

Effective Reversal of Edoxaban-associated Bleeding with Four-factor Prothrombin Complex Concentrate in a Rabbit Model of Acute Hemorrhage

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ABSTRACT

Background: Edoxaban is an oral, selective direct factor Xa inhibitor approved in Japan for venous thromboembolism prevention after orthopedic surgery. Data are lacking regarding reversal strategies for edoxaban; this study assessed whether four-factor prothrombin complex concentrate (Beriplex®/Kcentra®; CSL Behring GmbH, Marburg, Germany) can effectively reverse its effects on hemostasis using a previously described rabbit model.

Methods: The study comprised assessments of thrombin generation *in vitro*, pharmacokinetic parameters, and edoxaban reversal *in vivo*. In a blinded *in vivo* stage, a standardized kidney incision was performed in animals (n = 11 per group) randomized to receive vehicle + saline, edoxaban (1,200 µg/kg) + saline, or edoxaban (1,200 µg/kg) + four-factor prothrombin complex concentrate (50 IU/kg). Animals were monitored for treatment impact on hemostasis and coagulation parameters. Data are median (range). Statistical tests were adjusted for multiple testing.

Results: Edoxaban administration increased blood loss (30 [2 to 44] ml) and time to hemostasis (23 [8.5 to 30.0] min) compared with the control group (3 [1 to 8] ml and 3 [2.0 to 5.0] min, respectively). Biomarkers of coagulation (prothrombin time, activated partial thromboplastin time, whole blood clotting time) and thrombin generation parameters (*e.g.*, peak thrombin, endogenous thrombin potential, lag time) were also affected by edoxaban. Administration of four-factor prothrombin complex concentrate significantly reduced time to hemostasis (to 8 [6.5 to 14.0] min, observed $P < 0.0001$) and total blood loss (to 9 [4 to 22] ml, observed $P = 0.0050$) compared with the edoxaban + saline group. Of the biomarkers tested, prothrombin time, whole blood clotting time, and endogenous thrombin potential correlated best with clinical parameters.

Conclusion: In a rabbit model of hemostasis, four-factor prothrombin complex concentrate administration significantly decreased edoxaban-associated hemorrhage. (**ANESTHESIOLOGY 2015; 122:387-98**)

ALTHOUGH heparins and vitamin K antagonists (VKAs) are key components of prophylactic therapy for patients at risk of thromboembolic events, with established and well-understood monitoring and reversal strategies, these agents are not without significant clinical limitations. Heparin may result in heparin-induced thrombocytopenia,¹ and the response to a fixed dose of unfractionated heparin is unpredictable, partially owing to plasma protein binding.² Furthermore, heparins require parenteral administration, making them unsuitable for long-term therapy. VKAs have a slow onset and offset of action, interact with many foods/drugs, and require careful monitoring and dose adjustment to minimize the risk of thromboembolic and major bleeding events.³

The limitations of traditional anticoagulants have led to the development of newer oral anticoagulants (NOACs), which exert their pharmacological effect by targeting a single coagulation factor.⁴ Licensed NOACs include dabigatran, a direct thrombin inhibitor, and several direct factor Xa inhibitors (edoxaban, apixaban, and rivaroxaban). In randomized clinical trials, NOACs were at least as efficacious as older anticoagulant regimens for some indications, but

What We Already Know about This Topic

- Prothrombin complex concentrates reverse the anticoagulation effects of rivaroxaban in healthy volunteers and reduce both rivaroxaban- and dabigatran-associated blood loss in preclinical animal models
- However, little is known about whether they work with some of the newer target-specific anticoagulants, such as edoxaban

What This Article Tells Us That Is New

- In a rabbit model of acute hemorrhage, a four-factor prothrombin complex concentrate also significantly decreased edoxaban-associated bleeding and improved hemostatic activation

not others.⁵ NOACs have a rapid onset of action, a short half-life compared with VKAs, few food/drug interactions, and predictable anticoagulant effects, meaning that routine monitoring is not required.⁶ However, the risk of bleeding complications (including spontaneous and perioperative bleeding) is still an important consideration with these newer agents, and although selective reversal agents are in development, there are no clinically validated reversal strategies for NOACs to date.⁴

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Edoxaban is an oral, selective direct factor Xa inhibitor that is rapidly absorbed ($t_{\text{MAX}} = 1$ to 2 h), exhibits a terminal half-life of 10 to 14 h (Daiichi Sankyo Co. Ltd., Tokyo, Japan; data on file) and has predictable pharmacokinetic and pharmacodynamic profiles.⁷ A study conducted in healthy volunteers showed a correlation between prothrombin time (PT) and plasma levels of edoxaban,⁷ and in an *ex vivo* model, changes in the antithrombotic effect of edoxaban mirrored changes in clotting parameters.⁸ Edoxaban is currently licensed in Japan for the prophylaxis of venous thromboembolism after orthopedic surgery, based on the results of several trials—STARS J-5 (total hip replacement), STARS E-3 (total knee replacement), and STARS J-4 (hip fracture surgery). Pooled analysis of STARS J-5 and STARS E-3 demonstrated that edoxaban 30 mg once daily is superior to enoxaparin 20 mg twice daily for the prevention of deep vein thrombosis after total knee or hip replacement, without significantly increasing the incidence of major and clinically relevant nonmajor bleeds.⁹ Edoxaban is also in late-stage clinical development for the prevention of stroke in patients with atrial fibrillation and the treatment and secondary prevention of venous thromboembolism.^{10,11}

Although rapid reversal of NOACs may be necessary in patients with life-threatening bleeding or those requiring emergency surgery, there is a paucity of data on this topic. Prothrombin complex concentrates (PCCs) have been used to successfully reverse the anticoagulant effects of VKAs such as warfarin,^{12–14} and have also been suggested to be effective in reversing the effects of NOACs. However, existing data on NOAC reversal with PCCs are limited to preclinical and clinical studies of dabigatran and rivaroxaban,¹⁵ and a single edoxaban study on the reversal of PT prolongation *in vitro*.¹⁶

The aim of this study was to investigate the ability of a four-factor PCC (4F-PCC) to effectively reverse the effects of edoxaban using an *in vivo* rabbit model of acute bleeding.

Materials and Methods

The study was conducted between October 2012 and May 2013 using a rabbit model previously utilized to assess dabigatran reversal.¹⁷ Study procedures were approved by the local animal welfare authority (Regierungspräsidium Gießen, Hessen, Germany), and experimental animals received care in compliance with the European Convention on Animal Care.

