Effect of edoxaban on markers of coagulation in venous and shed blood compared with fondaparinux

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Summary

Edoxaban, an oral direct factor Xa (FXa) inhibitor, is in phase III clinical development for stroke prevention in atrial fibrillation and treatment of venous thromboembolism. The shed blood model allows for study of activated coagulation at a site of standardised tissue injury due to local release of tissue factor. The objective of this study was to evaluate the effect of three doses of edoxaban on markers of coagulation in shed and venous blood versus placebo and a standard prophylactic dose of fondaparinux. A total of 100 healthy male subjects were randomised to receive single doses of one of five treatments: subcutaneously administered fondaparinux 2.5 mg; orally administered edoxaban 30, 60, or 120 mg; or placebo. The primary objective was measurement of blood coagulation markers prothrombin fragment 1+2 (F_{1+2}) and thrombin-antithrombin (TAT) complex, and platelet activation marker β-thromboglobulin (β-TG), in venous and shed blood. Secondary objectives included pharmacokinetics, shed blood volume, and safety of edoxaban. Single doses of edoxaban caused rapid and significant decreases of F_{1+2}, TAT, and β-TG in the shed blood model, indicating inhibition of thrombin generation and platelet activation. Inhibition was significantly less for fondaparinux versus edoxaban. Baseline-corrected F_{1+2}, TAT, and β-TG values demonstrated sustained inhibition up to 24 hours for shed blood in the edoxaban groups but no significant inhibition in venous blood. Overall, edoxaban treatments were well tolerated. In conclusion, single oral doses of edoxaban 30, 60, or 120 mg caused rapid and sustained inhibition of coagulation up to 24 hours in the shed blood model.

Keywords

Coagulation markers, direct factor Xa inhibitor, edoxaban, fondaparinux, shed blood model

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Introduction

Investigations into new antithrombotic therapies have largely focused on factor Xa (FXa) as a therapeutic target (1, 2). FXa plays a pivotal role in the coagulation cascade, as it is generated at the convergence of the intrinsic and extrinsic pathways from direct activation of factor X by tissue factor (1). Small molecule direct FXa inhibitors have been developed that directly inhibit both free FXa and FXa bound to factor Va and phospholipids in the prothrombinase complex (3, 4). In contrast, indirect FXa inhibitors, like fondaparinux, only block free FXa and do not affect FXa in the prothrombinase complex (5–7).

Fondaparinux, an indirect FXa inhibitor, is approved for the prophylaxis and treatment of deep-vein thrombosis and pulmonary embolism. It is administered by subcutaneous injection and therefore rapidly and completely absorbed with absolute bioavailability of 100% (8, 9). Following a single subcutaneous dose of fondaparinux sodium 2.5 mg in young male subjects, maximum plasma concentration (C_{max}) of 0.34 mg/l was reached in approximately 2 hours (h). The elimination half-life of fondaparinux is 17–21 h; it is eliminated unchanged in urine in individuals with normal kidney function (8–11).

Edoxaban is a novel, direct, oral FXa inhibitor in clinical development for stroke prevention in atrial fibrillation (AF) and treatment of venous thromboembolism (VTE). Edoxaban has a rapid onset of action with approximately 50% oral bioavailability, terminal elimination half-life of 8–10 h, linear pharmacokinetics, and low intrasubject pharmacodynamic variability (12). In a phase II study of patients with AF, edoxaban 30 and 60 mg once daily demonstrated safety profiles similar to dose-adjusted warfarin (13). Similarly, in patients undergoing elective hip or knee arthroplasty, once-daily edoxaban reduced the risk of postoperative VTE in a dose-dependent manner with similar rates of bleeding across the dose range (14, 15).

The shed blood model allows for the study of thrombin generation and platelet activation in humans *in vivo* (16, 17). In contrast to measurement of bleeding times, the model involves incision of the skin with standardised devices and the collection of blood for measurement of parameters of coagulation (18). It has been used to study the effects of anticoagulants and other agents on thrombin

generation and platelet activation under physiologic conditions (18–22). The aim of this study was to investigate the effect of three doses of orally administered edoxaban compared with placebo and the standard prophylactic dose of subcutaneously administered fondaparinux on markers of thrombin generation and platelet activation in shed and venous blood, and on coagulation markers and anti-Xa activity in venous blood.

Secondary objectives included the evaluation of coagulation markers (activated partial thromboplastin time [aPTT] and prothrombin time [PT]), anti-Xa activity, pharmacokinetics, shed blood volume, and safety of orally administered edoxaban. Post-study assessments were performed 5–10 days after dosing and telephone follow-up for adverse event (AE) reporting was conducted 30 days post-dose.

Materials and methods

Study population

Healthy Caucasian males aged 18–55 years, with a body mass index (BMI) of 19–29 kg/m^2, were eligible. Exclusion criteria included any significant abnormal physical or laboratory finding; a history of significant cardiac, renal, hepatic, or gastrointestinal disease; use of concurrent medication during the study; or any hereditary deficiency of a blood clotting factor. Eligible patients provided signed informed consent. The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki.

