentration of 44 mg/mL. Aliquots were stored at −80°C and thawed at 37°C for 10 minutes prior to application.

Blood collection and *ex vivo* addition of haemostatic agents

Blood samples were collected into sodium citrate (Sarsted, Nuembrecht, Germany) at the following four time points: baseline (3 days before oral administration of dabigatran was started), 12 h after the last oral dose of DE, which represents trough levels of dabigatran (low dabigatran level), after the 90-minute dabigatran infusion, which represents peak levels of dabigatran (high dabigatran level) and 60 minutes post-injury (post-trauma), which was also 60 minutes after stopping the dabigatran infusion and induction of blunt trauma injury. Placebo (saline), PCC, aPCC, rFVIIa or aDabi-Fab was added *ex vivo* to each citrated whole blood sample from each time point. The concentration of PCC and aPCC added was equivalent to the plasma concentrations achieved with 30 U/kg and 60 U/kg; rFVIIa was similarly added to achieve plasma levels equivalent to those achieved with 90 μg/kg and 180 μg/kg. aDabi-Fab was added at a concentration to achieve plasma levels equivalent to 30 or 60 mg/kg.

Analytical methods including coagulation assays and thromboelastometry

Haemoglobin (Hb) concentrations were measured with a blood gas analyser (ABL500, Radiometer, Copenhagen, Denmark). Prothrombin time (PT, Innovin), aPTT (Actin FS) and fibrinogen concentration (thrombin reagent) were determined by standard laboratory methods using the appropriate tests (all from Dade Behring, Marburg, Germany) on a coagulometer (MC 4 plus, Merlin Medical, Lemgo, Germany). Dabigatran plasma concentration was determined using the diluted thrombin time (Hemoclot, HyphenBiomed, Neuville sur-Oise, France).

Coagulation was assessed in whole blood using a thromboelastometry device (ROTEM, Tem International GmbH, Munich, Germany) and the EXTEM assay. The following parameters were measured: clotting time (CT, s), clot formation time (CFT, s) and maximum clot firmness (MCF, mm).

Statistical analysis

Statistical analysis was performed using PASW 18 (SPSS, Chicago, IL, USA). For graphical purposes, GraphPad Prism (Version 6.0, GraphPad Software, Inc., La Jolla, CA, USA) was used. Differences between the control and intervention groups were analysed with a one-way analysis of variance (ANOVA), with the Dunnett *post hoc* test for multiple comparisons. 'Non-measurable' was entered for clot formation time (CFT) when the required clot amplitude of 20 mm was not reached within 4,000 seconds. Data are presented as mean ± SD. Statistical tests were performed two-tailed and *P*-values <0.05 were considered statistically significant.

Results

Five male German land-race pigs were included in this *ex vivo* study; the animals' bodyweights ranged between 37 and 42 kg.

Effects of oral administration of DE and intravenous infusion of dabigatran

All coagulation parameters were within reference ranges at baseline (grey dotted line in all figures). After three days of oral DE, the mean plasma concentration of dabigatran was 380 ± 106 ng/mL (low dabigatran, in Table 1). Laboratory coagulation parameters were prolonged compared with baseline: PT from 9 ± 1 to 25 ± 8 s and aPTT from 13 ± 1 to 22 ± 4 s (control, Figures 1A and 2A). Accordingly, the EXTEM variables CT and CFT were also substantially prolonged (control, Figure 3A and B). However, no effects of oral DE administration on clot strength (MCF) or concentration of haemoglobin, platelets or fibrinogen were observed (control, Figure 3C and Table 2).

Following the 90-minute infusion of dabigatran, the mean plasma concentration (activity) of dabigatran increased to 1423 ± 432 ng/mL. This supratherapeutic level was associated with a further prolongation of PT, aPTT, and the EXTEM variables CT and CFT (Figures 1B, 2B and 4). These changes in coagulation parameters were compounded by blood loss following trauma (total blood loss at 60 minutes 1978 ± 265 mL) and dilution following the infusion of crystalloids. Sixty minutes after trauma, four out of five animals had no measurable clot formation (EXTEM CFT ≥4,000 s), and clot strength (EXTEM MCF) had reduced to 11 ± 7 mm (Figure 5). At the same time, plasma fibrinogen concentration had decreased to 64 ± 12 mg/dL and the mean haemoglobin level had dropped to 4.5 ± 0.6 g/L (Table 2). In addition, further prolongation of PT and aPTT was seen (control, Figures 1C and 2C).

