Vascular Medicine

Edoxaban Effects on Bleeding Following Punch Biopsy and Reversal by a 4-Factor Prothrombin Complex Concentrate

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Background—The oral factor Xa inhibitor edoxaban has demonstrated safety and efficacy in stroke prevention in patients with atrial fibrillation and in the treatment and secondary prevention of venous thromboembolism. This study investigated the reversal of edoxaban's effects on bleeding measures and biomarkers by using a 4-factor prothrombin complex concentrate (4F-PCC).

Methods and Results—This was a phase 1 study conducted at a single site. This was a double-blind, randomized, placebo-controlled, 2-way crossover study to determine the reversal effect of descending doses of 4F-PCC on bleeding duration and bleeding volume following edoxaban treatment. A total of 110 subjects (17 in part 1, 93 in part 2) were treated. Intravenous administration of 4F-PCC 50, 25, or 10 IU/kg following administration of edoxaban (60 mg) dose-dependently reversed edoxaban's effects on bleeding duration and endogenous thrombin potential, with complete reversal at 50 IU/kg. Effects on prothrombin time were partially reversed at 50 IU/kg. A similar trend was seen for bleeding volume.

Conclusions—The 4F-PCC dose-dependently reversed the effects of edoxaban (60 mg), with complete reversal of bleeding duration and endogenous thrombin potential and partial reversal of prothrombin time following 50 IU/kg. Edoxaban alone and in combination with 4F-PCC was safe and well tolerated in these healthy subjects. A dose of 50 IU/kg 4F-PCC may be suitable for reversing edoxaban anticoagulation.

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Edoxaban is a once-daily, non–vitamin K oral anticoagulant (NOAC) that directly inhibits factor Xa,[1] and has demonstrated safety and efficacy in the prevention of stroke in patients with atrial fibrillation[2] and in the treatment and secondary prevention of venous thromboembolism.[3] Edoxaban has been approved in Japan for the prevention of stroke in atrial fibrillation, treatment and prevention of recurrent venous thromboembolism,[4] and also the prevention of venous thromboembolism following orthopedic surgery.[5] Edoxaban is currently under regulatory consideration in other countries. In comparison with warfarin, edoxaban has more rapid anticoagulant effects and a more predictable pharmacokinetic profile.[6,7] Additionally, unlike warfarin, anticoagulation with edoxaban does not require routine laboratory monitoring.[8]

Clinical Perspective on p 90

Reversal of the anticoagulant effects of NOACs, including edoxaban, is important in the event of clinically relevant bleeding, or when emergency intervention is called for in patients receiving a NOAC. A number of agents have been evaluated for the reversal of NOAC activity. These include a modified human factor Xa (andexanet alfa; PRT064445)[9]; a small molecule, rapid reversal agent that binds directly to NOACs (PER977)[10]; an antibody fragment specific to dabigatran (idarucizumab)[11,12]; and various prothrombin complex concentrates (PCCs).[13–16] PCCs are pooled plasma products that contain significant concentrations of 3 factors (II, IX, and X) or 4 factors (II, VII, IX, and X) and vitamin K–dependent proteins, and are under clinical investigation for the reversal of NOAC anticoagulation.[14]

In animal studies, hemostatic models have been effective for assessing the reversal of the anticoagulatory response of the NOAC.[17] To date, in human subjects, reversal of the NOAC has typically been confirmed by using biomarker end points including anti–Factor Xa activity, prothrombin time (PT), and endogenous thrombin potential (ETP), the area under the thrombin generation curve. In a clinical study in human subjects, a 50 IU/kg dose of a 4-factor PCC (4F-PCC) was shown to completely reverse the effects of a 20-mg twice-daily rivaroxaban dose by using PT and ETP.[13] Another clinical study compared 50 IU/kg doses of 3- and 4-factor PCCs for reversal of the effects of 20-mg twice-daily rivaroxaban, and demonstrated partial reversal of rivaroxaban effects on both ETP and PT. The 3-factor PCC showed greater reversal effects on ETP, and 4F-PCC showed greater reversal effects on PT.[15] In the absence of a reliable assessment for bleeding time, it is difficult to know which biomarker is optimal for evaluating PCC-mediated reversal of NOAC activity. Unfortunately, standard bleeding time is a platelet-dependent measure that is relatively insensitive to the anticoagulation activity of edoxaban.[18,19] Hence, it is critical to identify a bleeding model that is sensitive to the effects of NOACs and can be used to evaluate reversal by PCCs.

The 2-part study reported here demonstrated an edoxaban dose–dependent (60 and 180 mg edoxaban) prolongation of bleeding duration (BD) following punch biopsy (part 1; study design and results of part 1 can be found in online-only Data Supplement Materials) and evaluated the effects of a 4F-PCC on the anticoagulation activity of 60 mg edoxaban in healthy subjects as measured by BD, bleeding volume (BV), and ETP (part 2). The primary objective for part 1 was to determine the sensitivity and variability of BD and BV following single doses of 60 mg or 180 mg of edoxaban. Edoxaban doses investigated in part 1 of this study include the currently investigated clinical dose (60 mg) and a supratherapeutic dose (180 mg). This allowed us to establish the sensitivity of bleeding duration following punch biopsy to changes in edoxaban exposure. In part 2, reversal of anticoagulation achieved with a therapeutic dose of 60 mg of edoxaban was evaluated by using 50, 25, and 10 IU/kg 4F-PCC by measuring BD, BV, ETP, and PT as effect metrics. The highest dose of 4F-PCC (50 IU/kg) was selected based on a previous publication indicating that this dose reversed the effect of 20 mg of rivaroxaban[13] and based on safety concerns associated with administering higher doses of PCC to healthy subjects.[20]

Materials and Methods

Study Design and Objectives

This was a 2-part, single-center (Quintiles Phase I Unit, Overland Park, KS), phase 1 study (NCT02047565). The protocol was approved by an institutional review board (MidLands Independent Review Board, Overland Park, KS) and conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonisation. All subjects provided written informed consent prior to screening. The first patient was enrolled in the study on September 27, 2013, with the last patient discharged from the clinic on May 18, 2014. Part 1 was a 2-cohort, open-label, randomized, 2-sequence, 2-treatment, 2-way crossover study that established the punch biopsy procedure and determined the sensitivity and variability of BD and BV following single doses of edoxaban 60 mg (online-only Data
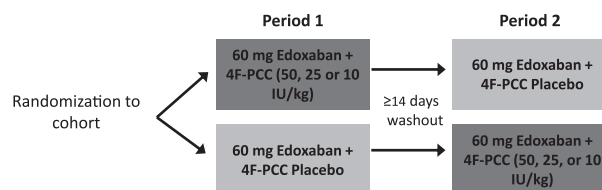

Figure 1. Study design for part 2. 4F-PCC indicates 4-factor prothrombin complex concentrate.

Supplement Figure I). Details of the methods used in part 1 are presented in online-only Data Supplement Materials.

Part 2 was a double-blind, randomized, placebo-controlled, 2-sequence, 2-period crossover study that determined the reversal effect of descending doses of 4F-PCC on edoxaban using BD, BV, ETP, and PT as metrics (Figure 1). The primary end point for assessing reversal was BD. Subjects were sequentially enrolled into 3 cohorts. Within each cohort, subjects were randomly assigned to receive edoxaban 60 mg followed by placebo or 4F-PCC in period 1 and the alternate treatment in period 2. A baseline punch biopsy was conducted on day –1. On day 1, after an overnight fast, each subject received a single, oral 60-mg dose of edoxaban followed by an infusion of placebo (0.9% sodium chloride injection, USP) or 4F-PCC (Beriplex P/N; CSL Behring GmbH, Marburg, Germany) according to the randomization schedule. Three doses of 4F-PCC (50, 25, and 10 IU/kg) were investigated in 3 separate cohorts in a dose-descending manner (Figure 1). Descending doses of 4F-PCC were studied until no reversal was observed. Infusions were administered at the maximum rate of 210 IU/min, lasted 15 to 20 minutes, and were timed to end 2.25 hours after the dose of edoxaban. No 4F-PCC administration exceeded the maximum allowable dose of 5000 IU.[21] On treatment days (day 1 of each period), punch biopsies were performed 30 minutes after the end of 4F-PCC or placebo infusion. Samples for determination of edoxaban plasma concentration and coagulation biomarkers were collected pre- and postdose on day 1 and on the morning of days 2 and 3. There was a washout period of at least 2 weeks between edoxaban dosing (day 1 of periods 1 and 2). Follow-up virology testing was conducted 12 days after discharge and 100 days after the last dose of treatment (data not presented here). Safety assessments included adverse events (AEs), clinical laboratory tests, fecal occult blood tests, vital signs, triplicate 12-lead ECGs, physical examination findings, and virology testing results. AEs were recorded throughout the study.