Study design

This was a randomised, open-label, five-parallel-group, placebo- and active-controlled, single-dose study conducted in a single centre by the Department of Clinical Pharmacology, Medical University of Vienna (Austria). A total of 100 subjects were studied as five groups of 20. Subjects were randomised in blocks of five using the randomisation code provided by Clinical Trial Services, to receive single doses of one of five treatments: fondaparinux (Arixtra®, GlaxoSmithKline, Research Triangle Park, NC, USA) 2.5 mg/0.5 ml administered subcutaneously; edoxaban (Daiichi Sankyo Co., Ltd., Tokyo, Japan) 30, 60, or 120 mg administered orally; or placebo administered orally. The edoxaban doses were selected based on previous clinical studies (12).

Study endpoints

The primary objective was to compare assays for thrombin generation: prothrombin fragment 1+2 (F_{1+2}) and thrombin-antithrombin (TAT); and for platelet activation: β-thromboglobulin (β-TG), in venous and shed blood for edoxaban, placebo, and fondaparinux. F_{1+2} is a polypeptide formed from prothrombin during its conversion to thrombin by the prothrombinase complex, composed of FXa and cofactor Va on membrane surfaces (23). TAT is formed following neutralisation of thrombin by antithrombin and reflects the *in vivo* thrombin generation processes (23). β-TG, a marker of platelet activation, is a protein released by platelet granules when platelets are activated (18).

Blood sampling

Shed blood

Shed blood samples for F_{1+2}, TAT, β-TG, and volume were collected at pre-dose and at 1.5, 5, 12, and 24 h post-dose; collections were performed as previously described by Wolzt et al. (18). Briefly, a sphygmomanometer was positioned on the upper arm and inflated to 45 mm Hg. Standardised disposable devices (Surgicutt®, ITC, Edison, NJ, USA) were used to make two incisions on the volar surface of the forearm, parallel to the antecubital crease; new incisions ~1 cm apart from one another were made for each blood sampling. The shed blood sample was collected directly from the edge of the incision for 4 minutes (min) using Gilson Pipetman® 200 µl air-displacement pipette (Gilson Inc., Middleton, WI, USA). The pipette tip was placed on the edge of the skin incision and no additional maneuvre applied. The blood was transferred immediately into ice-cooled plastic tubes containing 100 µl stop solution (100 mM EDTA, 30 µM indomethacin, 3.8% sodium citrate, 1,500 U/ml sodium heparin, and 1,000 U/ml aprotinin) to prevent further thrombin generation or platelet activation in the collection tube. Shed blood volume was determined from pre- and post-collection tube weights. The shed blood samples were centrifuged within 30 min of collection after the addition of 125 µl of phosphate buffered saline, at 10,000 g for 5 min at 4°C, and the plasma was separated and stored at –70°C until analysis. Shed blood was collected for 4 min post-incision because it has been demonstrated that F_{1+2} levels in shed blood increase exponentially and reach a plateau within this period (20).

Venous blood

Blood samples for F_{1+2}, TAT, β-TG, aPTT, PT, and anti-Xa activity were collected from fresh venipunctures at pre-dose and at 1.5, 5, 12, and 24 h post-dose. Blood samples (2 x 4.5 ml) for aPTT, PT, and anti-Xa activity were collected in 3.8% sodium citrate tubes. The samples were centrifuged at 1,500 g for 10 min at 4°C and the plasma was separated and stored at –20°C until analysis. Blood (4.5 ml) for F_{1+2}, TAT, and β-TG measurements was collected into ice-cooled plastic tubes containing stop solution at a 1:9 volume ratio to blood. Samples were centrifuged at 3,000 g for 10 min at 4°C and the separated plasma stored at –70°C until analysis.

Blood samples for plasma edoxaban concentrations were collected at pre-dose and at 1.5, 5, 12, and 24 h post-dose.

Bioanalytical methods

All assays were performed using methodology validated by the Clinical Institute for Laboratory Medicine at the Vienna General Hospital except where noted. F_{1+2} and TAT levels were determined using the Enzygnost® F_{1+2} and TAT microtest kits (Dade Behring GmbH, Marburg, Germany). β-TG concentrations were measured using the ASSERACHROM® β-TG test kit (Diagnostica Stago, Asnières, France). Anti-Xa activity was determined using the Rotachrom® STA® (Diagnostica Stago). The lower limits of quantification for the assays were 0.04 nM for F_{1+2}, 0.89 µg/l for TAT, and 7 ng/ml (in edoxaban units) for anti-Xa activity. The normal reference ranges were 0.4–1.1 nM for venous F_{1+2}, 1.0–4.1 µg/l for venous TAT, and 133.83–1186.57 IU/10^9 platelets for venous β-TG. For anti-Xa activity, calibration was performed with both STA® low-molecular-weight heparin (LMWH) calibrator and normal pooled plasma containing increasing amounts of edoxaban. All results were expressed in anti-Xa IU and ng edoxaban/ml; normal reference range for the linear edoxaban plasma concentration relation was 19–250 ng/ml (edoxaban units).