Measurements after haemostatic therapy *ex vivo*

Ex-vivo treatment with PCC

The effects of dabigatran anticoagulation, alone (after both oral and intravenous administration) and compounded by

Table 1 Plasma concentration (activity, measured by diluted thrombin time) of dabigatran (ng/mL) during the study

	Low dabigatran	High dabigatran	Post-trauma
Control	380 ± 106	1423 ± 432	1021 ± 238
PCC (30; 60 U/kg)	299 ± 101; 302 ±115	1276 ± 443; 1273 ± 479	814 ± 81; 801 ± 185
aPCC (30; 60 U/kg)	291 ± 108; 288 ± 107	1199 ± 452; 1188 ±449	785 ± 166; 795 ± 159
rFVIIa (90; 180 μg/kg)	270 ± 86; 267 ± 87	1266 ± 500; 1295 ± 496	766 ± 194; 797 ± 161
aDabi-Fab (30; 60 mg/kg)	0.0 ± 0.0; 0.0 ± 0.0	0.0 ± 0.0; 0.0 ± 0.0	0.0 ± 0.0; 0.0 ± 0.0

Because dabigatran etexilate had not been administered, plasma concentrations of dabigatran were not measured at baseline.
Data are shown as mean ± SD. PCC, prothrombin complex concentrate; aPCC, activated PCC; rFVIIa, recombinant activated factor VII; aDabi-Fab, antibody fragment to dabigatran.

trauma-induced coagulopathy, were reduced by PCC, as shown by significant decreases versus control in PT, CT and CFT (Figures 1, 3, 4 and 5). Increased effects were apparent with the higher concentration of PCC after intravenous administration of dabigatran (high plasma concentration of dabigatran) and, in addition to the effects on PT, CT and CFT, clot strength (EXTEM MCF) almost returned to baseline with both doses of PCC. A similar pattern was observed following trauma: PT, CT and CFT were significantly shortened by PCC, and clot strength was returned to levels close to baseline (Figures 1C and 5A-C). Despite approximately 80 to 90% reversal of the effects on these parameters, PCC treatment had no effect on aPTT at any time point (Figure 2A-C), and the plasma level (activity, measured by diluted thrombin time) of dabigatran was also unchanged by PCC.

Ex vivo treatment with aPCC

Dabigatran-induced coagulopathy, both after oral intake and infusion, was reversed by aPCC application, with very similar response patterns to those observed with PCC (Figures 1, 3, 4 and 5). Decreases in PT, CT and CFT were observed, and MCF returned close to baseline after intravenous dabigatran and trauma, although, as with PCC these parameters were not fully restored to baseline levels. Also as with PCC, aPCC had no significant effect on aPTT or levels of dabigatran.

Ex vivo treatment with rFVIIa

Ex-vivo addition of rFVIIa had no significant effect on any of the coagulation parameters at any time point. This was true for both doses of rFVIIa. In addition, rFVIIa had no impact on the plasma concentration of dabigatran at any time point.

Ex vivo treatment with aDabi-Fab

After oral DE and intravenous dabigatran, both concentrations of aDabi-Fab restored coagulation parameters to their baseline values or similar. Even after trauma, PT, aPTT, CT, and CFT were reversed to baseline values (Figures 1C, 2C and 5), although a small decrease in MCF was still apparent. The restoration of aPTT to baseline values (Figure 2A-C) distinguished aDabi-Fab from PCC and aPCC. In addition, the mean plasma concentration of dabigatran was below the limit of detection (that is, approximately zero) after the addition of both concentrations of aDabi-Fab (Table 1).

Discussion

This experimental animal trial is the first to show *in vitro* that PCC, aPCC and aDabi-Fab are effective for the reversal of dabigatran-induced coagulopathy in a model of anticoagulation and liver trauma. Coagulation parameters, which were all affected by dabigatran administration, were corrected most effectively by aDabi-Fab, but, this product is yet to be licensed for clinical use. It is therefore of potential clinical importance that this study also suggests that PCC and aPCC could potentially be used for emergency reversal of dabigatran. In contrast, rFVIIa had no significant impact on coagulation after oral DE or intravenous dabigatran administration, despite the use of relatively high-dose rFVIIa (equivalent to 180 μg/kg).

Theoretically, the use of prothrombin (a major component of PCC) may reverse the anticoagulation activity of dabigatran by providing thrombin for the transformation of fibrinogen to fibrin. PCCs and aPCCs are derived from human plasma and most contain the vitamin K-dependent coagulation factors II, VII, IX, and X. PCCs with low levels of factor VII (3-factor PCCs) are commonly used in the USA, but products with higher levels of factor VII (4-factor PCCs) are used more commonly outside the USA. Based on the results of an open-label, phase IIIb randomised controlled trial [18], the first 4-factor PCC (Kcentra, CSL Behring, Germany) has been recently licensed in the US for the urgent reversal of coagulation factor deficiency induced by vitamin K antagonist (for example, warfarin) therapy in adult patients with acute major bleeding [19,20]. Currently, there are two major indications for PCC: rapid reversal of oral anticoagulation (vitamin K antagonists) and deficiency of vitamin K-dependent coagulation factors in life-threatening bleeding. Unlike 4-factor or 3-factor PCCs, aPCC (FEIBA, Baxter, USA) contains activated factors and is indicated for haemophilia A and haemophilia B patients with inhibitors, either to control spontaneous