Subjects

Healthy men and women of nonchildbearing potential, aged 18 to 45 years, with a body mass index of 18 to 30 kg/m² and weighing ≤110 kg were eligible for study enrollment. Subjects were excluded if they had a history of unexplained syncope, had taken strong inhibitors or inducers of cytochrome P450 3A4/5 enzymes or P-glycoprotein within the past 28 days, or used any nonprescription drugs or herbal supplements with the exception of acetaminophen (up to 3 g/d) within the past 14 days. A history of minor, major, or gastrointestinal bleeding; a family history of coagulopathy; anticoagulant use; or sensitivity to any product used in the study were also conditions for exclusion. Subjects for whom surface blood vessels could not be visualized or who had a history or likelihood of forming keloid scars were excluded. In addition, subjects for part 2 were excluded if they had factor V Leiden mutation or any other predispositions for clotting or bleeding, had a known anaphylactic or severe systemic reaction to 4F-PCC or its components, or had a prolonged BD (>15 minutes) at baseline testing before randomization during period 1.

Blood Sample Collection

Blood samples for bioanalysis and biomarker analysis were obtained via an indwelling catheter or direct venipuncture (see online-only Data Supplement Table I for timing). For determination of edoxaban concentration, 3-mL samples were collected in heparinized Vacutainer
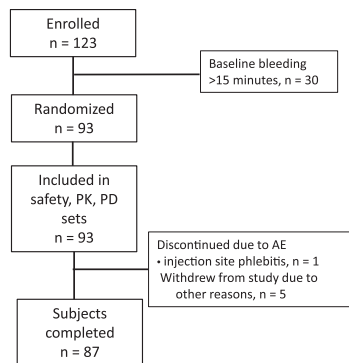
Figure 2. CONSORT diagram for part 2. AE indicates adverse event; PD, pharmacodynamics; and PK, pharmacokinetic.

tubes, plasma was separated from whole blood, and plasma samples were frozen at –20°C for shipment to Quintiles Biosciences, Inc. (Ithaca, NY) for analysis. Two 4.5-mL samples per subject were collected by direct venipuncture for biomarker analysis, plasma was separated from whole blood, divided into aliquots, and frozen at –70°C until assayed at MedPace Reference Laboratories (Cincinnati, OH).

Punch Biopsy

Following administration of local lidocaine without epinephrine, a disposable instrument was used to perform a punch biopsy (5 mm diameter, 5 mm depth) on the back of the thigh. BV was assessed at 2-minute intervals by converting the weight (1.05 g/mL) of blood absorbed onto preweighed filter paper discs. Evaporation was limited by placing saturated discs in a sealed, humidified, preweighed container. BD was defined as time of blood emergence to the end of bleeding. Standard methods to induce hemostasis were taken if bleeding continued for >25 minutes. For part 2, BD and BV were considered unevaluable if BD exceeded 25 minutes. However, other biomarker data from these subjects were used for statistical analysis.

Biomarker and Plasma Concentration Assessments

The maximum observed plasma drug concentration (C_{max}), time to reach maximum plasma concentration (t_{max}), area under the plasma concentration versus time curve from time 0 to the last quantifiable concentration, and the area under the plasma concentration versus time curve from time 0 to 24 hours were computed from the individual plasma concentrations of edoxaban versus time profile by using a noncompartmental approach. Assays for PT were performed by Medpace Reference Laboratories using validated methods. PT was measured using the thromboplastin preparation Neoplastin C1+ on a Stago platform (Diagnostica Stago, Parsippany, NJ). ETP was measured using Technothrombin TGA (Technoclone GmbH, Vienna, Austria) on a BioTex Flx 800 fluorometer (BioTek, Winooski, VT). The minimum observed activity value (A_{min}), maximum observed activity value (A_{max}), and time of maximum observed activity value (T_{max}) were calculated for PT and ETP. For ETP, the minimum percentage of change in observed activity relative to baseline was calculated ($\%\Delta A_{min}$).

Statistical Analysis

For part 2, a dose cohort size of 22 evaluable subjects was determined to have at least 80% power to demonstrate the 95% confidence interval (CI) for the BD ratio (posttreatment over baseline) to be within a 70% to 143% limit. Therefore, ≈30 subjects were randomized per dose cohort to attain 22 evaluable subjects.

   The BD and BV associated with punch biopsies at baseline and at ≈30 minutes after completion of each 4F-PCC infusion, as well as the the change from baseline, were summarized by treatment and cohort. For both part 1 and part 2, log-transformed BD values associated with each punch biopsy, as well as the change from baseline,

were analyzed by treatment with the use of a linear mixed model with time as a fixed effect and subject as a random effect. PT and ETP at each time point and change from baseline were summarized by treatment; statistical comparisons were performed at ≈2.75 hours after edoxaban dosing.

   In part 2, the correlation of treatment to BD was analyzed by using an analysis of covariance model for crossover design with baseline values as a covariate. Least-squares means and 95% CIs were estimated for between-treatment group ratio (4F-PCC/placebo) and within-treatment group ratio (posttreatment/baseline), and *P* values were determined for the treatment effect. Least squares means and 95% CIs were estimated for posttreatment/baseline.

   The assessment of complete reversal was based on the 95% CI of the least squares means ratio following 4F-PCC treatment over baseline falling within the interval of 70% to 143%. Percent reversal was calculated post hoc according to the following formulas using the group mean values:

$$\left(\text{Placebo effect} \; - \; 4F-PCC\;\text{effect}\right) / \text{placebo} \; * \; 100$$

Where for BD and BV:

$$\text{Placebo effect} = \text{Ratio (post-treatment/baseline)} * 100 \; - \; 100,$$
$$\text{for edoxaban} + \text{placebo}$$

$$4F\text{-}PCC \text{ effect} = \text{Ratio (post-treatment/baseline)} * 100 \; - \; 100,$$
$$\text{for edoxaban} + 4F-PCC$$

and for ETP and PT:

$$\text{Placebo effect} = \text{Ratio (postdose/predose)} * 100 \; - \; 100,$$
$$\text{for edoxaban} + \text{placebo}$$

$$4F\text{-}PCC \text{ effect} = \text{Ratio (postdose/predose)} * 100 - 100,$$
$$\text{for edoxaban} \; + \; 4F-PCC$$

Results

A total of 110 subjects were treated in the study; 17 subjects in part 1 (online-only Data Supplement Figure II), and 93 subjects in part 2 (Figure 2). In part 2, cohort 1, 31 subjects received placebo and 33 subjects received 4F-PCC 50 IU/kg; in cohort 2, 28 subjects received placebo and 28 received 4F-PCC 25 IU/kg; in cohort 3, 30 subjects received placebo and 30 subjects received 4F-PCC 10 IU/kg. The majority of subjects enrolled in the study were male and white, with a mean age of 30 years and a body mass index of 25 kg/m². Subject demographics were similar in part 1 and part 2 (online-only Data Supplement Table II). Results from part 1 of the study are provided in online-only Data Supplement Text.