aPTT measurements were performed using the STA® aPTT (Diagnostica Stago) on a fully automated STA® Hemostasis Analyzer (Diagnostica Stago, Inc., Parsippany, NJ, USA). The normal reference range for venous aPTT was <43 seconds. PT was measured using the STA® Neoplastin Plus (Diagnostica Stago) on a STA® Hemostasis Analyzer (Diagnostica Stago, Inc.). The normal reference ranges for PT with the reagent Neoplastin Plus are >70% and <15.6 seconds. Venous anti-Xa activity analyzed at the Laboratoire Claude Levy, Paris, France, used edoxaban as the assay standard.

Plasma samples were prepared by liquid-liquid extraction, and plasma edoxaban concentrations were measured by a validated LC-MS/MS method at BioDynamic Research Ltd. (BioDynamics Report no. DPC/10; Rushden, UK). The lower limit of quantification of edoxaban in plasma was 1 ng/ml and the upper limit was 500 ng/ml.

Safety

Safety evaluations on human volunteers included AEs, vital signs, 12-lead electrocardiogram (ECG), clinical laboratory evaluations, PT, fecal occult blood, body weight, and physical examination.

Statistical analysis

A previous study evaluating the effects of ximelagatran, an oral direct thrombin inhibitor, r-hirudin, a parenteral direct thrombin inhibitor, and enoxaparin on thrombin generation and platelet function in healthy male subjects using a shed blood model suggested that study groups comprising 20 subjects are predicted to detect a 50% reduction in shed blood F_{1+2} or TAT concentrations from baseline with >80% power (24). Based on the Sarich 2003 study, the sample size was calculated to be 20 subjects, which was considered adequate to attain the objectives of this study.

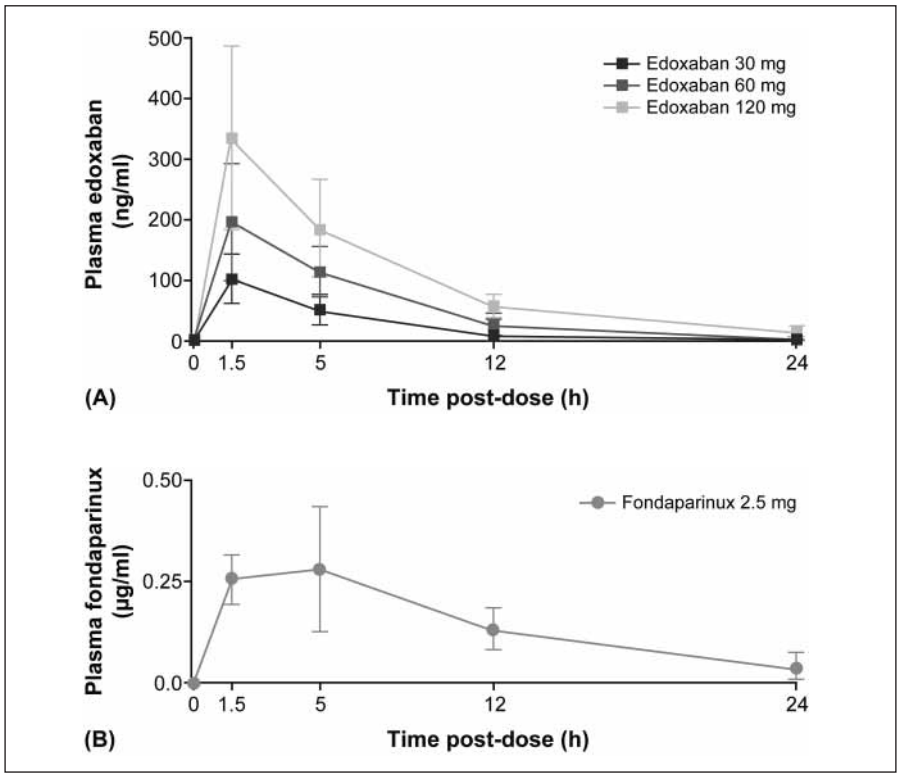

Figure 1: Arithmetic mean (SD) plasma concentrations vs. time for (A) oral edoxaban 30, 60, and 120 mg (measured by high performance liquid chromatography) and (B) subcutaneous fondaparinux 2.5 mg (measured as anti-Xa activity with fondaparinux as calibrator) (n = 20/treatment group).