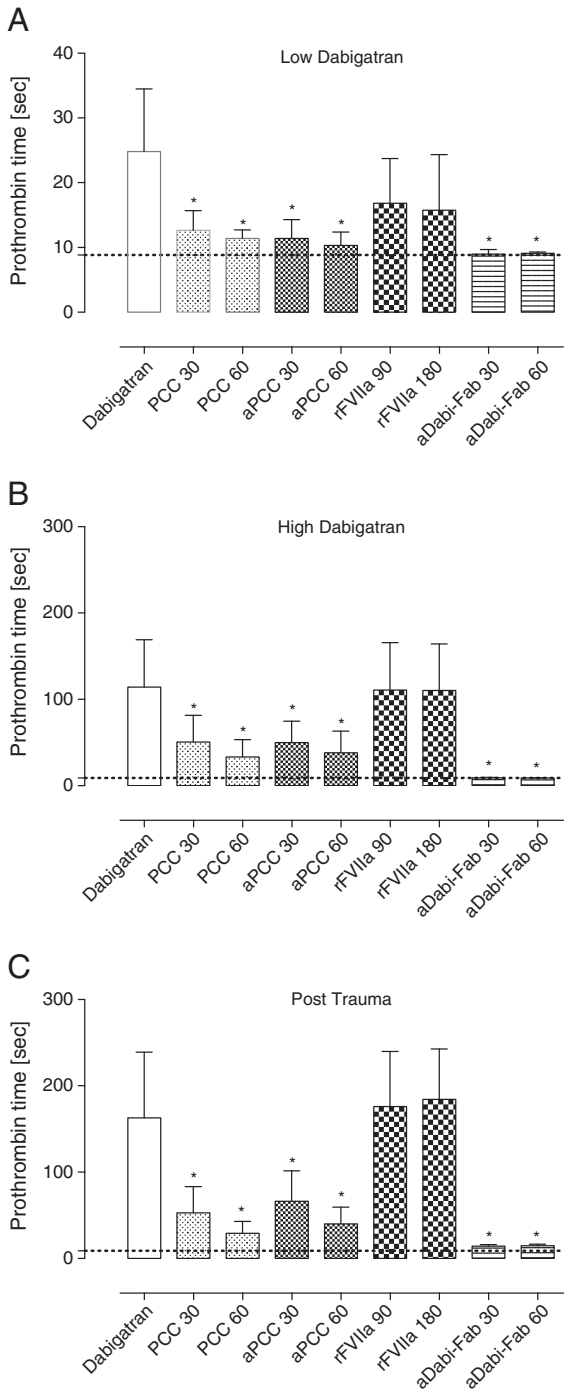

Figure 1 Prothrombin time at the low dabigatran (A), high dabigatran (B) and post-trauma (C) time points. Prothrombin time was obtained on a coagulometer using Innovin to determine the effect of *ex vivo* haemostatic therapy at various time points. Grey dotted lines indicate mean baseline values. $^{*}P$ <0.05 versus control. PCC, prothrombin complex concentrate; aPCC, activated PCC; rFVIIa, recombinant activated factor VII; aDabi-Fab, antibody fragment to dabigatran.