Study Agents

4F-PCC (Beriplex® P/N [known as Kcentra® in the United States]; CSL Behring GmbH, Marburg, Germany;

lot number 59070111), containing factors II, VII, IX, and X and coagulation proteins C and S,* was reconstituted in water for injection (B. Braun, Melsungen, Germany) and prepared fresh on the day of use. The diluent was injected into the 4F-PCC vial at the volume instructed by the label, resulting in a concentration of 29 IU/ml of factor IX. Storage of the reconstituted solution did not exceed 8 h at room temperature. Edoxaban tosylate hydrate (Daiichi Sankyo Co. Ltd.; lot number MH414A) was reconstituted in a vehicle of 5% (weight/volume) glucose solution (Glucosteril® 5%, Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany). The edoxaban solution was prepared fresh on the day of use by adding 5% glucose solution to edoxaban and sonicating the mixture until edoxaban was dissolved.

Animals

Female Chinchilla Bastard rabbits (Bauer, Neuental, Germany) 3 to 4 months of age (2.5 to 3.5 kg weight) were used. Animals were housed in individual wire-steel cages at 21 to 23°C and 50% relative humidity under a 12h/12h light-darkness cycle. The animals were fed rabbit pellets (Deukanin, Deutsche Tiernahrung Cremer GmbH & Co. KG, Duesseldorf, Germany) *ad libitum* and had free access to tap water.

Study Design

The study included three separate phases: *in vitro* assessment of thrombin generation, pharmacokinetic evaluation, and *in vivo* assessment of reversal of edoxaban-mediated impairment of hemostasis (fig. 1).

Study Endpoints

The ability of 4F-PCC to reverse the effects of edoxaban was evaluated using the *in vivo* primary endpoints of volume of blood loss and time to hemostasis after kidney incision. The effects of treatment on markers of coagulation, including PT, activated partial thromboplastin time (aPTT), whole blood clotting time (WBCT), and thrombin generation were also assessed as secondary endpoints. Evaluation of *in vivo* hemostasis was conducted in two stages (fig. 1): (1) escalating doses of 4F-PCC were investigated for their effects on bleeding in edoxaban-treated rabbits using an unblinded, nonrandomized design; (2) in the blinded, randomized stage of the *in vivo* evaluation of edoxaban reversal, the animals were randomly allocated to study groups based on computer-generated pseudo-random numbers. The groups were distributed within and between racks in a manner that allowed equalization of environmental influences across the study. Assessment of blood loss and time to hemostasis was performed by the same people throughout the study to ensure consistency and reproducibility. The inclusion of the blinded, randomized study stage ensured the objectivity of the assessments.

* CSL Behring: Kcentra® Highlights of Prescribing Information. December 2013. Available at: <http://labeling.cslbehring.com/PI/US/Kcentra/EN/Kcentra-Prescribing-Information.pdf>. Accessed September 3, 2014.

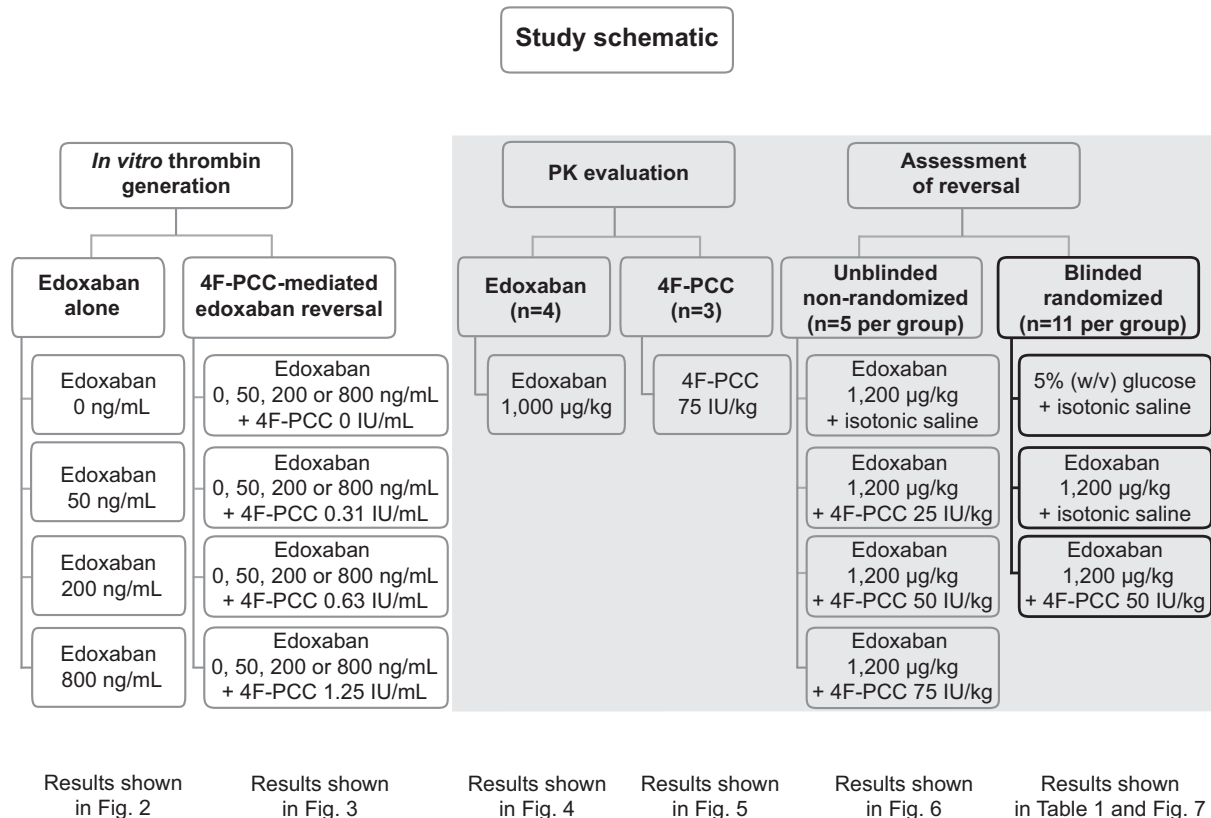


Fig. 1. Study schematic. Shading indicates *in vivo* stages of the study; 4F-PCC = four-factor prothrombin complex concentrate; IU = international unit; n = number of animals used in each group; PK = pharmacokinetic.

Sample Size Calculation

The sample size of the blinded/randomized *in vivo* stage was based on results obtained in the unblinded/nonrandomized study stage. The observed residual SD of log-transformed blood loss values was 0.60 and the observed effect of 50 IU/kg 4F-PCC compared with no 4F-PCC at the 1,200 µg/kg edoxaban dose was a reduction in log blood loss by 1.0. To reproduce this result at a two-sided significance level of 0.05 with a power of 95%, a sample size of 11 animals per group was required. Sample sizes for the other study phases were chosen based on previous experience.