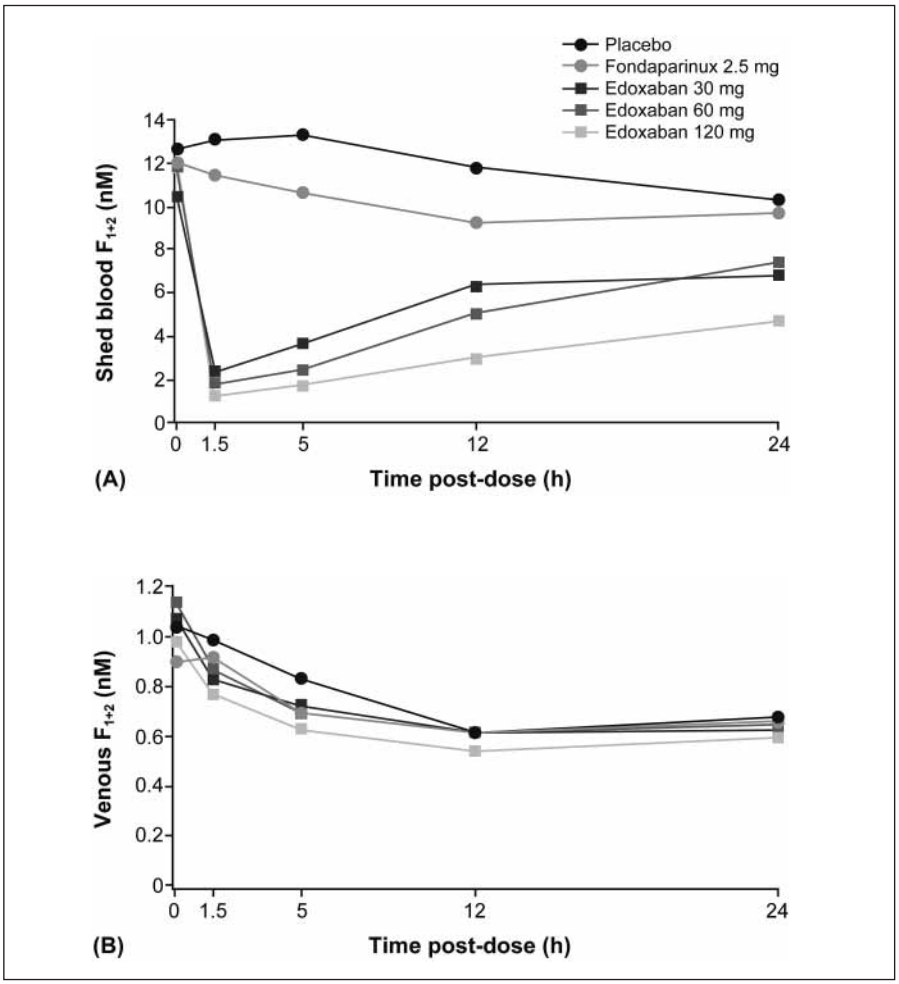

Figure 2: Geometric mean absolute values of (A) shed blood and (B) venous blood F_{1+2} concentrations (n = 20/treatment group). Shed blood F_{1+2} concentrations remained statistically significantly reduced compared with baseline at all time points post-dose through 24 h for all oral edoxaban doses ($p < 0.0001$). Following subcutaneous fondaparinux 2.5 mg, shed blood F_{1+2} levels were reduced at 5 h post-dose and dropped to minimum values at 12 h post-dose. In venous blood, reductions in F_{1+2} were not significant for the three edoxaban doses and fondaparinux. F_{1+2} = prothrombin fragment 1 + 2. (A) $P \leq 0.001$ for comparison between edoxaban 30, 60 and 120 mg vs. placebo at 1.5 h and between edoxaban 120 mg vs. placebo at 12 h. $P \leq 0.001$ for comparison between edoxaban 30, 60 and 120 mg vs. fondaparinux 2.5 mg at 1.5 h and between edoxaban 120 mg vs. fondaparinux at 12 h. $P \leq 0.05$ for comparison between edoxaban 60 mg vs. placebo at 12 h and between edoxaban 120 mg vs. edoxaban 60 mg at 12 h. $P \leq 0.01$ for comparison between edoxaban 120 mg and edoxaban 30 mg at 12 h.

Descriptive statistics for pharmacodynamics, pharmacokinetics, and safety data were determined using SAS® software Version 8.2 (Cary, NC, USA). A repeated-measures analysis of variance (ANOVA) was used to compare treatments for the changes from baseline (pre-dose) and between time points. For anti-Xa activity, log-transformed absolute values were used. The factors for the ANOVA were subject, treatment, time, treatment*time, and random error. For each comparison, the 95% confidence interval of the difference was calculated using the residual variance from the ANOVA.

Results

Subject demographics

Of the 100 male subjects randomised to fondaparinux, edoxaban, or placebo between May and August 2004, 92 completed the study. Eight subjects who did not complete the study participated in the treatment phase and post-study assessments, but were not accessible for the follow-up telephone call: one subject in the fondaparinux group, three subjects in the placebo group, and two subjects each in the edoxaban 30- and 60-mg groups. There were no major differences in subject demographics and baseline characteristics. The mean age was 28 years; mean body weight was 77 kg; and mean BMI was 24 kg/m^2.