Part 2

Based on lower intrasubject variability associated with BD in comparison with BV, BD was selected as the primary end point in part 2 (online-only Data Supplement Materials). Pharmacokinetic results for edoxaban (online-only Data Supplement Table III) and the 4 factors in the PCC (FII, FVII, FIX, and FX) in part 2 (data not shown) were consistent with those reported previously. Administration of 4F-PCC 50 IU/kg resulted in complete reversal of the effects of edoxaban on BD, as indicated by a ratio of postdose to baseline
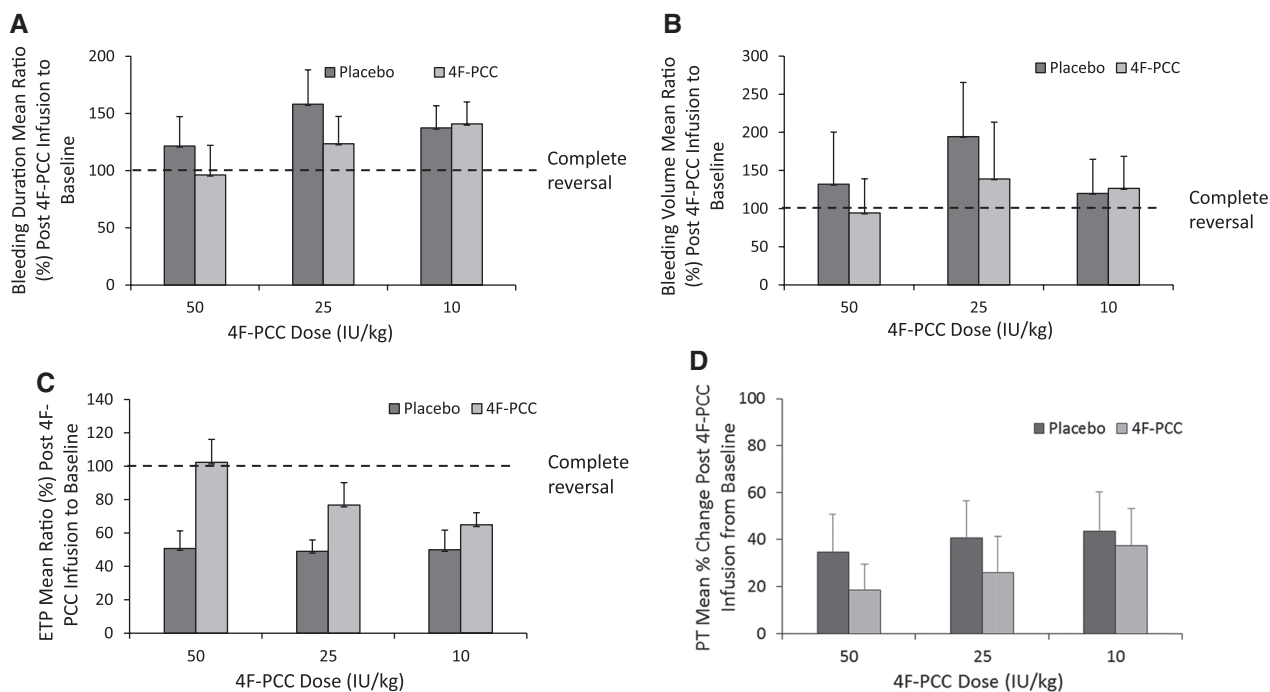
Figure 3. Effect of treatment with 4F-PCC or placebo on the mean ratio post–4F-PCC infusion to baseline from bleeding duration (minutes) (**A**), baseline bleeding volume (mL) (**B**), ETP (nM×min) (**C**), and for mean percent change in PT (seconds) (**D**). Error bars represent upper limit of the 95% CI. CI indicates confidence interval; ETP, endogenous thrombin potential; 4F-PCC, 4-factor prothrombin complex concentrate; and PT, prothrombin time.

least squares mean values for BD near unity, and containment of the ratio of the postdose to baseline least squares mean values within the predefined limits of 70% to 143% (Figure 3B). Additionally, there was an ≈21% decrease in baseline normalized postdose BD values following 4F-PCC 50 IU/kg versus placebo treatment (mean ratio 4F-PCC versus placebo, 79.2; 95% CI, 61.5–101.9). Administration of 4F-PCC 25 IU/kg resulted in partial reversal of the effects of edoxaban on BD. Bleeding duration values at the 4F-PCC end of infusion relative to baseline were similar for both placebo and 4F-PCC 10 IU/kg, indicating no reversal of edoxaban effect by the lowest tested dose of 4F-PCC (Figure 3A). A similar trend was present for BV; coadministration of 4F-PCC 50 IU/kg resulted in complete reversal of the effects of edoxaban on BV (mean ratio 4F-PCC versus placebo, 94.2; 95% CI, 63.9–139.0; Figure 3B), whereas 4F-PCC 25 IU/kg produced partial reversal, and the 4F-PCC 10 IU/kg dose produced no reversal.

After administration of edoxaban followed by placebo infusion, the mean ETP at 2.75 hours post–edoxaban dosing was reduced by ≈50% in comparison with baseline in all cohorts (Figure 3C). Administration of 4F-PCC 50 IU/kg after edoxaban dosing resulted in a complete reversal of the effects of edoxaban on ETP at 2.75 hours after edoxaban administration; 4F-PCC 25 IU/kg produced partial reversal, and 4F-PCC 10 IU/kg did not reverse effects on ETP (Figure 3C, Tables 1 and 2, and online-only Data Supplement Table IV). These results were consistent with the effects on BD. At later time points, ETP values increased above baseline dose-dependently with increasing 4F-PCC dose (Figure 4, Tables 1 and 2, and online-only Data Supplement Table IV [see $\%\Delta A_{min}$]).

Although a complete reversal of ETP effects occurred following the administration of 4F-PCC 50 IU/kg, this dose did not result in a complete reversal of the effects of edoxaban administration on PT (Figure 5). However, a 4F-PCC dose response was evident, with the higher concentrations of 4F-PCC producing a greater reduction in PT prolongation than the lowest dose (Figure 3D). As shown in Figure 6, there was a dose-dependent response of the 4F-PCC on each measure. However, the percentage of reversal achieved by 4F-PCC 50 and 25 IU/kg was similar for BD, BV, and ETP, whereas PT generally indicated a lower percentage of reversal for all doses.

Prothrombin fragment 1+2 (F_{1+2}) showed a transient and dose-dependent increase around the time of infusion, but all F_{1+2} values returned to near baseline by 24 hours postdose. No significant changes were observed in D-dimer at any time point (online-only Data Supplement Figures III and IV).

Safety

A total of 8 (7.3%) subjects discontinued early from the study. There were 3 discontinuations due to AEs; 2 subjects from part 1, one because of a pilonidal cyst and one because of a prolonged activated partial thromboplastin time secondary to an inhibitor present in his plasma that developed following a viral infection that occurred 5 days after receiving treatment. The elevated activated partial thromboplastin time returned to a normal range at a later follow-up. One subject in part 2 withdrew owing to phlebitis at the catheter site for blood draws. A total of 5 subjects from part 2 withdrew for other reasons. All AEs leading to discontinuation resolved, were considered to be mild in severity, and none were considered to be related to study drug administration.

Table 1. Comparisons of Bleeding Duration and Bleeding Volume in Response to Infusion of Placebo or PCC 50, 25, or 10 IU/kg at 2.75 Hours Following Edoxaban Treatment: Comparison Posttreatment With Baseline by Treatment Group

Dose	Baseline, n	Baseline Geometric/LS Mean	Postdose, n	Postdose Geometric LS Mean	Ratio Postdose to Baseline	95% CI (*P* Value)
BD						
50 IU/kg						
4F-PCC	27	12.0	26	11.5	96.3	75.9–122.1 (0.7451)
Placebo	25	12.1	22	14.7	122	100.4–147.2 (0.0455)
25 IU/kg						
4F-PCC	28	9.62	24	11.9	124	103.6–147.4 (0.0208)
Placebo	28	9.34	26	14.8	158	132.9–188.1 (<0.0001)
10 IU/kg						
4F-PCC	30	11.1	26	15.6	141	123.8–160.1 (<0.0001)
Placebo	30	11.3	25	15.5	137	120.3–156.7 (<0.0001)
BV						
50 IU/kg						
4F-PCC	27	2.60	26	2.45	94.2	63.9–139.0 (0.7553)
Placebo	25	2.34	22	3.08	132	86.9–200.4 (0.1843)
25 IU/kg						
4F-PCC	28	1.73	24	2.40	139	90.4–213.3 (0.1281)
Placebo	28	1.79	26	3.49	194	142.5–265.4 (0.0002)
10 IU/kg						
4F-PCC	30	2.26	26	2.86	126	94.9–168.5 (0.1054)
Placebo	30	2.00	25	2.39	120	87.3–164.6 (0.2509)

BD indicates bleeding duration; BV, bleeding volume; CI, confidence interval; LS, least squares; 4F-PCC, 4-factor prothrombin complex concentrate.

No deaths, serious AEs, or thromboembolic events occurred during the study. The most common treatment-emergent AEs during part 1 were upper respiratory tract infection (n=3) and nausea (n=2). The most common treatment-emergent AEs during part 2 were headache (n=6), nasal congestion and upper respiratory tract infection (n=3), and abdominal discomfort, nausea, contusion, excoriation, and procedural pain (2 subjects each). Most AEs in part 1 and part 2 were mild in intensity with the exception of moderate contusion and moderate muscle strain in 1 subject in part 2, which were not considered related to the study drug administration. In part 1, 1 subject had prolonged activated partial thromboplastin time secondary to an inhibitor present in his plasma following a viral infection. No other treatment-emergent AEs were reported for hematology, serum chemistry, urinalysis, and coagulation variables in part 1 or part 2.

