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 IU/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. 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. 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. 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); 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, 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, MA).

The six CFCs tested were NovoSeven® 90, 270, and 500 μg/kg (rFVIIa; NovoNordisk, Bagsvaerd, 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 and current label recommendations. 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., 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

<table>
<thead>
<tr>
<th>Product</th>
<th>Availability</th>
<th>Clotting Factors</th>
<th>Anticoagulant Proteins</th>
<th>Heparin*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three-factor PCCs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bebulin VH13</td>
<td>U.S.</td>
<td>24–38 IU/ml</td>
<td>&lt;5 IU/ml</td>
<td>NA</td>
</tr>
<tr>
<td>Profilnine SD11</td>
<td>U.S.</td>
<td>NMT</td>
<td>NMT 100 U/100</td>
<td>NA</td>
</tr>
<tr>
<td>Four-factor PCCs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beriplex P/N12</td>
<td>Europe, Canada, U.S. as Kcentra</td>
<td>20–48 IU/ml</td>
<td>10–25 IU/ml</td>
<td>22–60 IU/ml</td>
</tr>
<tr>
<td>Octaplex13</td>
<td>Europe, Canada</td>
<td>14–38 IU/ml</td>
<td>9–24 IU/ml</td>
<td>18–30 IU/ml</td>
</tr>
<tr>
<td>rFVIIa</td>
<td>NovoSeven14</td>
<td>None†</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Activated PCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEIBA NF15</td>
<td>U.S., Europe</td>
<td>1.3 U</td>
<td>0.9 U/‡</td>
<td>1.1 U/U</td>
</tr>
</tbody>
</table>

Bebulin (Baxter BioScience, Westlake Village, CA); Beriplex (CSL Behring, Marburg, Germany); FEIBA (Baxter AG, Vienna, Austria); NovoSeven (NovoNordisk, Bagsvaerd, 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, 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·kg⁻¹·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.

Plasma concentrations of dabigatran in the rat model were determined by the clotting-based Hemoclot thrombin inhibitor assay (HYPHEN BioMed, Neuville sur-Oise, France). 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). 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. 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, whereas dabigatran 600 and 1,000 ng/ml were supratherapeutic concentrations that might be observed in patients with bleeding events. 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 (Thrombinscope BV, Maastricht, The Netherlands). Thrombin generation curves were calculated using thrombinscope software (version 3.0.0.29; Thrombinscope 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 Etxilate**

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 427.4 ± 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, Bagsværden, Denmark); Octaplex (Octapharma AG, Lachen, Switzerland); Profilnine (Grifols Biologicals Inc., Los Angeles, CA).](image-url)
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. 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 $585 \pm 50$ 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

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Dabigatran + Vehicle (n = 7)</th>
<th>Dabigatran + Beriplex 35 IU/kg (n = 5)</th>
<th>Dabigatran + Octaplex 40 IU/kg (n = 5)</th>
<th>Dabigatran + NovoSeven 270 μg/kg (n = 5)</th>
<th>Dabigatran + FEIBA 100 IU/kg (n = 5)</th>
<th>Dabigatran + Profilnine 50 IU/kg (n = 5)</th>
<th>Dabigatran + Bebulin 60 IU/kg (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predose*</td>
<td>585 ± 50 (100)</td>
<td>575 ± 123 (100)</td>
<td>735 ± 170 (100)</td>
<td>600 ± 109 (100)</td>
<td>472 ± 88 (100)</td>
<td>525 ± 42 (100)</td>
<td>443 ± 74 (100)</td>
</tr>
<tr>
<td>5 min after CFC</td>
<td>516 ± 63 (88)</td>
<td>474 ± 108 (82)</td>
<td>604 ± 149 (82)</td>
<td>498 ± 108 (83)</td>
<td>352 ± 70 (75)</td>
<td>410 ± 46 (78)</td>
<td>348 ± 51 (79)</td>
</tr>
<tr>
<td>15 min after CFC</td>
<td>408 ± 53 (70)</td>
<td>367 ± 90 (64)</td>
<td>458 ± 128 (62)</td>
<td>403 ± 90 (67)</td>
<td>278 ± 68 (59)</td>
<td>289 ± 45 (55)</td>
<td>216 ± 73 (49)</td>
</tr>
<tr>
<td>30 min after CFC</td>
<td>206 ± 57 (35)</td>
<td>295 ± 73 (51)</td>
<td>392 ± 105 (53)</td>
<td>305 ± 85 (51)</td>
<td>222 ± 53 (47)</td>
<td>172 ± 36 (33)</td>
<td>186 ± 62 (42)</td>
</tr>
</tbody>
</table>

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). \* \* \* \* \< 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 TTT (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). Octaplex and FEIBA showed a trend for increase in aPTT, although this was not statistically significant (fig. 3B). 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 Profilnine 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 Profilnine.

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.824–35 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
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, Beriplex 50 IU/kg reduced blood loss induced by dabigatran. In addition, in a murine model, Beriplex 100 IU/kg prevented intracerebral hematoma expansion.

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

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**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, Bagsvaerd, Denmark); Octaplex (Octapharma AG, Lachen, Switzerland); Profilnine (Grifols Biologicals Inc., Los Angeles, CA).

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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. Eerenberg et al. 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.

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, Bagsvaerd, Denmark); Octaplex (Octapharma AG, Lachen, Switzerland); Profilnine (Grifols Biologicals Inc., Los Angeles, CA).
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 intrinsic pathway as reflected by changes in the PT/international normalized ratio. Furthermore, the use of ECT or TT assays bypasses the additional prothrombin concentrations, 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. 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.

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. 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. 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. 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. 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. 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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