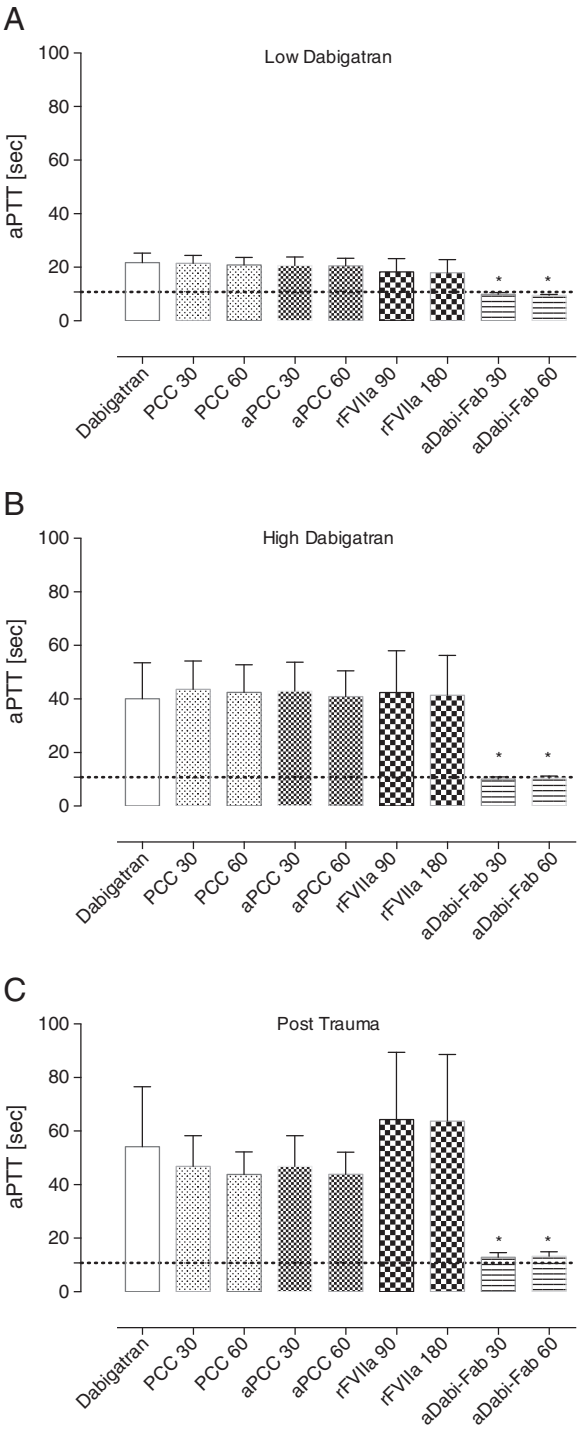

Figure 2 aPTT at the low dabigatran (A), high dabigatran (B) and post-trauma (C) time-points. Activated partial thromboplastin time (aPTT) was obtained on a coagulometer to determine the effect of hemostatic therapy at various time points. Grey dotted lines indicate mean baseline values. Data are shown as mean ± SD. $^{*}P$ <0.05 versus control. PCC, prothrombin complex concentrate; aPCC, activated PCC; rFVIIa, recombinant activated factor VII; aDabi-Fab, antibody fragment to dabigatran.