Edoxaban pharmacokinetics and fondaparinux pharmacodynamics

The plasma edoxaban concentration versus time profile is presented in ▶Figure 1A. The plasma concentrations following oral administration of edoxaban demonstrated increased exposure proportional for 30- and 60-mg doses, but reduced exposure relative to dose for the 120-mg dose, which was also associated with greater variability at the earlier time point. The plasma concentrations of fondaparinux as measured by anti-Xa activity (Rotachrom), using fondaparinux as a calibrator, are presented in ▶Figure 1B. The peak concentrations ranged from 0.17–0.42 at 1.5 h post-dose and were consistent with exposure profiles from other studies (10).

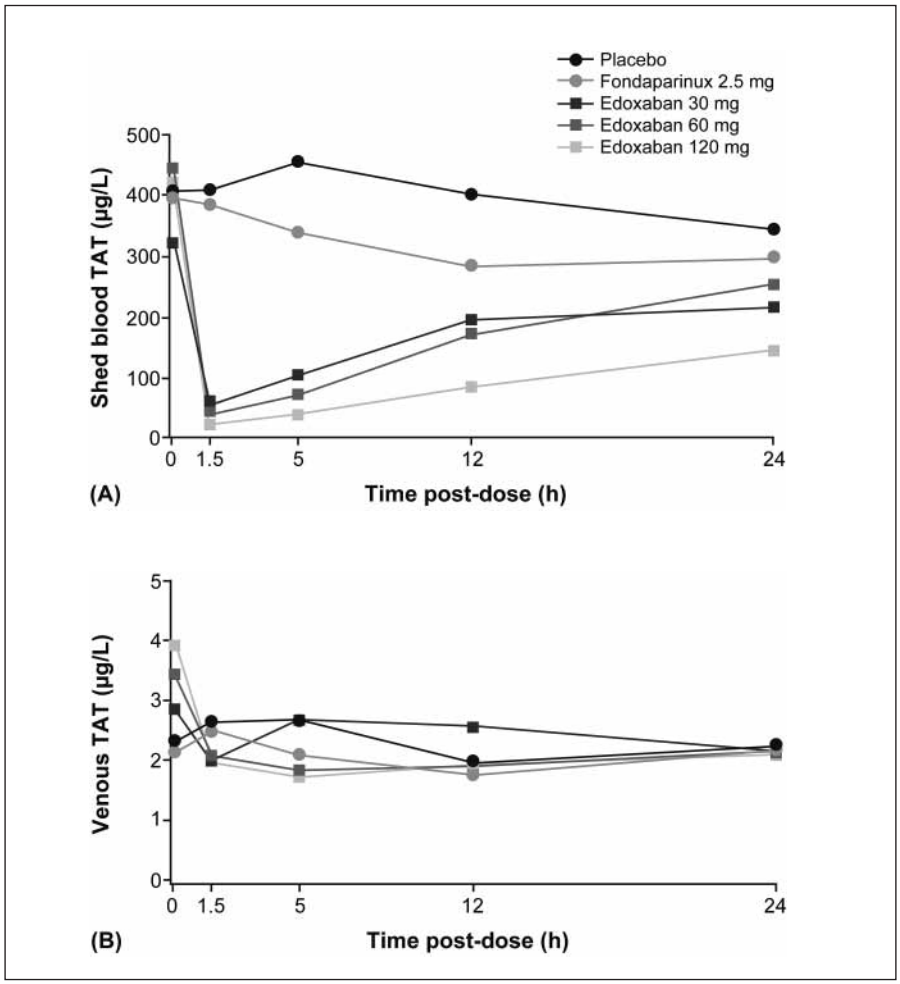

Figure 3: Geometric mean absolute values of (A) shed blood and (B) venous blood TAT concentrations (n = 20/treatment group). Shed blood TAT levels remained statistically significantly reduced compared with baseline at all time points post-dose through 24 h for all oral edoxaban doses ($p < 0.0001$ for 60 and 120 mg at all time points, and for 30 mg at 1.5 and 5 h; $p < 0.01$ for 30 mg at 12 and 24 h). Shed blood TAT levels remained significantly reduced compared with baseline only at 12 and 24 h post-dose with subcutaneous fondaparinux 2.5 mg ($p < 0.05$). Venous TAT concentrations were nonsignificantly reduced with edoxaban. TAT = thrombin-antithrombin. (A) $P \leq 0.01$ for comparison between edoxaban 30 mg vs. placebo at 1.5 h and between edoxaban 120 mg vs. fondaparinux at 12 h. $P \leq 0.001$ for comparison between edoxaban 60 and 120 mg vs. placebo at 1.5 h and between edoxaban 120 mg vs. placebo at 12 h. $P \leq 0.001$ for comparison between edoxaban 30, 60 and 120 mg vs. fondaparinux at 1.5 h. $P \leq 0.05$ for comparison between edoxaban 60 mg vs. placebo and between edoxaban 120 mg vs. edoxaban 30 mg at 12 h.