Procedures

In Vitro Study Phase. Rabbit plasma (n = 1) was spiked with edoxaban in order to achieve final plasma levels of 50, 200, and 800 ng/mL, and with 4F-PCC to achieve final plasma levels of 0.31, 0.63, and 1.25 IU/mL. Plasma levels of edoxaban reflected the expected clinical levels (approx. 200 ng/mL)^{7,18} and multiples thereof; plasma levels of 4F-PCC were selected based on the recommended clinical dose for VKA reversal (25, 35, and 50 IU factor IX/kg body weight for pretreatment international normalized ratios of 2 to 4, 4 to 6, and >6, respectively)* and previous experience regarding anticoagulant reversal by 4F-PCC in a rabbit model.¹⁷

Pharmacokinetic Evaluation. A single iv bolus of 1,000 µg/kg edoxaban was administered to rabbits (n = 4) *via* a lateral ear vein. Blood samples were collected, using a syringe, in awake, restrained animals at baseline (t = 0 min) and at multiple time points postdosing (5, 15, 30, 45, 60, 90, 120, 240, 360, 480, and 1,440 min, or until plasma edoxaban levels were no longer detectable) for determination of edoxaban plasma levels and thrombin generation.

In an additional pharmacokinetic study, rabbits (n = 3) received a single iv administration of 4F-PCC 75 IU/kg (infusion rate: 1 mL/10 s). Blood samples were collected predose and at 1 min, 8, 24, 48, and 72 h postdosing for determination of the pharmacokinetic parameters of each coagulation factor contained in 4F-PCC.

In Vitro Study Phase. Animals were anesthetized using 5 mg/kg iv ketamine and 0.5 mg/kg iv xylazine 2%. Animals were intubated and ventilated over the study period and inhaled anesthesia was maintained with isoflurane (Isofluran CP®, CP-Pharma Handelsgesellschaft mbH, Burgdorf, Germany). After a 20-min stabilization period, a carotid artery catheter (22G needle; B Braun) was applied for blood sampling purposes. The catheter was fixed by a ligature around the artery and flushed with saline after each blood sampling.

1. In the unblinded, nonrandomized *in vivo* study stage, animals (n = 5 per group) received a single iv

administration of edoxaban 1,200 µg/kg with the aim of inducing a clear bleeding signal after kidney incision. At 5 min after edoxaban administration, the animals received an iv administration of 4F-PCC 25, 50, or 75 IU/kg, selected based on the recommended clinical dose for VKA reversal* and multiples thereof. Negative-control animals (n = 5) received an iv bolus of isotonic saline instead of 4F-PCC.

- Under fully blinded conditions, animals (n = 11 per group) were randomized to receive one of three interventions as iv boluses. The placebo group was administered vehicle only (5% [weight/volume] glucose [1.92 ml]) + saline (0.9% [weight/volume] isotonic saline [5.52 ml]); the other two groups were administered either edoxaban 1,200 µg/kg + saline, or edoxaban 1,200 µg/kg + 4F-PCC 50 IU/kg.

Kidney Incision

Kidney incision was performed as described previously.¹⁷ Briefly, at 5 min after edoxaban administration, animals received iv 4F-PCC at the doses described above. At 5 min after 4F-PCC dosing, a standardized kidney injury (scalpel incision 15 mm long × 5 mm deep) was created at the lateral kidney pole. Blood loss and time to hemostasis were then monitored over a 30-min observation period. Blood loss was defined as the volume of blood collected by syringe from the kidney-incision site. Time to hemostasis was defined as the time taken from the kidney incision to the cessation of observable bleeding or oozing. Blood samples were collected at baseline, before 4F-PCC administration ($t = 3$ min), just before kidney incision ($t = 8$ min), and at the end of the observation period ($t = 40$ min).

Assessments

Edoxaban Plasma Levels. Edoxaban plasma levels were determined based on factor Xa inhibition assessed by a colorimetric test (STA-Liquid Anti-Xa, Diagnostica Stago S.A.S., Asnières-sur-Seine, France) using a Behring Coagulation System® (Siemens AG, Erlangen, Germany).

Plasma Levels of Factors II, VII, IX, and X. 4F-PCC plasma levels were determined based on the human factor II, VII, IX, and X antigen levels, as assessed using respective enzyme-linked immunosorbent assay (ELISA) methods (ELISA kit for human factors II, VII, and X obtained from Biozol, Eching, Germany; ELISA kit for human factor IX obtained from Kordia Laboratory Supplies, Leiden, The Netherlands) in 10% citrate plasma.

PT and aPTT. PT and aPTT were measured using a Behring Coagulation System® (Siemens AG) employing the Thromborel S (Siemens AG) and actin FSL (Siemens AG) reagents, respectively.

Thrombin Generation. Thrombin generation was determined using a calibrated automated thrombogram in platelet-poor plasma after addition of phospholipids containing 5 pM tissue factor (Diagnostica Stago S.A.S.).

WBCT. WBCT was determined manually in a glass vial kept at 37°C using a water bath.

Statistical Analysis

For the blinded, randomized stage of the *in vivo* study, study groups were compared using Welch two-sample *t* test (for heterogeneous variances) applied to the log-transformed data. Time to hemostasis was compared between the groups using the log-rank test. Exploratory *P* values of the statistical tests were reported (Fisher evidential *P* values). In addition, for the variables of primary interest (blood loss and time to hemostasis, six comparisons) and for the secondary variables (36 comparisons), we separately applied a Bonferroni–Holm correction *post hoc* at a significance level of 5% (*i.e.*, the smallest in a set of *m* *P* values was significant if $P \leq 0.05/m$, the second smallest if $P \leq 0.05/(m-1)$, and so on until the first *P* value was not significant). In the results section, “significant” always means statistically significant at the 5% level after Bonferroni–Holm adjustment.

Data from the nonblinded stages of this study were analyzed using descriptive statistics only. Mean (SD) or median (range) are reported. Pharmacokinetic parameters were calculated separately for each animal using noncompartmental analysis after subtracting the predose measurement. Specifically, the area under the curve from the first to the last positive plasma concentration value was calculated by interpolating downwards logarithmically and upwards linearly. Terminal half-life was estimated by linearly regressing the terminal log-transformed plasma concentration values *versus* time. The number of terminal points included in the regression model was determined by maximizing the adjusted *R*-squared value. Using the estimated terminal half-life, the area under the curve was extrapolated to infinity from the predicted concentration at the last positive observation. Extrapolation back to time zero was done logarithmically using the first two positive plasma concentration values. Clearance was calculated as dose divided by area under the curve from zero to infinity. The geometric means of the individual parameter values are reported.

Sample size calculation and statistical analysis was done with SAS/STAT® version 9.3 (Cary, NC). Results were documented using GraphPad Prism® version 5.04 (La Jolla, CA).

Results

Effects on Thrombin Generation In Vitro

After extrinsic activation of thrombin generation, the addition of edoxaban to rabbit plasma *in vitro* resulted in a concentration-dependent decrease in peak thrombin generation and endogenous thrombin potential (ETP) as well as a concentration-dependent increase in lag time and time to peak (figs. 2, A–E).