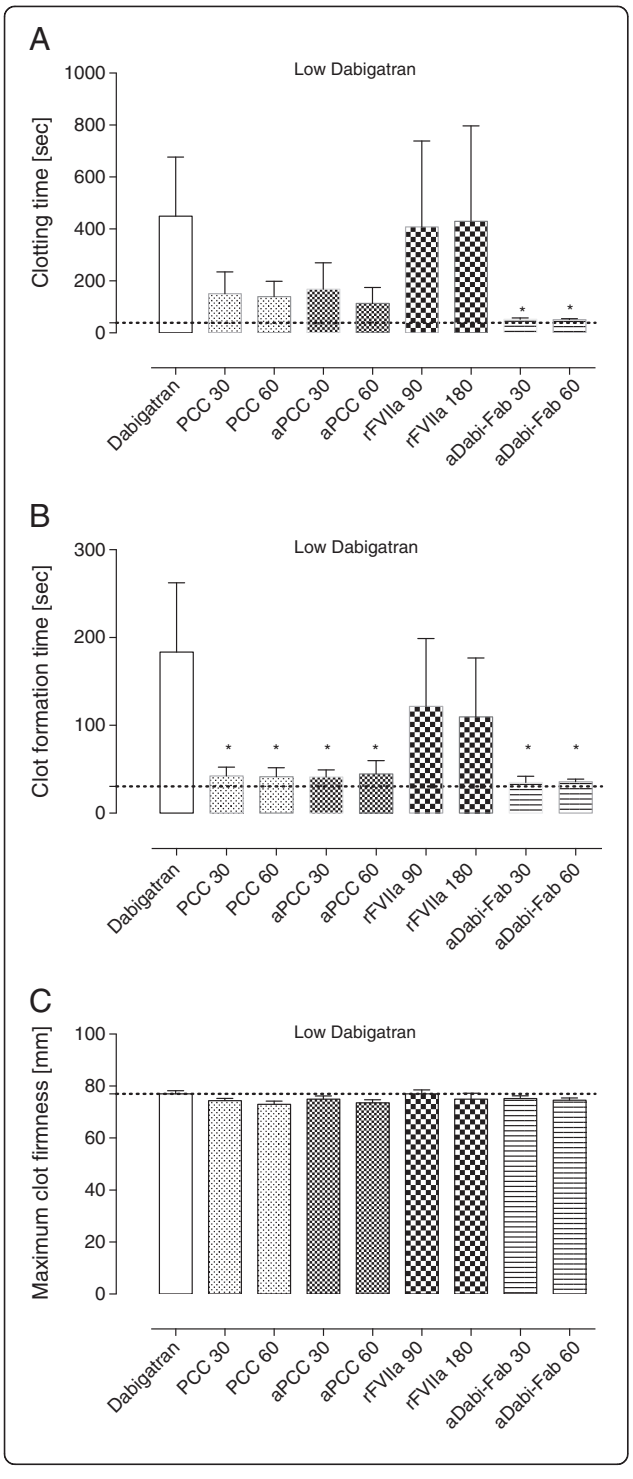

Figure 3 Thromboelastometry parameters after oral administration of dabigatran etexilate. The ROTEM coagulation analyzer (TEM international, Munich, Germany) was used for thromboelastometry. For activation the ExTEM reagent containing tissue factor as starting reagent was used according to the manufacturer instructions. **(A)** The clotting time (CT) represents the initiation of clot formation and corresponds to the reaction time. **(B)** clot formation time (CFT) reflects the coagulation time until 20 mm of amplitude are reached. **(C)** The maximum clot firmness (MCF) reflects the strength of a resulting clot. Data are shown as mean ± SD. $^{*}P$ <0.05 versus control. PCC, prothrombin complex concentrate; aPCC, activated PCC; rFVIIa, recombinant activated factor VII; aDabi-Fab, antibody fragment to dabigatran.

bleeding episodes or for use during surgical interventions [21].

PCC has been successfully used to terminate serious bleeding in pre-clinical studies [22-24]. Evidence from pre-clinical studies also supports a potential role for the use of PCC to reverse the effects of dabigatran [11-13]. In a rabbit model of anticoagulation associated with dabigatran, the use of PCC significantly reduced blood loss in a dose-dependent manner following a standardised kidney incision [11]. Furthermore, in a murine model of intracerebral haemorrhage, animals receiving dabigatran twice daily showed an expansion of intracerebral haematoma on magnetic resonance imaging [13]. The use of PCC (up to 100 U/kg) was associated with dose-dependent prevention of haematoma growth and also reversal of prolonged tail bleeding time. In another murine study, administration of PCC in combination with rFVIIa or aPCC prior to tail tip resection significantly reduced bleeding time but had no significant impact on blood loss, although the dose of PCC used in this study was low (14.3 U/kg) [12].

Few data exist on the use of PCC for the reversal of the anticoagulant effects of dabigatran in humans. In one study, following oral administration of dabigatran to 10 healthy volunteers, the capacity of thrombin generation was evaluated *ex vivo*. The intake of dabigatran was shown to affect the kinetics of thrombin generation, with a prolongation of the lag time and time to peak [14]. The addition of PCC significantly increased the endogenous thrombin potential, although no influence on the lag time was observed. In contrast to this study, in a trial of six healthy volunteers, PCC did not reverse the prolongation of the aPTT resulting from standard oral DE doses of 150 mg twice daily [15].

Although the investigators in the latter study used a different PCC (Cofact; Sanquin Blood Supply, Amsterdam, the Netherlands) to the PCC used in this study (Beriplex P/N 250, CSL Behring GmbH, Marburg, Germany), we have previously shown the two PCCs to have a similar pattern of thrombin generation [25]. Thus, discrepancy between studies regarding the effect of PCC on plasma

Table 2 Haematological parameters and fibrinogen concentration during the study

	Baseline	Low dabigatran	High dabigatran	Post-trauma
Haemoglobin (g/L)	10.5 ± 0.7	10.2 ± 0.8	9.5 ± 0.4	4.5 ± 0.7
Platelets (× 10^3/μL)	388 ± 51	403 ± 36	355 ± 66	202 ± 50
Fibrinogen (mg/dL)	161 ± 16	135 ± 13	118 ± 19	64 ± 12

Data are shown as mean ± SD.

coagulation assays raises the question of which coagulation test is adequate for monitoring the potential of a PCC to reverse the anticoagulant effects of dabigatran. The reversal of the prolonged PT, but not prolonged aPTT by PCC/aPCC in this study highlights the need for an assay that is sensitive not only to the anticoagulant used, but also to the effects of the reversal agent. In the case of warfarin, the PT (or international normalized ratio) is dependent on the vitamin K-dependent coagulation factors [26] and therefore serves not only as a sensitive measure of the effects of anticoagulation therapy, but also of its reversal by PCCs or aPCC. Conversely, PT is an insensitive measure of the effects of therapeutic doses of dabigatran [26-28]. However, as shown in this study, a high concentration of the drug can prolong the PT, thus enabling the dabigatran-prolonged PT to be reversed by PCC and aPCC. aPTT is more sensitive than PT to therapeutic levels of dabigatran [26,28], but the lack of effects of PCCs and aPCCs on aPTT suggests that this test is not suitable for monitoring reversal with these products. For confirmation of reversal of dabigatran therapy in the presence of trauma-related bleeding, well-designed studies with appropriate (sensitive) coagulation assays are urgently warranted.