Discussion

In a study evaluating the safety and efficacy of recombinant-activated factor VII for the reversal of bleeding in healthy subjects, warfarin-treated patients had both prolonged BD and BV relative to baseline following a punch biopsy procedure.[22] Based on the presence of large coefficients of variation for both BD and BV the methodology was shown to have a high degree of variability in these previous studies.[22,23] To address this issue, in part 1 of the current study, the punch biopsy method was rigorously standardized during the training cohort, establishing a procedure followed throughout part 2. To maintain this level of standardization, the numbers of physicians performing the biopsies and measuring BV were limited, and further, the same physicians performed all biopsies for any given subject with only a few exceptions. As a result, better coefficients of variation were obtained in this study (26%–35% for BD and 36%–38% for

Table 2.   Comparisons of Bleeding Duration and Bleeding Volume in Response to Infusion of Placebo or PCC 50, 25, or 10 IU/kg at 2.75 Hours Following Edoxaban Treatment: Comparison Between 4F-PCC and Placebo by 4F-PCC Dose

Dose	4F-PCC, n	4F-PCC Geometric LS Mean	Placebo, n	Placebo Geometric LS Mean	Ratio 4F-PCC to Baseline	95% CI (P Value)
BD						
50 IU/kg	25	95.4	21	120.5	79.2	61.5–101.9 (0.0680)
25 IU/kg	24	126	26	160	78.8	65.4–94.9 (0.0140)
10 IU/kg	26	144	25	143	101	87.9–115.0 (0.9308)
BV						
50 IU/kg	25	91.6	21	120	76.3	56.8–102.5 (0.0703)
25 IU/kg	24	140	26	204	68.6	50.8–92.7 (0.0161)
10 IU/kg	26	137	25	117	117	86.8–156.4 (0.2970)

BD indicates bleeding duration; BV, bleeding volume; CI, confidence interval; LS, least squares; 4F-PCC, 4-factor prothrombin complex concentrate.

BV) in comparison with the warfarin study. As expected, BD had less variation than BV.

In part 1, both BD and BV were shown to increase with edoxaban treatment in a dose-dependent manner, indicating the sensitivity of both BD and BV to edoxaban exposures at the target clinical and supratherapeutic doses. This is in contrast to the standard bleeding time measures, which are highly platelet dependent and not sufficiently sensitive for assessing the effects of NOACs.
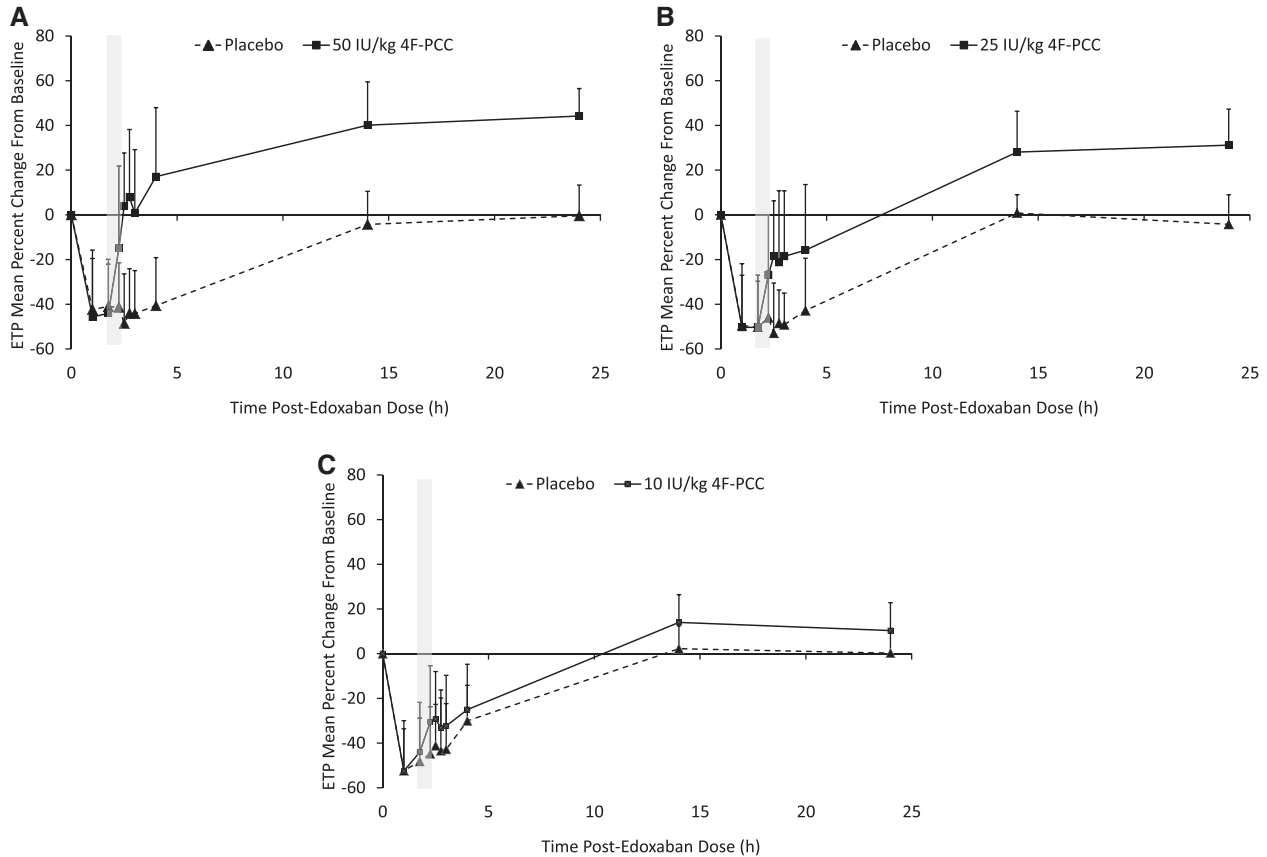

Figure 4. Time course of ETP on mean percent change from baseline for treatment with 50 IU/kg (**A**), 25 IU/kg (**B**), or 10 IU/kg 4F-PCC or placebo (**C**). Error bars represent standard deviation. Shaded bar indicates 4F-PCC infusion. ETP indicates endogenous thrombin potential; and 4F-PCC, 4-factor prothrombin complex concentrate.
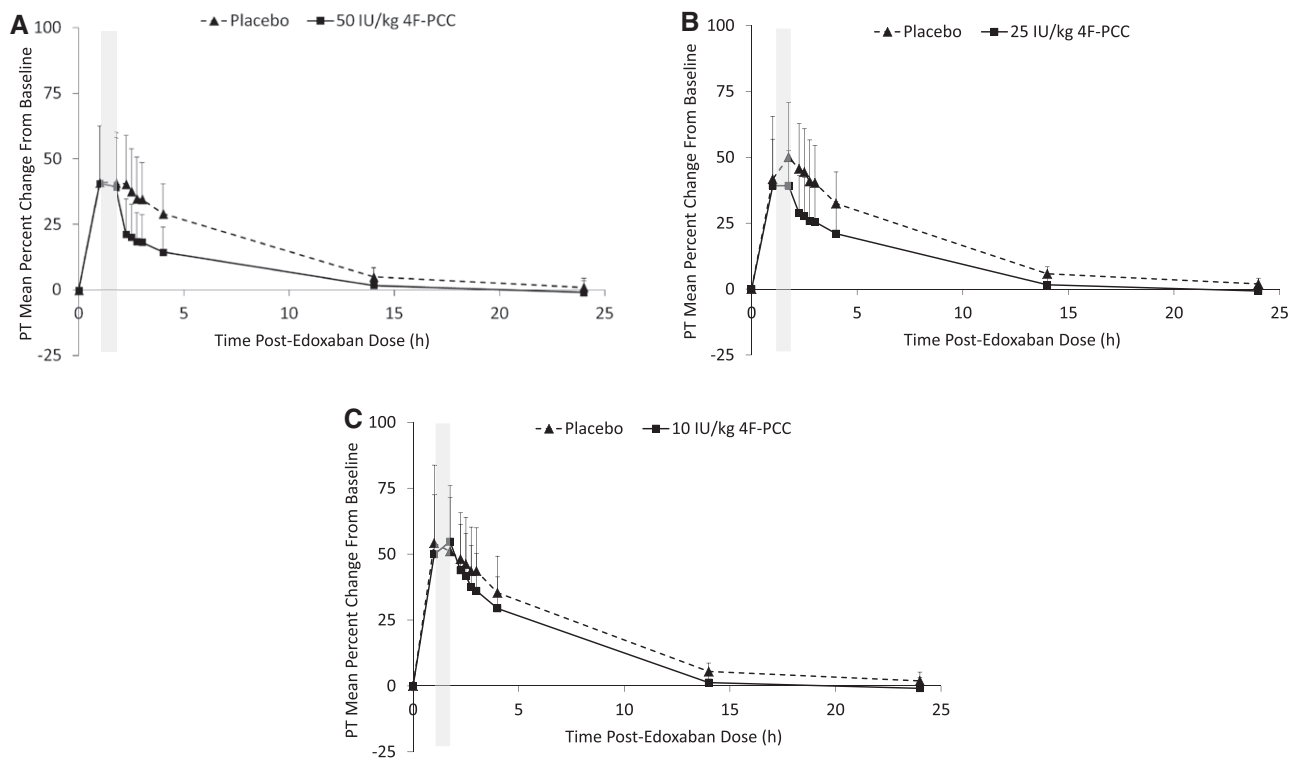
Figure 5. Time course of PT on mean percent change from baseline for treatment with 50 IU/kg (**A**), 25 IU/kg (**B**), or 10 IU/kg 4F-PCC or placebo (**C**). Error bars represent standard deviation. Shaded bar indicates 4F-PCC infusion. 4F-PCC indicates 4-factor prothrombin complex concentrate; and PT, prothrombin time.