Pharmacodynamics

Shed blood and venous F_{1+2}

Single oral doses of edoxaban 30–120 mg produced rapid and marked dose-related reductions in shed blood concentrations of F_{1+2}, a marker of thrombin generation, at 1.5 h post-dose; thereafter, F_{1+2} increased in a dose-related manner (▶Fig. 2A). At 1.5 h post-dose, F_{1+2} concentrations fell by 78%, 85%, and 90% below baseline (pre-dose) with the edoxaban 30-, 60-, and 120-mg doses, respectively. Shed blood F_{1+2} concentrations remained statistically significantly reduced compared with baseline at all time points post-dose through 24 h for all edoxaban doses ($p < 0.0001$). Following subcutaneous fondaparinux, shed blood F_{1+2} levels were reduced at 5 h post-dose and dropped to minimum values at 12 h post-dose, corresponding to a decrease of 23% below baseline. No change was observed with placebo. In venous blood, reductions in F_{1+2} were not significant, and were similar across the three edoxaban doses (▶Fig. 2B). Similar reductions in venous F_{1+2} were seen following placebo and subcutaneous fondaparinux.

Shed blood and venous TAT

Shed blood concentrations of TAT, another marker of thrombin generation, were significantly reduced with a marked dose-related effect following single oral doses of edoxaban 30–120 mg at 1.5 h post-dose; levels thereafter increased in a dose-related manner (▶Fig. 3A). At 1.5 h post-dose, TAT levels fell by 82%, 91%, and 95% compared with baseline at the edoxaban 30-, 60-, and 120-mg doses, respectively. Shed blood TAT levels remained statistically significantly reduced compared with baseline at all time points post-dose through 24 h for all edoxaban doses ($p < 0.0001$ for 60 mg and 120 mg at all time points, and for 30 mg at 1.5 and 5 h; $p < 0.01$ for 30 mg at 12 and 24 h). Following subcutaneous fondaparinux, shed blood TAT levels declined from 5–24 h post-dose, with minimum values at 12 h corresponding to a 28% reduction. Shed blood TAT levels remained significantly reduced compared with baseline only at 12 and 24 h post-dose with fondaparinux ($p < 0.05$). No change was observed following placebo.

There was no apparent effect on venous TAT concentrations following edoxaban 30 mg, whereas with edoxaban 60 and 120 mg, TAT levels were reduced at 1.5 h post-dose and then remained unchanged up to 24 h post-dose, ranging between 37–56% below