The subsequent addition of 4F-PCC to edoxaban-containing plasma at concentrations up to 1.25 IU/ml

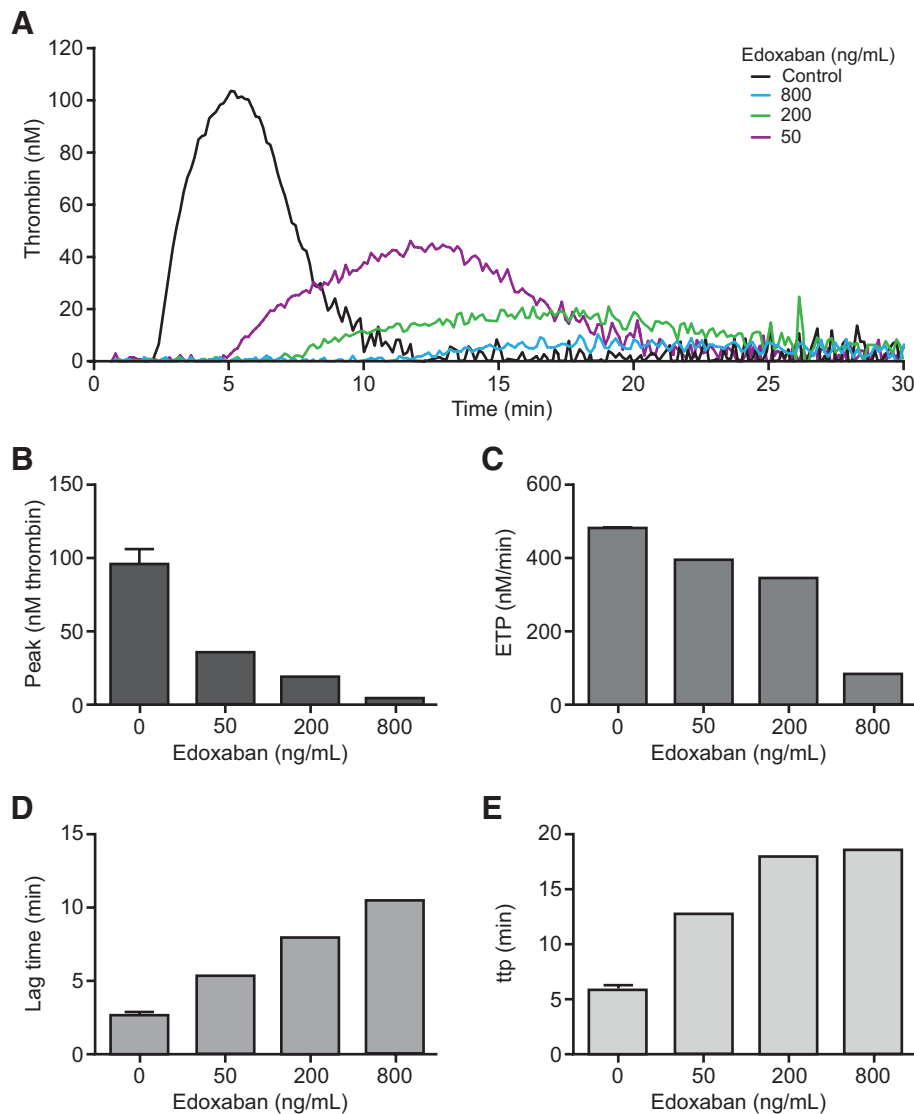


Fig. 2. Thrombin generation after addition of edoxaban to rabbit plasma *in vitro* (extrinsic activation). (A) Thrombin generation; (B) peak thrombin generation; (C) endogenous thrombin potential (ETP); (D) lag time; (E) time to peak (ttp). Mean \pm SD of $n = 4$ for edoxaban 0 ng/ml samples, otherwise $n = 1$.

resulted in a concentration-dependent recovery of peak thrombin generation and ETP. While peak thrombin levels could only be fully recovered at the lower edoxaban and higher 4F-PCC concentrations (fig. 3A), the decrease in ETP could be fully reversed even at the highest (800 ng/ml) edoxaban concentration (fig. 3B). For the lower two doses of edoxaban (*i.e.*, 50 and 200 ng/ml), 4F-PCC induced an overcorrection of ETP values to above control levels. No overcorrection was observed with the highest edoxaban dose. Edoxaban-induced changes in lag time and time to peak could not be reversed by the addition of 4F-PCC (data not shown).

Pharmacokinetics and Pharmacodynamics In Vitro

After a single iv bolus dose of edoxaban 1,000 $\mu\text{g/kg}$, the maximum plasma level of edoxaban ($C_{\text{MAX}} = 593 \text{ ng/ml}$), a

level corresponding to about twice the C_{MAX} seen in healthy volunteers after oral administration,^{7,18} was obtained by 5-min postdosing (the first sampling time point) (fig. 4A). This was followed by rapid clearance ($3,248 \text{ ml kg}^{-1} \text{ h}^{-1}$), with a terminal half-life ($t_{1/2\beta}$) of approximately 23 min. Since edoxaban levels were no longer detectable at 240-min postdosing, no further time samples were analyzed. The effects of edoxaban on thrombin generation followed the time course of edoxaban plasma levels, as indicated by the decrease in peak thrombin generation and ETP after extrinsic activation (fig. 4B).

In a separate pharmacokinetic evaluation, after a single iv bolus dose of 4F-PCC, plasma levels of coagulation factors II, VII, IX, and X were detectable up to 24 h (factor VII) or 48 h (factors II, IX, X), with the longest retention seen for factor X followed by factors IX, II, and VII (fig. 5).