Because the punch biopsy procedure is more invasive, it may be more representative of clinical bleeding situations; however, the extent to which post–punch biopsy bleeding extrapolates to clinical bleeding situations is unknown at this time.

In part 2, the effectiveness of a 4F-PCC was evaluated at descending doses for reversal of BD prolongation following a single dose of edoxaban 60 mg. Secondary end points included BV, ETP, and PT. The effects of edoxaban on BD were completely reversed by 4F-PCC 50 IU/kg. Partial reversal of the effects of edoxaban was achieved with 4F-PCC 25 IU/kg, and no reversal was observed after the administration of 4F-PCC 10 IU/kg.

In the event of surgery, the need for a reversal agent for edoxaban is less pressing than for warfarin. Because the pharmacological effects of edoxaban decrease as the drug is eliminated, the relatively short half-life of edoxaban (10–14 hours)[24,25] provides the option of waiting until the time of the next scheduled dose before initiating nonemergency surgery. This is in contrast to warfarin, for which the pharmacodynamic effects persist for days after drug discontinuation.[7] However, when the need for surgery is urgent, 4F-PCC 50 IU/kg appears suitable for reversal of the effects of a therapeutic dose of edoxaban (60 mg).

When considering reversal of clinical relevant bleeding, the greatest concern is intracranial hemorrhage, which occurs less frequently with edoxaban than with warfarin.[2] The results of this study demonstrate that 4F-PCC 50 IU/kg provides rapid reversal of prolonged bleeding following edoxaban. Additionally, 4F-PCC is readily available and widely marketed. Therefore, this represents an important option for rapid treatment in the unlikely event of an intracranial hemorrhage or other clinically relevant bleeds associated with edoxaban treatment.

The effect of 4F-PCC on ETP was similar to the effects on BD and BV, suggesting that ETP is an appropriate surrogate biomarker for assessing the effect on bleeding by using a punch biopsy method. In contrast, PT was less sensitive than ETP to the effects of the 4F-PCC studied here, demonstrating only partial reversal even at the highest dose of 4F-PCC studied. These findings are in agreement with those of Levi et al,[15] who showed that, for a 4F-PCC, PT was less sensitive than ETP in detecting the reversal of rivaroxaban effects, and they are consistent with preclinical data showing the partial reversal of the effects of edoxaban on PT in vitro.[26] Infusion of a 4F-PCC alters the levels of coagulation factors II, VII, IX, and X relative to other components of the clotting cascade in blood and plasma in healthy subjects. This may have unexpected effects on the performance of PT assays standardized to perform in blood from healthy or warfarin-treated subjects. Thus, the apparent insensitivity of PT to reversal may be an assay artifact. Alternatively, this insensitivity may reflect an actual difference in the clinical relevance of PT and thrombin generation assays in the context of NOAC therapy.

At later time points following 4F-PCC infusion, ETP increased above baseline in a 4F-PCC dose-dependent manner. This increase may be due to the presence of the factors of the prothrombin complex in the 4F-PCC. Although an early transient increase in F_{1+2} was observed before punch biopsy, the peak concentrations
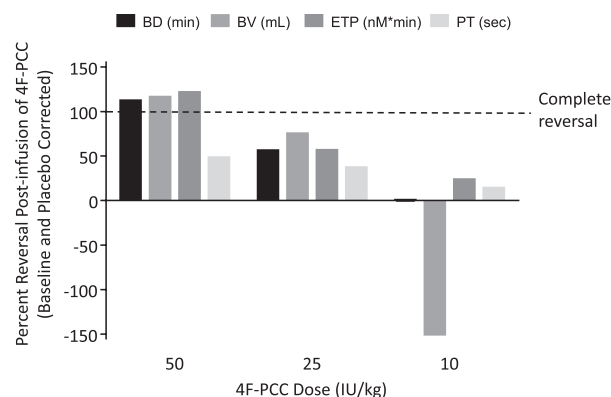
Figure 6. Percent reversal postinfusion 4F-PCC. BD indicates bleeding duration; BV, bleeding volume; ETP, endogenous thrombin potential; and 4F-PCC, 4-factor prothrombin complex concentrate.

were consistent with the amount of F_{1+2} in 4F-PCC,[27] and no late procoagulant effects were seen for either F_{1+2} or D-dimr. These results may suggest that a procoagulant state was not induced in the healthy subjects studied here, but it cannot be ruled out. The clinical implications of the ETP overshoot at later time points are unclear and warrant further investigation.

Variations in the mean effect of edoxaban 60 mg and placebo were present across various 4F-PCC dose cohorts in part 2 of the study. Although we cannot rule out individual variability in edoxaban exposure, the mean pharmacokinetic parameter appeared comparable across cohorts, and the ETP data demonstrated a consistent edoxaban response across cohorts. This suggests that the punch biopsy procedure has greater inherent variability than thrombin generation, even under a rigorously standardized protocol. A period effect for BD was observed in this study, with consistently longer baseline BD in period 2, irrespective of washout period and preceding treatment (with or without 4F-PCC). Therefore, statistical comparisons were performed by using the day −1 values from each corresponding period as the baseline values and analyzed by the baseline covariate adjusted analysis. The reason for the observed period effect was unclear. However, this result suggests that future studies should be performed by using a parallel group design to avoid the complications associated with the period effect.

In conclusion, because of the sensitivity of punch biopsy BD and BV to edoxaban dosing and the acceptable variability of these measures, bleeding following punch biopsy can be used as an assessment for evaluating the reversal of the edoxaban effect. The 4F-PCC dose-dependently reversed the effects of edoxaban on BD, with complete reversal observed following 50 IU/kg, partial reversal observed following 25 IU/kg, and no reversal observed following 10 IU/kg. These results suggest that 4F-PCC represents a readily available, widely marketed option in cases where rapid reversal of edoxaban anticoagulation is essential. A 4F-PCC 50 IU/kg dose appears to be appropriate for reversal of the effects of a therapeutic dose of edoxaban in the case of clinically relevant bleeding or an urgent need for surgery.

Acknowledgments

Sources of Funding

Disclosures

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CLINICAL PERSPECTIVE

The oral factor Xa inhibitor edoxaban has demonstrated safety and efficacy in stroke prevention in patients with atrial fibrillation and treatment and secondary prevention of venous thromboembolism. Reversal of anticoagulant effects of non–vitamin K oral anticoagulants, including edoxaban, is important in the event of clinically relevant bleeding, or when emergency intervention is called for in patients receiving a non–vitamin K oral anticoagulant. In this phase 1, double-blind, randomized, placebo-controlled, 2-way crossover single-site study, the reversal of edoxaban's effects on bleeding duration and bleeding volume following punch biopsy were investigated by using descending doses of a 4-factor prothrombin complex concentrate (4F-PCC). Intravenous administration of 4F-PCC 50, 25, or 10 IU/kg following edoxaban 60 mg dose-dependently reversed edoxaban's effects on bleeding duration and endogenous thrombin potential, with complete reversal at 50 IU/kg. A similar trend was seen for bleeding volume. The effects on prothrombin time were partially reversed at 50 IU/kg. The 4F-PCC dose-dependently reversed the effects of edoxaban (60 mg), with complete reversal of bleeding duration and endogenous thrombin potential and partial reversal of prothrombin time following 50 IU/kg. Punch biopsy was shown to have acceptable sensitivity and variability to assess the reversal of edoxaban's effects. Further, these results suggest that 4F-PCC represents a readily available, widely marketed option in cases where rapid reversal of edoxaban anticoagulation is essential. A 4F-PCC 50 IU/kg dose appears to be appropriate for reversal of the effects of a therapeutic dose of edoxaban in the case of clinically relevant bleeding or an urgent need for surgery.