In this study, we used thromboelastometry to monitor the effects of dabigatran. In the trauma setting, delays in the detection of coagulopathy may influence outcome; in contrast to the conventional coagulation tests, which are associated with slower turnaround times, thromboelastometry allows rapid assessment of a patient's coagulopathy. Based on findings from retrospective studies, thromboelastometry appears to be a useful tool to guide PCC therapy in patients with traumatic coagulopathy [29,30]. The EXTEM assay is similar to PT in that it assesses tissue factor-initiated extrinsic coagulation, making it the most suitable thromboelastometric assay for investigating PCC or aPCC reversal of dabigatran. There has been little investigation of the effects of dabigatran on viscoelastic coagulation parameters but, consistent with the present findings, prolonged activated clotting time with rapid thromboelastography was seen in several patients taking dabigatran [31]. We found that PCC, aPCC and aDabi-Fab reversed the anticoagulant effects of dabigatran as shown by improvements in CT and CFT. It is important to note that the decrease in MCF observed after dabigatran infusion was mainly attributable to a decrease in the amount of thrombin available for the conversion of fibrinogen to fibrin, as opposed to insufficient clot substrate (fibrinogen, platelets). Once sufficient thrombin becomes available through addition of PCC, aPCC or aDabi-Fab, transformation of fibrinogen to fibrin is restored and clot formation is no longer impaired. Overall, thromboelastometry may be useful in the detection of coagulopathy associated with dabigatran and in monitoring the effects of reversal therapy.

A drawback of PCC use is the potential risk of thromboembolic complications [24]. We have shown in an experimental animal model of liver injury that high levels (50 U/kg) of PCC may increase the risk for disseminated intravascular coagulation, and this was explained by an imbalance of pro- and anti-coagulant proteins [24]. However, there has been a suggestion that the procoagulant effects of high-dose PCC are reduced in the presence of dabigatran [32]; the safety profile of PCC or aPCC for the reversal of dabigatran remains to be characterised.

rFVIIa is approved to treat haemophilia patients with inhibiting antibodies against coagulation factors VIII or IX [33]. The use of this agent in patients with bleeding trauma who are resistant to conventional haemostatic therapy, even as a last resort, may be considered as surprising given that a large randomised controlled trial (CONTROL) was stopped after an analysis predicted that the likelihood of a successful outcome concerning the primary endpoints (mortality and morbidity) was very low [34]. In the present study, rFVIIa had no effect on reversal of any coagulation parameters. This is consistent with previous data demonstrating lack of effect of rFVIIa on dabigatran-induced bleeding in a murine model of haemorrhagic stroke [13]. These findings may be explained by the mechanism of action of rFVIIa: it influences the kinetics of thrombin generation, but it does not increase the concentration of prothrombin.

The results of this *in vitro* study have shown the specific antidote to dabigatran, aDabi-Fab, to have the greatest impact on all coagulation variables at all measured time points. Dabigatran, even at supratherapeutic concentrations (1,400 to 1,500 ng/mL, compared with the normal therapeutic range in humans of 50 to 250 ng/mL), was completely neutralised after the addition of aDabi-Fab. This is in line with results from a previous rat model of anticoagulation in which a single bolus injection of aDabi-Fab completely reversed the anticoagulant activity of