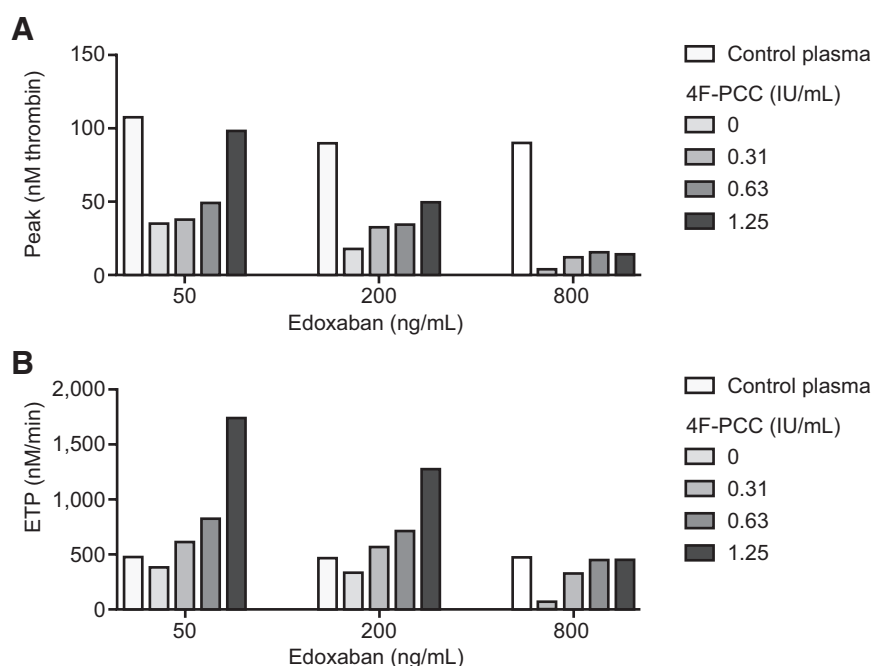


Fig. 3. Thrombin generation (extrinsic activation) after addition of 0–1.25 IU/ml 4F-PCC to edoxaban-containing rabbit plasma *in vitro*. (A) Peak thrombin generation; (B) endogenous thrombin potential (ETP). No edoxaban was added to control plasma. 4F-PCC = four-factor prothrombin complex concentrate; IU = international unit.

Effects on Bleeding Diathesis In Vitro

Results from a previous study found time to hemostasis and total blood loss after standardized kidney incision in isotonic saline-administered rabbits to be 1.8 to 6 min and 1.0 to 7.2 ml, respectively.¹⁷ In the unblinded, nonrandomized *in vivo* study stage, a single iv administration of edoxaban 1,200 µg/kg resulted in a marked delay in time to hemostasis (mean 20.2 min; fig. 6A) and marked increase in total blood loss (mean 25.6 ml; fig. 6B) after standardized kidney incision compared with the previous study's values (note: no untreated control group was used in this stage of the study).

Subsequent iv administration of 4F-PCC 50 or 75 IU/kg at 5 min after edoxaban administration dose-dependently reversed the edoxaban-induced increases in bleeding parameters; no effect was observed with 4F-PCC 25 IU/kg. At a 4F-PCC dose of 50 IU/kg, reductions of greater than 50% were achieved for both time to hemostasis (fig. 6A) and total blood loss (fig. 6B) compared with the edoxaban + saline rabbits (*i.e.*, no 4F-PCC). On the basis of these findings, the 50 IU/kg dose of 4F-PCC was chosen for the blinded, randomized stage of the study.

In the blinded, randomized stage of the study, time to hemostasis was lower in all animals in the vehicle-administered control group (median 3 min; range, 2.0 to 5.0) than in animals treated with edoxaban 1,200 µg/kg alone (median 23 min; range, 8.5 to 30.0) (fig. 7A). Administration of 4F-PCC 50 IU/kg to edoxaban-treated animals resulted in a significantly reduced time to hemostasis (median 8 min; range, 6.5 to 14.0) relative to the absence of 4F-PCC treatment. The estimated probability that administration of

4F-PCC shortened the time to hemostasis in edoxaban-treated animals was 96%.

In the absence of 4F-PCC, administration of edoxaban 1,200 µg/kg resulted in a significant increase in blood loss (median 30 ml; range, 2 to 44) compared with the vehicle control group (median 3 ml; range, 1 to 8; fig. 7B). Administration of 4F-PCC 50 IU/kg partially but significantly reversed the effect of edoxaban to a median blood loss of 9 ml (range, 4 to 22).

Biomarkers of Hemostasis

As expected, based on the *in vitro* and *in vivo* results, the thrombin generation markers ETP and peak thrombin level (extrinsic activation) were reduced, and the lag time prolonged by administration of edoxaban compared with controls (changes in ETP were significant at 40 min post-edoxaban; changes in peak thrombin levels and lag time were significant at 3, 8, and 40 min post-edoxaban; *t* = 8 min values reported in table 1). A significant reversal of the effect of edoxaban on ETP was observed shortly after 4F-PCC administration (*t* = 8 min; mean [SD]: 666 [199] nM/min; table 1) and at *t* = 40 min (mean [SD]: 868 [188] nM/min; *P* < 0.0001); post-4F-PCC values were above baseline values (493 [88] nM/min; table 1), indicating overcorrection of the ETP at 5 to 35 min post-4F-PCC dosing. The effect of edoxaban on peak thrombin generation was significantly reversed by 4F-PCC at *t* = 40 min (mean [SD]: 38 [12] nM; *P* = 0.0006) but not at *t* = 8 min (mean [SD]: 24 [8] nM; table 1). In addition, the increase in lag time induced by edoxaban was

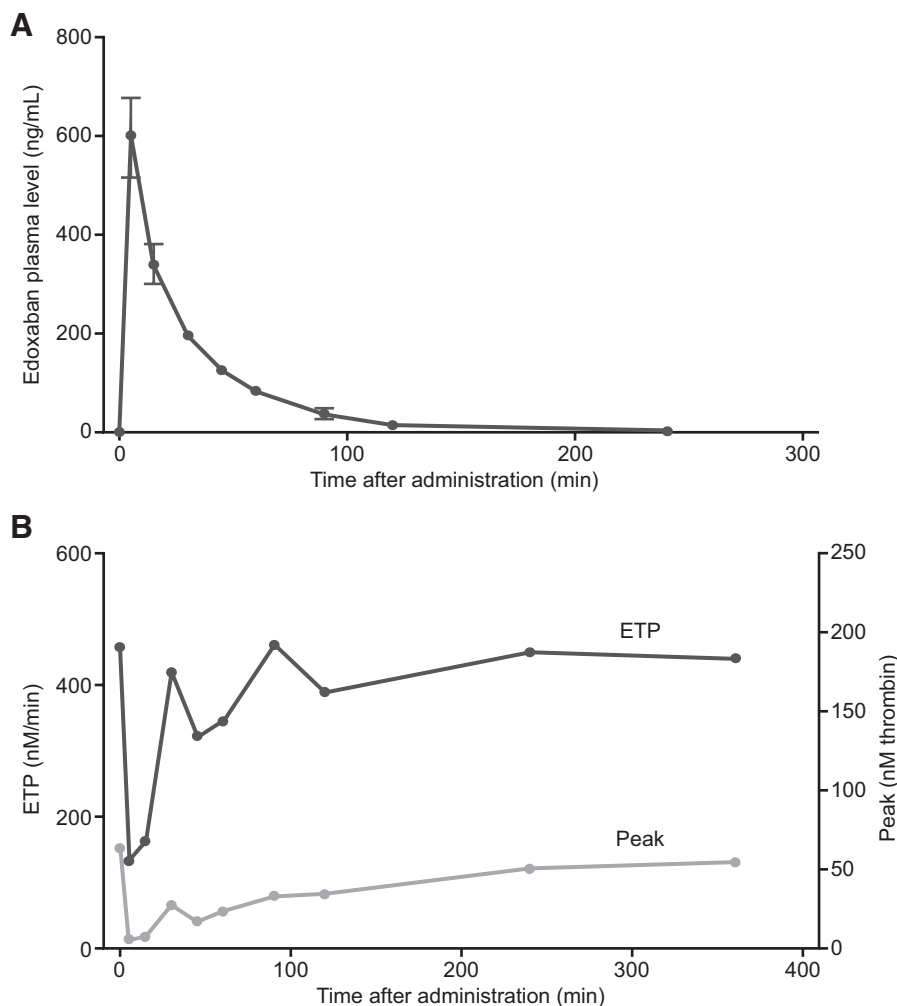


Fig. 4. Edoxaban plasma levels, mean \pm SD (A), peak thrombin generation and endogenous thrombin potential (ETP) (B) after single intravenous bolus administration of edoxaban 1,000 μ g/kg to rabbits. N = 4 for A; n = 1 for B.