Supplemental Material

Part 1 study design

Part 1 was a 2-cohort, open-label, randomized, 2-sequence, 2-treatment, 2-way crossover study that established the punch biopsy procedure and determined the sensitivity and variability of BD and BV following single doses of edoxaban (**Data Supplement Figure I**). Two cohorts were enrolled. The first cohort was used for study center training on the punch biopsy procedure. Cohort 2 was used to determine the sensitivity, variability, reproducibility, and effect size for BD and BV following single doses of edoxaban 60 and 180 mg.

Sample size in part 1 was not determined based on statistical consideration. In part 1, the correlation between BD and BV with each biomarker was assessed at the 5% level, as well as the correlation between BD, BV, biomarkers, and plasma edoxaban peak exposure.

Screening occurred within 30 days of study initiation. In period 1, following screening and safety assessments, subjects underwent a baseline punch biopsy. After an overnight fast, subjects were randomized to receive a single oral dose of either edoxaban 60 mg or a supratherapeutic dose of edoxaban 180 mg. A punch biopsy was taken 2.75 hours after dosing and BV and BD were measured. After a 1-week washout period, subjects received the alternate dose of edoxaban in period 2 and assessments were repeated. Safety follow-up was conducted approximately 12 days after discharge.

Bioanalytical methods

Edoxaban plasma concentrations were analyzed using a validated liquid chromatography-mass spectrometry method, with upper and lower limits of quantification of 0.074 ng/ml and 382 ng/ml, respectively. FII, FVII, and FX were determined by one-stage

clotting assay using Thromborel® S as activator reagent, FIX was determined by a one-stage clotting assay using Pathromtin SL as the activator reagent, and protein C was measured by chromogenic assay Berichrom® Protein C (all Siemens Healthcare Diagnostics, Marburg, Germany), all using the BCSXP Analyzer (Siemens Healthcare Diagnostics, Eschborn, Germany). Protein S was determined using a commercially available enzyme immunoassay (Affinity BiologicalsINC, Ontario, Canada) on the BEP® III Processor (Siemens Healthcare Diagnostics, Eschborn, Germany).

Part 1 results

Both edoxaban doses of 60 and 180 mg significantly increased both BD (60 mg: 9.7 min predose vs 14.1 min postdose, 95% CI 100.91 to 211.10, $P=0.05$; 180 mg: 9.79 min vs 17.38 min, 95% CI 128.0 to 246.3, $P=0.005$) and BV (60 mg: 1.9 mL predose vs 2.4 mL postdose, 95% CI 84.5 to 185.7, $P=0.22$; 180 mg: 1.5 mL vs 2.9 mL, 95% CI 122.5 to 300.4, $P=0.01$) relative to baseline. Irrespective of treatment, a period effect was also present, with increased baseline BD in period 2 relative to period 1 (17.2 minutes vs 8.45 minutes, respectively, for 60-mg edoxaban and 15.8 minutes vs 6.62 minutes, respectively, for 180-mg edoxaban).

Pharmacokinetic results for edoxaban treatment were consistent with those reported previously (**Data Supplement Table III**).[1,2] There were dose-dependent increases in BD and BV with edoxaban doses of 60 and 180 mg. Intrasubject variability associated with BD (35% for edoxaban 60 mg and 26% for edoxaban 180 mg) was lower than for BV (37.5% for edoxaban 60 mg and 35.7% for edoxaban 180 mg), therefore BD was selected as the primary endpoint in Part 2.

Correlations

2

Thrombin generation lag was significantly correlated with BD ($P=0.04$) and there was a trend towards a correlation between ETP (as a measure of thrombin generation) and BD ($P=0.07$). No other parameters of thrombin generation were significant at the 5% or 10% level. PT and aPTT were not significantly correlated with BD, but showed a trend towards significance at the 5% level ($P<0.1$). Neither factor Xa ($P=0.64$) nor intrinsic factor Xa ($P=0.07$) were shown to correlate with BD. There was no significant correlation between edoxaban C_{max} and BD or BV.

Supplemental References

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Data Supplement Table I: Blood draw schedule

	Day -1	Pre-dose	0.5	1	1.75	2	2.25	2.5	2.75	3	4	6	10	14	Day 2 24	Day 3 48
						Day 1 (hours)										
Part 1																
Edoxaban plasma concentrations		X	X	X		X				X	X	X	X	X	X	X
Biomarker assessment	X	X		X		X			X		X	X		X	X	X
Part 2																
Edoxaban plasma concentrations		X	X	X		X				X	X	X	X	X	X	X
PT/ETP		X		X	X		X	X	X	X	X			X	X	X
F1+2, D-dimer		X							X		X			X	X	

ETP, endogenous thrombin potential; F1+2, prothrombin fragment 1 + 2; PT, prothrombin time.

4

Data Supplement Table II: Subject demographics

	Overall (N = 110)	Part 1 (n = 17)	Part 2 (n = 93)
Sex, n (%)			
Male	77 (70.0)	13 (76.5)	64 (68.8)
Female	33 (30.0)	4 (23.5)	29 (31.2)
Race, n (%)			
White	64 (58.2)	11 (64.7)	53 (57.0)
Black/African American	41 (37.3)		
American Indian/Alaskan Native	2 (1.8)		
Asian	1 (0.9)		
Native Hawaiian/ Other Pacific Islander	1 (0.9)		
Other	1 (0.9)		
Ethnicity, n (%)			
Hispanic/Latino	7(6.4)		
Non-Hispanic/Latino	103 (93.6)		
Age (years)			
Mean ± SD	30.4 ± 7.8	29.0	30.6
Weight (kg)			
Mean ± SD	75.9 ± 12.1	76.2	75.8
Body Mass Index (kg/m^2)			
Mean ± SD	25.0 ± 3.1	24.7	25.02

SD = standard deviation

Data Supplement Table III: Pharmacokinetic parameters of edoxaban

	C_{max}, ng/ml	t_{max}, h	$AUC_{(0-24)}$, ng*h/mL	$AUC_{(0-last)}$, ng*h/mL
Part 1				
Edoxaban 60 mg (n =10)	294 (116)	1.70 (0.83)	1590 (323)	1670 (337)
Edoxaban 180 mg (n = 12)	554 (182)	1.58 (0.76)	3140 (1120)	3480 (1140)
Part 2				
Edoxaban 60 mg + Placebo (n = 31)	234 (93.1)	1.3 (0.7)	1330 (336)	1420 (343)
Edoxaban 60 mg + 50 IU/kg 4F-PCC (n = 33)	209 (88.3)	1.3 (1.0)	1230 (287)	1340 (304)
Edoxaban 60 mg + Placebo (n = 28)	249 (90.8)	1.6 (1.1)	1390 (376)	1460 (383)
Edoxaban 60 mg + 25 IU/kg 4F-PCC (n = 28)	251 (94.2)	1.3 (0.9)	1310 (343)	1400 (347)
Edoxaban 60 mg + Placebo (n = 30)	318 (111)	1.2 (0.8)	1610 (370)	1700 (385)
Edoxaban 60 mg + 10 IU/kg 4F-PCC (n = 30)	315 (100)	1.4 (1.1)	1630 (363)	1720 (390)

All values presented as arithmetic mean (standard deviation).

AUC, area under the plasma concentration vs time curve; $AUC_{(0-24)}$, AUC from time 0 to 24 hours; $AUC_{(0-last)}$, AUC from time 0 to the last quantifiable concentration; C_{max}, maximum observed plasma drug concentration; 4F-PCC, 4-factor prothrombin complex concentrate; t_{max}, time to reach maximum plasma concentration.

Supplemental Table IV: Pharmacodynamic parameters of endogenous thrombin potential in Part 2.