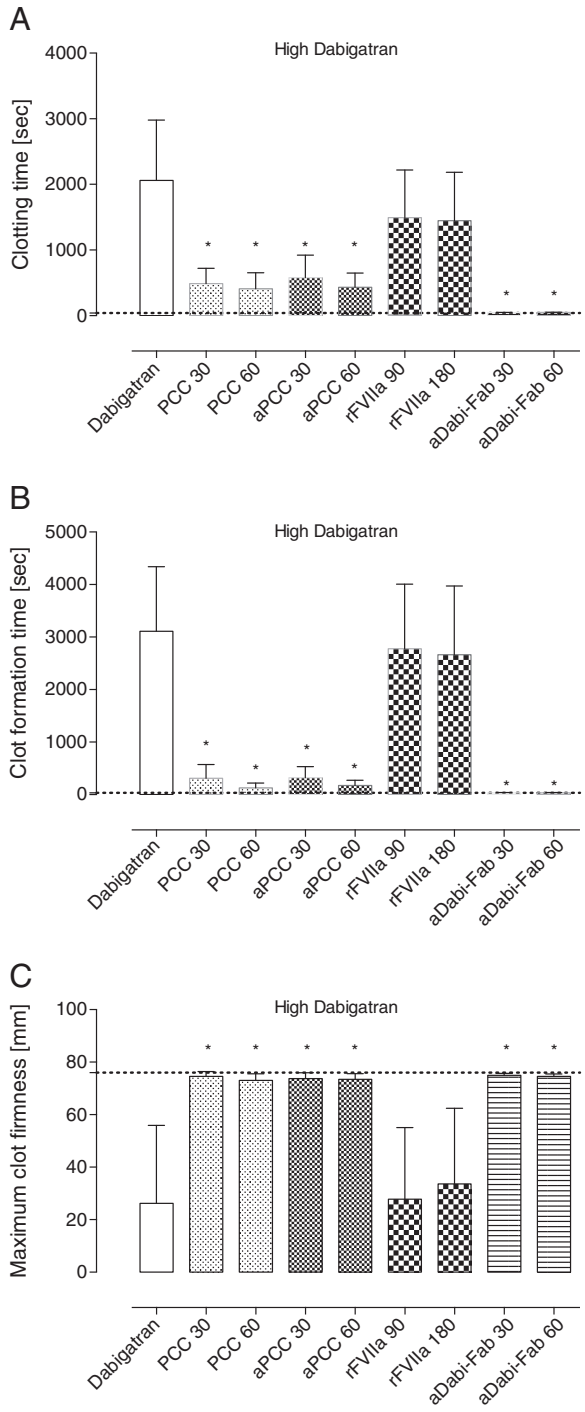

Figure 4 Thromboelastometry parameters after intravenous administration of dabigatran. After 90 minutes of intravenous infusion of dabigatran, the EXTEM assay was performed using a ROTEM device. Data are shown as mean ± SD. $^{*}P$ <0.05 versus control. PCC, prothrombin complex concentrate; aPCC, activated PCC; rFVIIa, recombinant activated factor VII; aDabi-Fab, antibody fragment to dabigatran.

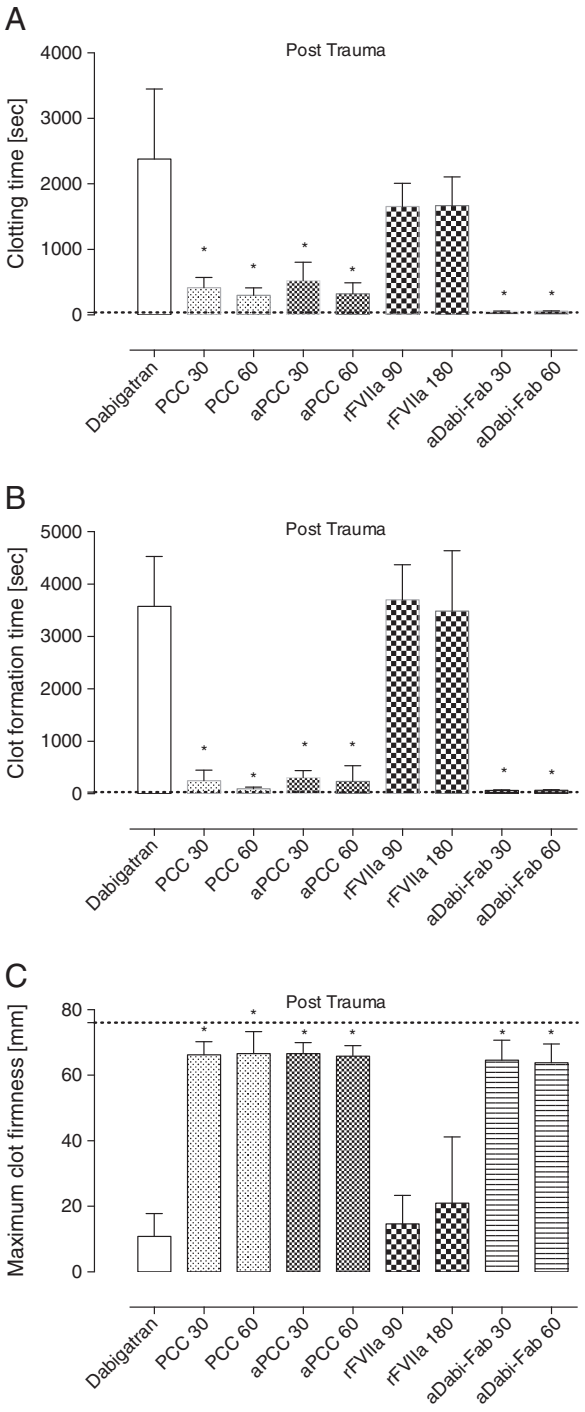

Figure 5 Thromboelastometry parameters 60 minutes after trauma. Sixty minutes after stopping the dabigatran infusion and the infliction of blunt liver injury trauma, a ROTEM device was used to measure the impact of dabigatran reversal. Data are shown as the mean ± SD. $^{*}P$ <0.05 versus control. PCC, prothrombin complex concentrate; aPCC, activated PCC; rFVIIa, recombinant activated factor VII; aDabi-Fab, antibody fragment to dabigatran.