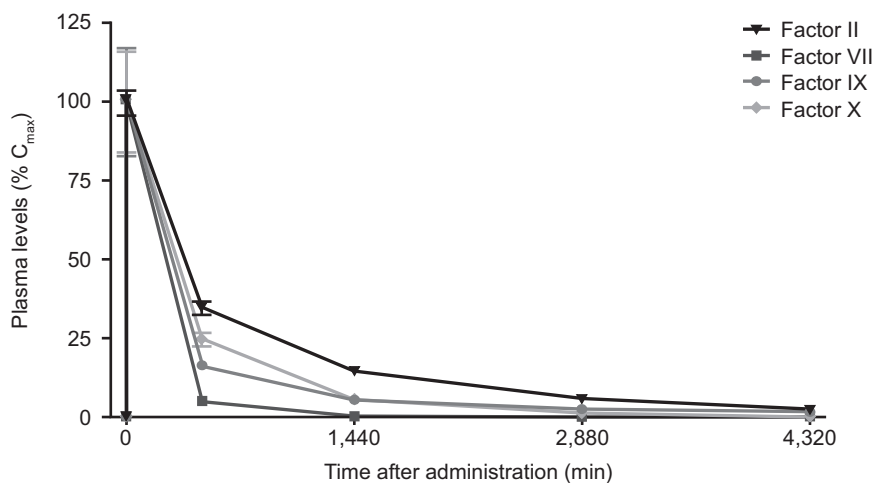


Fig. 5. Coagulation factor levels after single intravenous bolus administration of 4F-PCC 75 IU/kg to rabbits, mean \pm SD (n = 3). 4F-PCC = four-factor prothrombin complex concentrate; IU = international unit.

also significantly reversed by 4F-PCC at $t = 8$ min (mean [SD]: 5.8 [0.6]; table 1) and $t = 40$ min (mean [SD]: 4.4 [0.4]; $P < 0.0001$).

At all time points, PT, WBCT, and aPTT were significantly prolonged in animals exposed to edoxaban 1,200 μ g/kg compared with untreated controls (data only shown

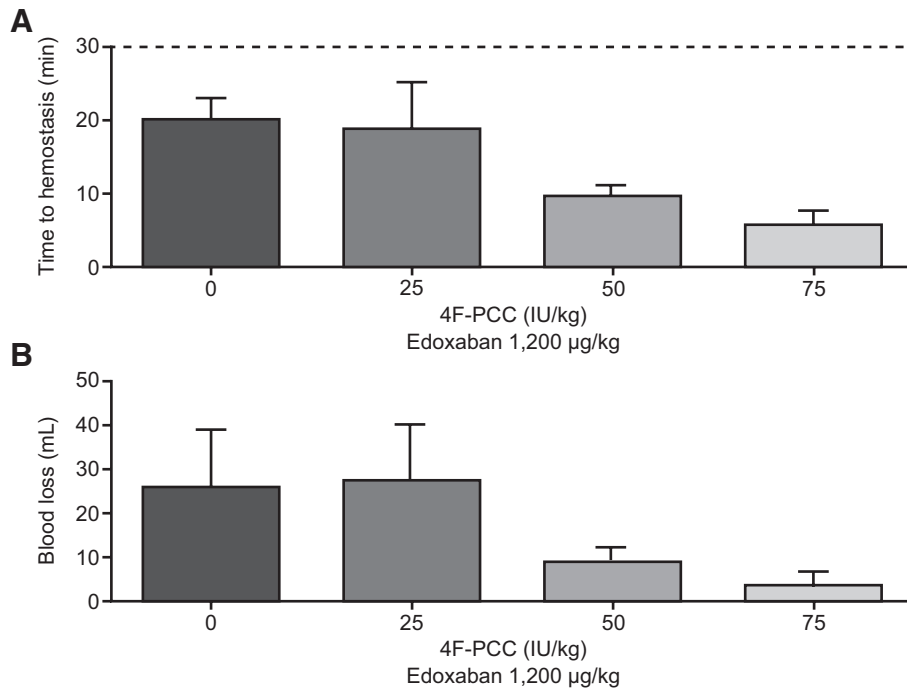


Fig. 6. Effect of 4F-PCC dose on time to hemostasis (A) and blood loss (B) after single administration of edoxaban 1,200 µg/kg and subsequent administration of 4F-PCC 0 to 75 IU/kg in a rabbit model of hemostasis. In both panels, data are mean \pm SD ($n = 5$). In panel A, the dotted line indicates the maximum observation period of 30 min. Historical values of time to hemostasis and total blood loss after standardized kidney incision in isotonic saline-administered rabbits are approximately 1.8 to 6 min and 1.0 to 7.2 ml, respectively. 4F-PCC = four-factor prothrombin complex concentrate; IU = international unit.