	Cohort 1		Cohort 2		Cohort 3	
	Edoxaban 60 mg + Placebo	**Edoxaban 60 mg + 50 IU/kg 4F-PCC**	**Edoxaban 60 mg + Placebo**	**Edoxaban 60 mg + 25 IU/kg 4F-PCC**	**Edoxaban 60 mg + Placebo**	**Edoxaban 60 mg + 10 IU/kg 4F-PCC**
	n = 31	**n = 33**	**n = 28**	**n = 28**	**n = 30**	**n = 30**
A_{min} (nM*min)	1395 (818)	1750 (1000)	1230 (729)	1500 (969)	1280 (700)	1560 (76.3)
A_{max} (nM*min)	2930 (720)	5900 (720)	3910 (576)	5020 (609)	3990 (621)	4630 (747)
T_{max} (h)	19.7 (5.95)	16.1 (6.96)	17.1 (5.78)	17.6 (4.88)	19.0 (5.09)	17.70 (4.89)
ΔA_{min} (%)	-64.4 (19.0)	-56.4 (22.4)	-68.5 (16.0)	-61.3 (23.4)	-66.8 (17.7)	-61.6 (16.7)
%ΔA_{min} (%)	3.15 (11.3)	50.3 (10.2)	3.48 (7.32)	35.8 (15.8)	5.21 (9.14)	16.8 (11.2)

All values presented as arithmetic mean (standard deviation).

%ΔA_{min}, percent change in the minimum observed activity value; 4F-PCC, 4-factor prothrombin complex concentrate; A_{min}, the minimum observed activity value; A_{max}, the maximum observed activity value; ΔA_{min}, change in the minimum observed activity value; T_{max}, the time of the maximum observed activity.

Data Supplement Figure I: Study design for part 1
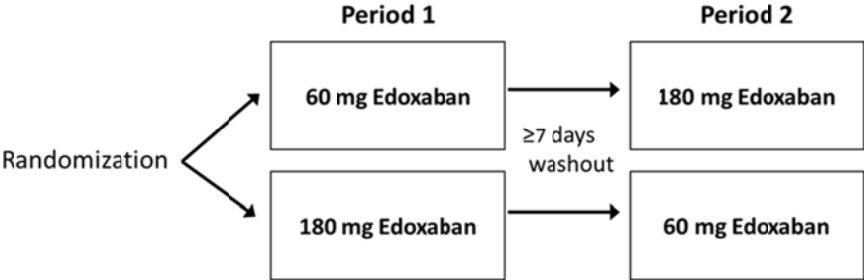
Data Supplement Figure II: CONSORT diagram, part 1. AE, adverse event; aPTT, activated partial thromboplastin time; PD, pharmacodynamics; PK, pharmacokinetic.
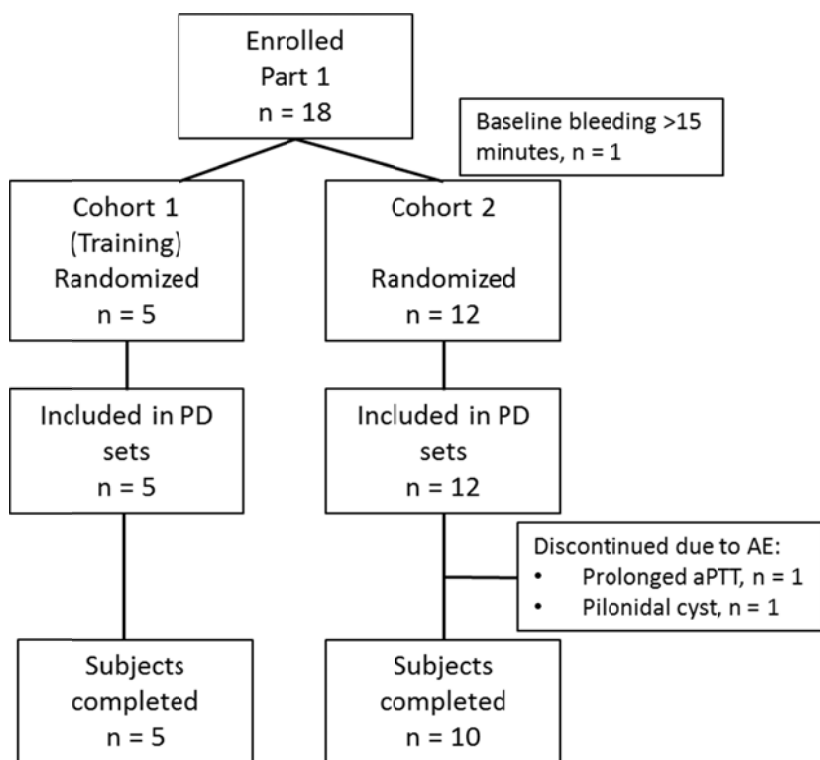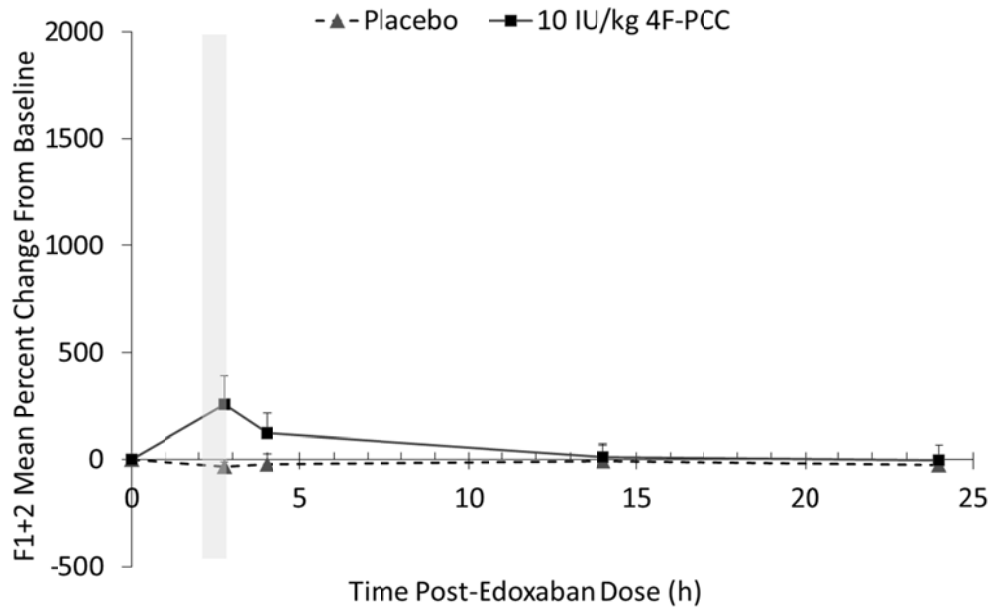
Data Supplement Figure III: Time course of F1+2 mean percent change from baseline for treatment with **(A)** 50 IU/kg, **(B)** 25 IU/kg, or **(C)** 10 IU/kg 4F-PCC or placebo. Error bars represent standard deviation. 4F-PCC, 4-factor prothrombin complex concentrate; F1+2, prothrombin fragment 1 + 2.