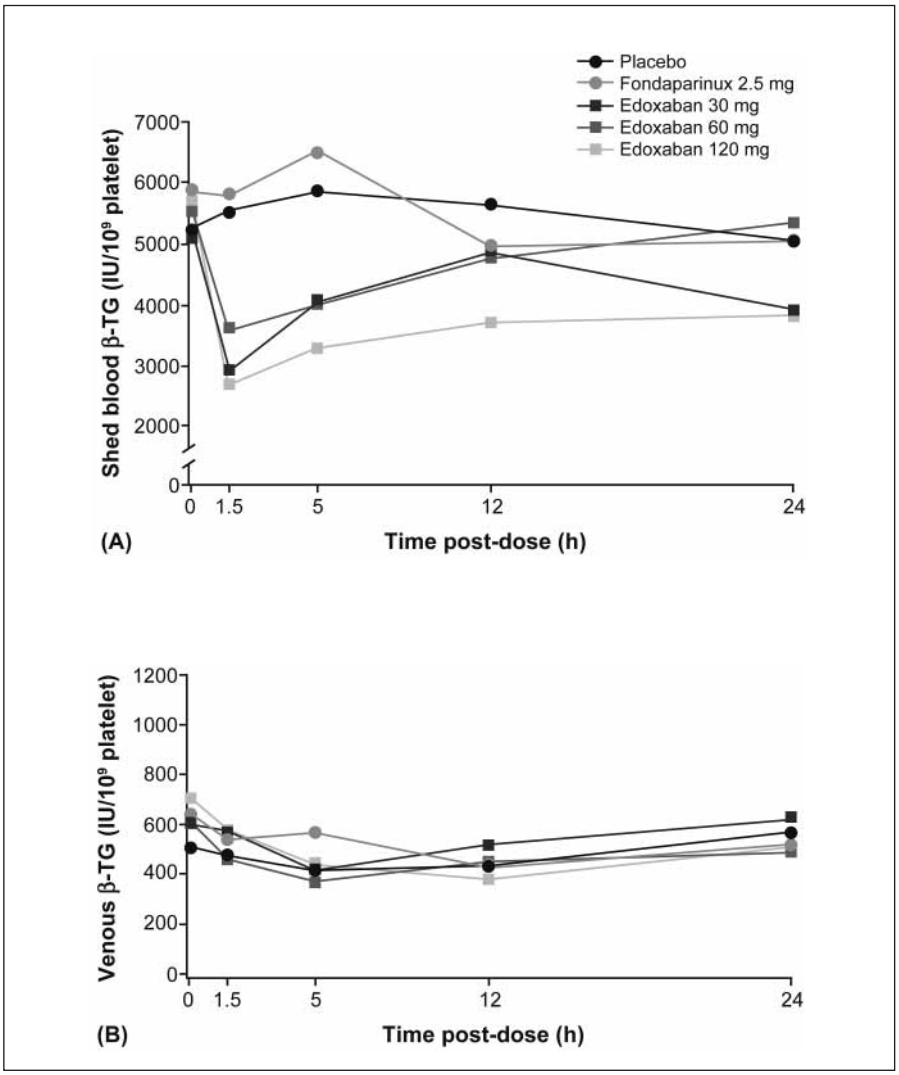

Figure 4: Geometric mean absolute values of (A) shed blood and (B) venous blood β-TG concentrations (n = 20/treatment group). Reduction in β-TG was statistically significant compared with baseline at 1.5 and 5 h post-dose at the edoxaban 30-mg ($p < 0.0001$ and $p = 0.013$, respectively) and 60-mg ($p < 0.0001$ and $p< 0.001$, respectively) doses and at all time points at the 120-mg dose ($p < 0.001$). Subcutaneous fondaparinux 2.5 mg caused non-significant reductions in shed blood β-TG levels. Edoxaban produced a non-significant reduction in venous β-TG, and a similar reduction was also observed following fondaparinux. β-TG = β-thromboglobulin. (A) $P \leq 0.01$ for comparison between edoxaban 30 mg vs. placebo at 1.5 h and between edoxaban 120 mg vs. placebo at 12 h. $P \leq 0.05$ for comparison between edoxaban 60 mg vs. placebo at 1.5 h and between edoxaban 120 mg vs. edoxaban 30 and 60 mg at 12 h. $P \leq 0.001$ for comparison between edoxaban 120 mg vs. placebo at 1.5 h and between edoxaban 120 mg vs. fondaparinux at 1.5 h.

baseline (►Fig. 3B). No reduction in venous TAT levels was observed following placebo and fondaparinux.

Shed blood and venous β-TG

Single oral doses of edoxaban 30–120 mg produced rapid declines in shed blood concentrations of β-TG, a marker of platelet activation, at 1.5 h post-dose; levels then increased in a dose-related manner (►Fig. 4A). β-TG levels at 1.5 h post-dose fell by 44%, 35%, and 53% below baseline at the edoxaban 30-, 60-, and 120-mg doses, respectively. The reduction in β-TG was statistically significant compared with baseline at 1.5 and 5 h post-dose at the edoxaban 30-mg ($p < 0.0001$ and $p = 0.013$, respectively) and 60-mg ($p < 0.0001$ and $p < 0.001$, respectively) doses, and at all time points at the 120-mg dose ($p < 0.001$). Subcutaneous fondaparinux caused non-significant reductions in shed blood β-TG levels at 12 and 24 h post-dose (14–16% below baseline). No significant change was observed following placebo.

Edoxaban produced a non-significant reduction in venous β-TG concentrations that generally did not vary by dose, and a similar reduction was also observed following fondaparinux (►Fig. 4B). No reduction in venous β-TG levels was observed following placebo.

Venous anti-Xa activity

Single oral doses of edoxaban 30–120 mg produced rapid dose-dependent increases in anti-Xa activity, with the maximum response occurring at 1.5 h post-dose. Mean maximum values of anti-Xa activity expressed in LMWH anti-Xa units were 1.40, 2.52, and 5.99 IU/ml at the edoxaban 30-, 60-, and 120-mg doses, respectively. Individual anti-Xa activity remained above zero at 24 h post-dose at the 60- and 120-mg doses. No anti-Xa activity was observed with placebo. Single doses of fondaparinux caused a steady induction of anti-Xa activity, with maximum values of 0.25 and 0.26 IU/ml at 1.5 and 5 h post-dose, respectively, although the