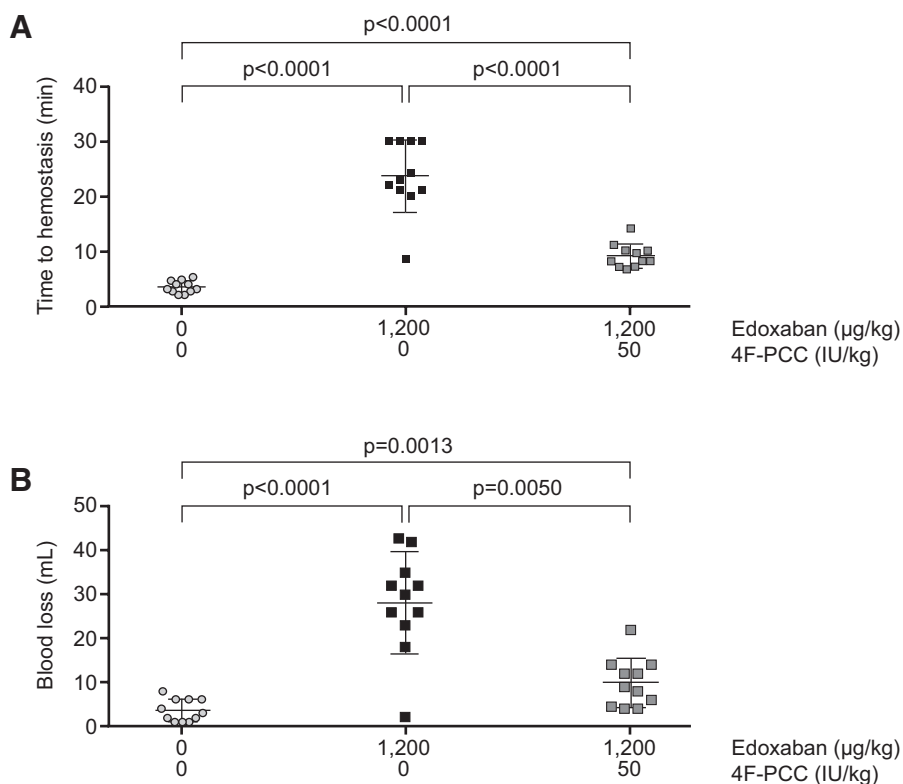


Fig. 7. Time to hemostasis (A), and blood loss (B) after standardized kidney incision in the presence and absence of edoxaban 1,200 µg/kg, with or without subsequent administration of 50 IU/kg 4F-PCC. In both panels, data are individual values and mean \pm SD ($n = 11$). 4F-PCC = four-factor prothrombin complex concentrate; IU = international unit.

Table 1. Biomarkers from the Randomized, Blinded *In Vivo* Evaluation ($t = 8$ min)

Parameter	Group 1: 5% (w/v) Glucose Saline	Group 2: Edoxaban 1,200 µg/kg Saline	<i>P</i> Value (Group 2 vs. Group 1)	Group 3: Edoxaban 1,200 µg/kg 4F-PCC 50 IU/kg	<i>P</i> Value (Group 3 vs. Group 2)
ETP, nM/min	493 ± 88	343 ± 125	0.0117	666 ± 199	0.0006*
Peak thrombin, nM	111 ± 37	20 ± 6.17	<0.0001*	24 ± 8	0.24
WBCT, s	189 ± 41	381 ± 58	<0.0001*	286 ± 26	0.0001*
aPTT, s	19.9 ± 3.3	35.2 ± 4.6	<0.0001*	35.0 ± 2.8	1.0
PT, s	9.7 ± 0.9	17.4 ± 1.7	<0.0001*	13.5 ± 0.7	<0.0001*
Lag-time, min	2.6 ± 0.4	9.5 ± 1.5	<0.0001*	5.8 ± 0.6	<0.0001*
Plasma edoxaban,† ng/ml	0 ± 0.0	655.3 ± 85.5		690.1 ± 81.8	

A blood sample was taken at 5 min after 4F-PCC administration (8 min after edoxaban administration). Values are mean ± SD.

* Statistically significant at the 5% level after Bonferroni–Holm adjustment. † Baseline corrected.

aPTT = activated partial thromboplastin time; ETP = endogenous thrombin potential (area under the thrombin generation assay curve); 4F-PCC = four-factor prothrombin complex concentrate; PT = prothrombin time; WBCT = whole blood clotting time.

for $t = 8$ min in table 1). Treatment with 4F-PCC resulted in a significant reduction in PT and WBCT at 5 min after 4F-PCC administration ($t = 8$ min) compared with edoxaban alone; however, levels were not fully reversed relative to control values. Comparisons between edoxaban-treated groups with and without 4F-PCC treatment were also statistically significant for PT ($P < 0.0001$) but not for aPTT ($P = 0.0184$) or WBCT ($P = 0.12$) at the 40-min time point (data not shown).

Discussion

In this preclinical study conducted in rabbits, 4F-PCC dose-dependently improved measures of hemostasis affected by edoxaban, with the 50 IU/kg dose showing statistically significant reductions in bleeding relative to the FXa inhibitor alone.

The mechanism of anticoagulant reversal for NOACs differs from that involved in the reversal of VKAs. Whereas PCCs are used in VKA-treated patients to replace vitamin K-dependent coagulation factors that are deficient as a result of anticoagulation therapy, their use in NOAC-treated patients aims to counteract direct factor IIa or Xa inhibition by augmenting physiological levels of vitamin K-dependent factors. The results of the current study add to a limited body of mostly preclinical evidence on the latter approach (reviewed in Dickneite and Hoffman).¹⁵ Notably, there is a paucity of data in healthy volunteers and on NOAC reversal in patients with clinically relevant bleeding.¹⁵

Regarding the existing clinical and preclinical data on PCC reversal of NOAC-induced coagulopathy, a trial in healthy volunteers found that administration of the 4F-PCC Beriplex® or the 3F-PCC Profilnine® SD (Grifols Biologicals Inc., Los Angeles, CA) was able to reverse rivaroxaban-induced changes in PT and ETP, but did not normalize aPTT or thrombin generation lag time.¹⁹ Another 4F-PCC (Cofact®; Sanquin; Amsterdam, the Netherlands) completely restored PT and ETP after rivaroxaban administration to healthy volunteers, but did not normalize the changes in coagulation parameters (e.g., aPTT) caused by

dabigatran.²⁰ In contrast, an *ex vivo* study showed that Beriplex® significantly reversed dabigatran-induced effects on PT.²¹ In a small *ex vivo* study, the 4F-PCC Kanokad® (LFB, Courtaboeuf, France) and an activated PCC (FEIBA® [Factor VIII Inhibitor Bypassing Activity]; Baxter Healthcare Corp, Westlake Village, CA) reversed rivaroxaban-induced inhibition of ETP; other markers of hemostasis were not significantly affected by these two products after rivaroxaban administration.²² Dabigatran reversal was also assessed in this *ex vivo* study. Kanokad® reversed dabigatran-induced effects on the thrombin generation parameters peak and ETP, but not lag time, which could only be restored using FEIBA®.²² *In vitro* studies have also evaluated Cofact® or Beriplex® for rivaroxaban or dabigatran reversal, respectively.^{23,24} Thrombin generation results in these studies varied depending on the assay parameters and matrices used.^{23,24}