dabigatran. This reversal was maintained for approximately 25 minutes despite continued infusion of dabigatran [16]. The increased potency of aDabi-Fab in comparison with PCC, aPCC and rFVIIa is undoubtedly related to their different mechanisms of action. The aDabi-Fab binds directly to dabigatran in plasma or whole blood and inactivates it, thus all coagulation measurements are returned to baseline. In contrast, PCC, aPCC and rFVIIa do not bind to dabigatran. Instead, these drugs overcome the anticoagulant activity by increasing the availability of substrate for coagulation.

There are some limitations of our study that need to be acknowledged. Haemostatic agents and aDabi-Fab were supplemented *ex vivo*. This approach was taken mainly for ethical reasons: it enabled investigation of a variety of haemostatic agents with a small number of animals. As a consequence, however, the effects of treatment on blood loss could not be measured and it is uncertain to what extent our observations may be predictive of clinical dabigatran reversal. This uncertainty was in part mitigated by the use of intravenous dabigatran in its active form rather than the orally administered prodrug DE. Thus, possible species-related differences in drug absorption and conversion to active form cannot have influenced the present data. However, there remains a possibility of species differences in aspects such as the tissue factor/rFVIIa complex, and the aPTT/PT responses to dabigatran [35]. Another consideration regarding applicability to the clinical setting is the plasma level of dabigatran. Mean levels at the low dabigatran timepoint were slightly above the normal therapeutic range, with higher values with high dabigatran and post-trauma. Although our study would have been improved by matching more closely the normal therapeutic range of dabigatran levels, it seems reasonable to assume that if reversal is successful at supratherapeutic concentrations, it would also be successful at lower, therapeutic levels. Clinical data from human patients receiving dabigatran therapy are clearly needed to confirm the efficacy, optimal dosing and safety of PCC, aPCC and aDabi-Fab for the reversal of dabigatran.

Conclusion

In summary, dabigatran-induced coagulopathy was reversed by *ex vivo* administration of two different doses of exogenous PCC and aPCC in this porcine model of anticoagulation and blunt liver injury. In contrast, *ex vivo* rFVIIa had no significant effect on coagulation parameters after administration of dabigatran. Unlike PCC or aPCC, *ex vivo* addition of aDabi-Fab provided complete neutralisation of dabigatran as well as full reversal of its effects. However, aDabi-Fab is still in development and is therefore not yet available for clinical use. Although further data investigating the effects of aDabi-Fab and PCCs are urgently needed, the administration of PCC or aPCC could potentially be considered as a therapeutic option to control life-threatening bleeding among patients under treatment with dabigatran.

Key messages

- PCC and aPCC effectively reverse dabigatran-induced coagulopathy in an anticoagulation/liver trauma model.
- rFVIIa has no significant effect on coagulation in this setting.
- aDabi-Fab provides the most effective reversal of the anticoagulant effects of dabigatran.
- Thromboelastometry variables may help to guide therapy in patients receiving dabigatran.
- Until aDabi-Fab becomes available, administration of PCC or aPCC might be a reasonable intervention for dabigatran-treated patients with life-threatening bleeding.

Abbreviations

aDabi-Fab: antibody fragment to dabigatran; ANOVA: analysis of variance; aPCC: activated prothrombin complex concentrate; aPTT: activated partial thromboplastin time; CFT: clot formation time; CT: clotting time; DE: dabigatran etexilate; DOAC: direct oral anticoagulant; ECG: electrocardiography; Hb: haemoglobin; MCF: maximum clot firmness; PCC: prothrombin complex concentrate; PT: prothrombin time; rFVIIa: recombinant activated factor VII.

Competing interests

OG has received research funding from Novo Nordisk, Biotest, CSL Behring, Nycomed. He has also received honoraria for consultancy and/or travel support from Bayer Healthcare, Boehringer Ingelheim and CSL Behring. RR has received honoraria for lectures and consultancy from CSL Behring and Novo Nordisk. JvR is an employee of Boehringer Ingelheim Pharma GmbH & Co., Germany. HS has received research funding from Boehringer Ingelheim and honoraria for consultancy from Bayer.

Authors' contributions

OG conceived and conducted the experimental laboratory work and drafted the manuscript. RR, HS and JvR helped to draft the manuscript. All authors read and approved the final manuscript.

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