A
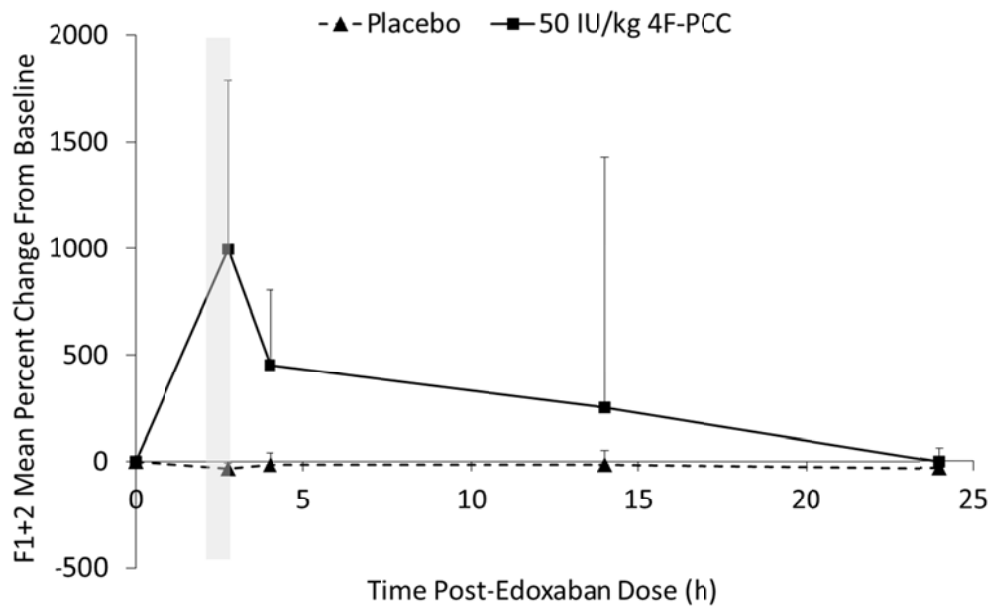
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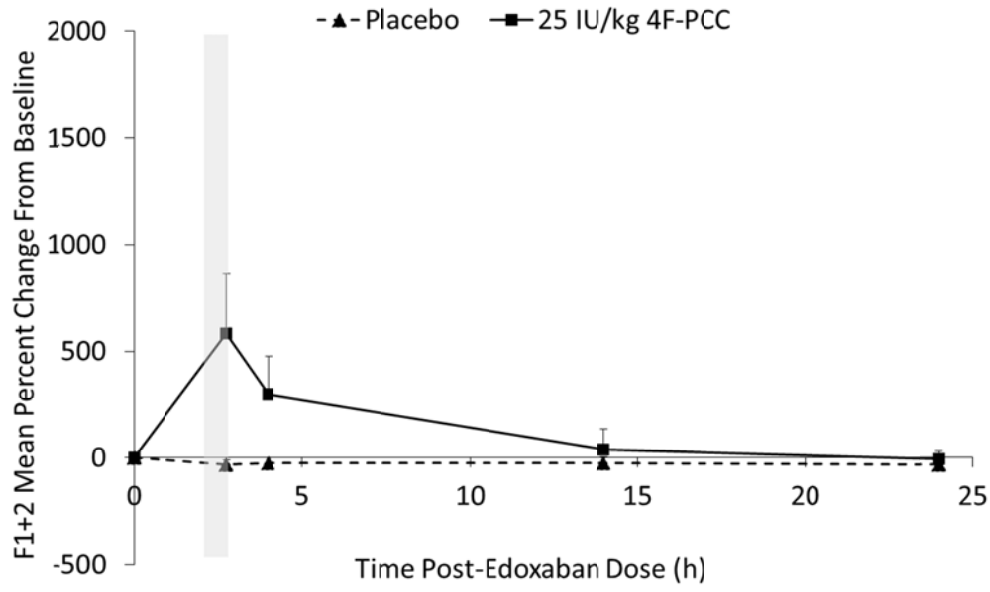
Data Supplement Figure IV: Time course of D-dimer mean percent change from baseline for treatment with **(A)** 50 IU/kg, **(B)** 25 IU/kg, or **(C)** 10 IU/kg 4F-PCC or placebo. Error bars represent standard deviation. 4F-PCC, 4-factor prothrombin complex concentrate.

A

B

C

American Heart Association®
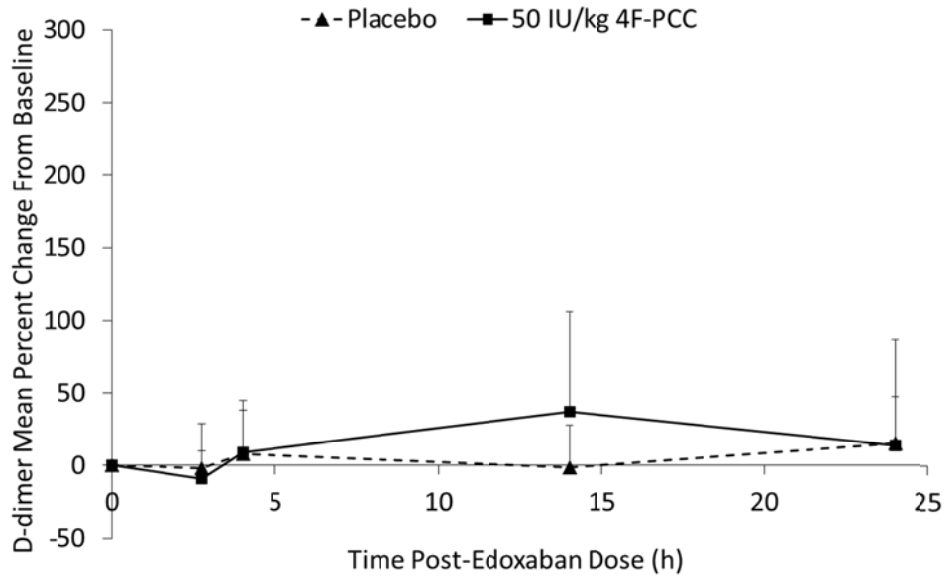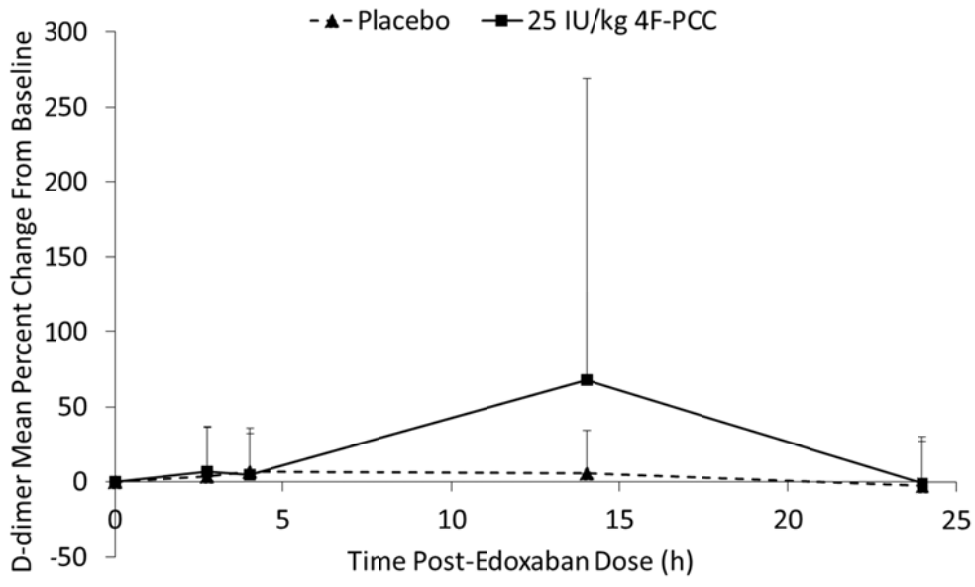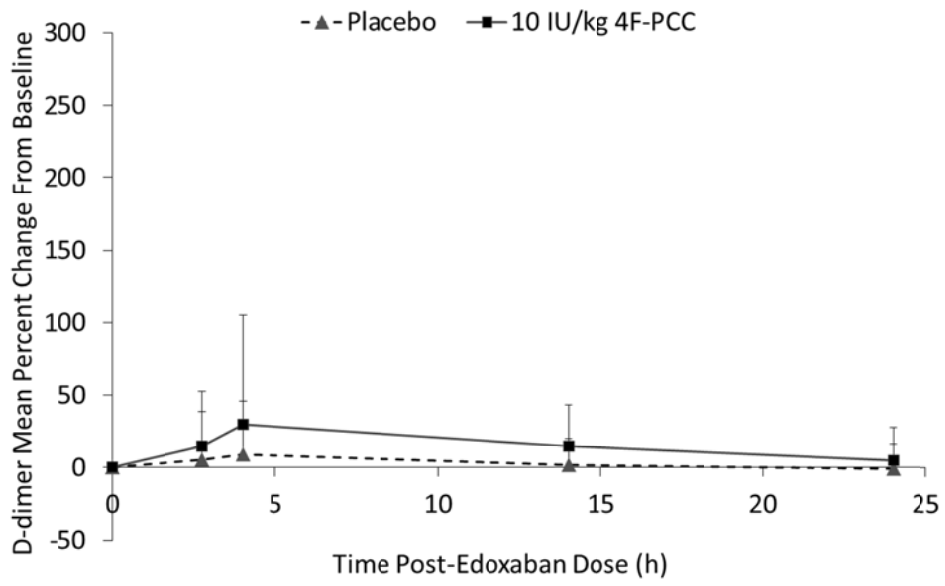
Edoxaban Effects on Bleeding Following Punch Biopsy and Reversal by a 4-Factor Prothrombin Complex Concentrate

Hamim Zahir, Karen S. Brown, Alexander G. Vandell, Madhuri Desai, Jen-Fue Maa, Victor Dishy, Barbara Lomeli, Annette Feussner, Wenqin Feng, Ling He, Michael A. Grosso, Hans J. Lanz and Elliott M. Antman

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