Interpretation of the results of previous studies is challenging for a number of reasons. First, some preclinical studies reported a reduction in bleeding after reversal with PCCs, whereas others did not. Second, the effect of PCCs on various laboratory parameters is inconsistent among both preclinical and clinical studies, and in some cases inconsistent among individual parameters within a study, highlighting the unsuitability of standard coagulation assays to predict effective restoration of hemostasis in NOAC-treated animals or patients. The aPTT and PT are relatively insensitive to dabigatran concentration, and both assays can give varying results depending on the reagent used.^{25–27} Similarly, the sensitivity of the aPTT and PT assays to rivaroxaban depends on the type of assay and reagent used.^{25,28,29} Third, in preclinical studies there is frequently a lack of robust correlation between clinical hemostasis and laboratory parameters. While the precise reason(s) for these discrepancies is/are unknown, potential explanations include inter-study differences in design, methodologies, bleeding models, and NOAC and reversal agent types and doses used. Some authors have speculated that this lack of predictive power may stem from the fact that standard coagulation assays reflect systemic clotting rather than local hemostasis at the

injury site.³⁰ Of note, in the current study, PT, WBCT, and ETP showed the best correlation with restoration of hemostasis, suggesting that these biomarkers might prove to be useful surrogates of 4F-PCC-mediated edoxaban anticoagulation reversal. However, it remains to be demonstrated whether these findings are applicable to other NOACs. In addition, other biomarkers that were not assessed as part of the current study, for example, thromboelastography parameters, may turn out to be valuable. Finally, no reversal studies have yet been published in a large animal model with significant injuries. While such a study may provide interesting evidence, the relevance of a large animal model to assess NOAC reversal is unknown and would first need to be validated, particularly considering the variable sensitivity of animal models to factor Xa inhibition.^{31–35}

Current management guidelines for the reversal of NOAC-associated bleeding suggest a number of strategies.^{36–40} In cases of life-threatening bleeding, the use of a nonactivated PCC is proposed; other hemostatic products such as recombinant factor VIIa (rFVIIa) and activated PCCs (*e.g.*, FEIBA®), might also be considered. The potential for a greater prothrombotic effect with activated PCCs and rFVIIa compared with nonactivated PCCs has led to a preference for the latter in consensus guidance for rivaroxaban reversal.⁴⁰ Porcine models of dilutional/trauma-induced coagulopathy suggest that high dose 4F-PCC or 4F-PCC plus fibrinogen concentrate reduces mortality and blood loss, but may be associated with a thromboembolic risk.^{41,42} These results need to be considered within a wider clinical context, as pharmacovigilance data show that the incidence of treatment-related thromboembolic events after administration of 4F-PCCs is extremely low (21 cases/>600,000 applications).⁴³ Nevertheless, no direct comparative clinical studies of rFVIIa or activated PCCs *versus* nonactivated PCCs have been published, and future safety-focused studies of the potential thrombotic risk of PCC treatment are warranted. Selective NOAC reversal agents, for example, antibody fragments that target factor IIa inhibitors⁴⁴ and a specific antidote to factor Xa inhibitors,⁴⁵ are currently in development. However, general reversal agents are still likely to have a clinical role regardless of the future availability of more selective reversal agents.

Several strengths and limitations of this study should be mentioned. The pivotal portion of the study was performed using a randomized, placebo-controlled, fully blinded design, enabling a rigorous evaluation of the effect of 4F-PCC on edoxaban reversal. In addition, we used an established model that has been used previously to demonstrate the effectiveness of a PCC in reversing the effects of dabigatran on bleeding.¹⁷ Furthermore, edoxaban-induced factor Xa inhibition and anticoagulant effect have been shown to be similar in rabbits and humans, highlighting the pharmacological relevance of this preclinical model.³¹ Conversely, the extent to which these animal data can be extrapolated to a clinical situation is limited, particularly considering that a supratherapeutic

dose of edoxaban was given in order to ensure a measurable difference in bleeding. It should also be noted that the observation period of this study was limited to 40 min; therefore, we were not able to follow-up the potential course of ETP levels at time points exceeding 35-min post-4F-PCC dosing. In addition, the current study design focused on the investigation of the efficacy of 4F-PCC for reversal of edoxaban-associated bleeding. The potential occurrence of adverse events (*e.g.*, thrombosis) as a result of anticoagulant reversal with 4F-PCC was not investigated and would need to be assessed as part of appropriately designed future studies. Previous preclinical studies of 4F-PCC-mediated NOAC reversal in rabbits indicate that there is no prothrombotic risk associated with 4-PCC use in rabbits at doses used in the current study.⁴⁶ Furthermore, the presence of an anticoagulant may further decrease the prothrombotic potential of 4F-PCC;⁴⁶ however, this would need to be confirmed for edoxaban. Therefore, further evaluation of PCC in edoxaban-treated healthy subjects and patients with bleeding complications is required.

This study assessed the effects of 4F-PCC, administered before kidney incision, on edoxaban-associated bleeding. This method allows a comparison of edoxaban effects and reversal with a previously published study of dabigatran reversal.¹⁷ While this model best reflects the need for rapid anticoagulant reversal before urgent surgical procedures, assessing the effectiveness of 4F-PCC treatment in cases of edoxaban-related acute bleeding would require a slightly modified study design, whereby 4F-PCC would be administered postinjury. Based on the half-lives of 4F-PCC coagulation factors in this preclinical model (2 to 20 h), similar plasma levels would be observed throughout the study, irrespective of the timing of 4F-PCC administration and kidney incision. Therefore, we would not necessarily expect any marked difference in outcomes based on the sequence of 4F-PCC dosing and kidney incision. Furthermore, this study evaluated an off-label use of this 4F-PCC, which is currently indicated for urgent VKA reversal in adult patients with acute major bleeding or requiring an urgent surgery/invasive procedure.*

The half-lives of the coagulation factors contained in 4F-PCC were shorter in this study than values observed in humans (5.0 to 60.4 h).^{*} This is consistent with the observation that, in general, elimination of human protein is faster in animals than in humans.^{47,48} As the effects of 4F-PCC on hemostasis were only evaluated up to 30 min in our study (*i.e.*, within one half-life), these differences would probably not impact the study conclusions or affect the relevance of the study model to a human setting. It is possible that the longer factor half-lives in humans would allow for an increased efficacy of reversal; however, a longer half-life may also represent a potential procoagulatory risk and further clinical investigations are warranted.

In conclusion, this 4F-PCC effectively reduced edoxaban-associated bleeding in a rabbit model of hemostasis. There

was a statistically significant reduction in blood loss and time to hemostasis compared with anticoagulated animals that did not receive 4F-PCC. Coagulation test abnormalities were partially normalized, and a number of laboratory parameters (PT, WBCT, and ETP) were identified as potential biomarkers of anticoagulant reversal. 4F-PCC might be an option for urgent reversal of the anticoagulant effects of edoxaban. However, clinical trial data in edoxaban-treated patients are required to confirm these observations.

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Competing Interests

Eva Herzog, Franz Kaspereit, Wilfried Krege, Baerbel Doerr, Jochen Mueller-Cohrs, Ingo Pragst, and Gerhard Dickneite are employees of CSL Behring, Marburg, Germany. Gerhard Dickneite owns CSL Behring stock. Yoshiyuki Morishima is an employee of Daiichi Sankyo, Tokyo, Japan.

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