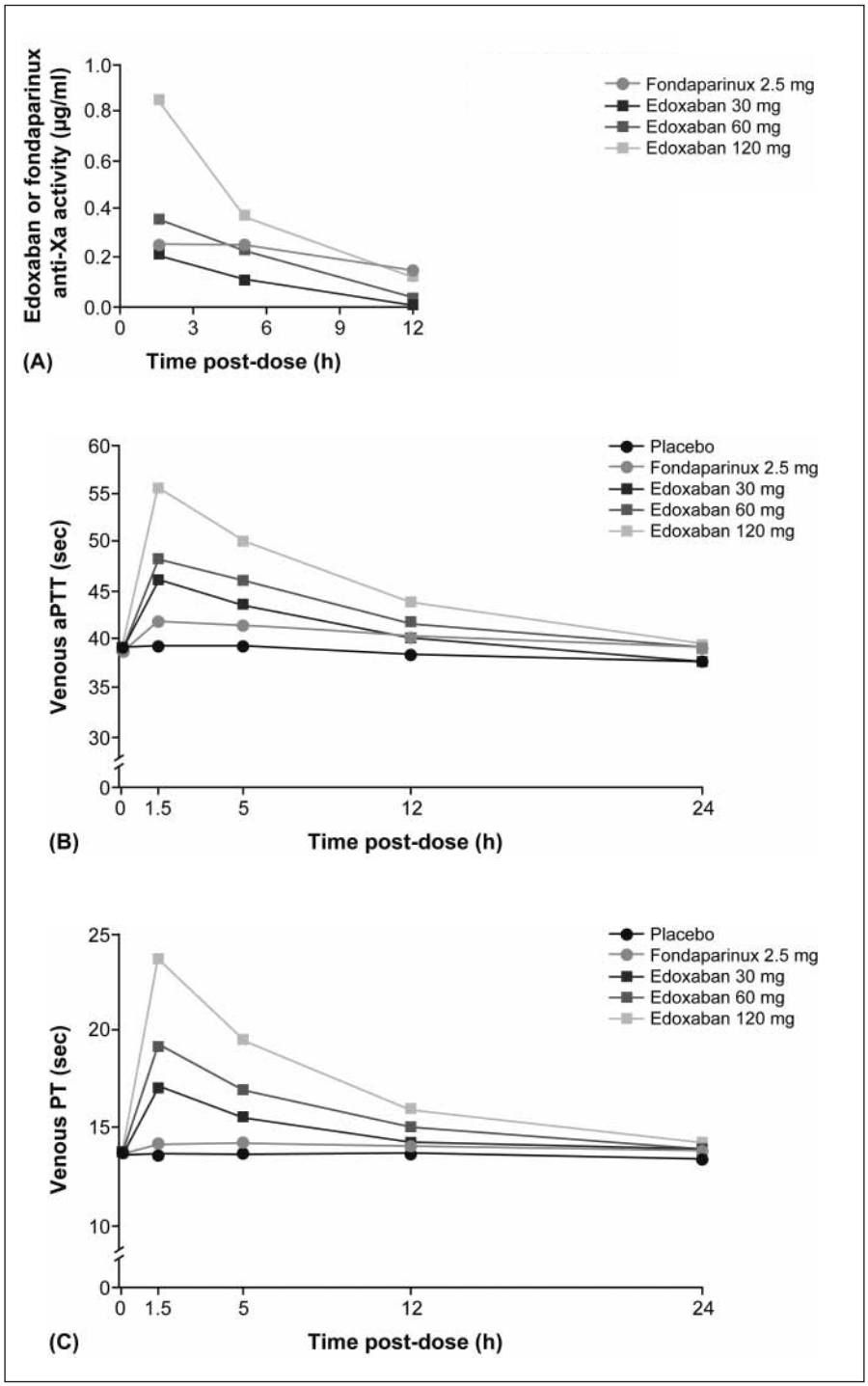

Figure 5: Geometric mean absolute values of venous (A) anti-Xa activity*, (B) aPTT, and (C) PT. In (A), anti-Xa activity is expressed in µg/ml to allow comparison between anticoagulants. Oral edoxaban 30–120 mg produced rapid dose-dependent increases in anti-Xa activity, with the maximum response occurring at 1.5 h post-dose. Single doses of subcutaneous fondaparinux 2.5 mg caused a steady induction of anti-Xa activity; maximum response was significantly less compared with the lowest edoxaban dose ($p < 0.0001$). Edoxaban prolonged aPTT and PT in a dose-dependent manner. aPTT = activated partial thromboplastin time; PT = prothrombin time. *The geometric mean values were not calculable at 24 h. Individual anti-Xa activity values had generally fallen to zero by 24 h post-dose at the 30 mg dose level, but remained slightly above baseline at the 60- and 120-mg dose levels.

maximum response was significantly less compared with the lowest edoxaban dose ($p < 0.0001$) (▶Fig. 5A).

Venous aPTT and PT

Following single oral doses of edoxaban 30–120 mg, rapid and marked dose-related prolongation of aPTT and PT was observed, with maximum prolongation occurring at 1.5 h post-dose (▶Fig. 5B and C). At the 30-mg dose, the prolongation of aPTT and PT relative to baseline was approximately 20% and 25%, respectively, which increased to approximately 40% and 71% at the 120-mg dose. The effects on aPTT and PT were prolonged, with values returning close to baseline by 24 h. Fondaparinux caused significant prolongation of aPTT compared with baseline at 1.5 and 5 h post-dose (p

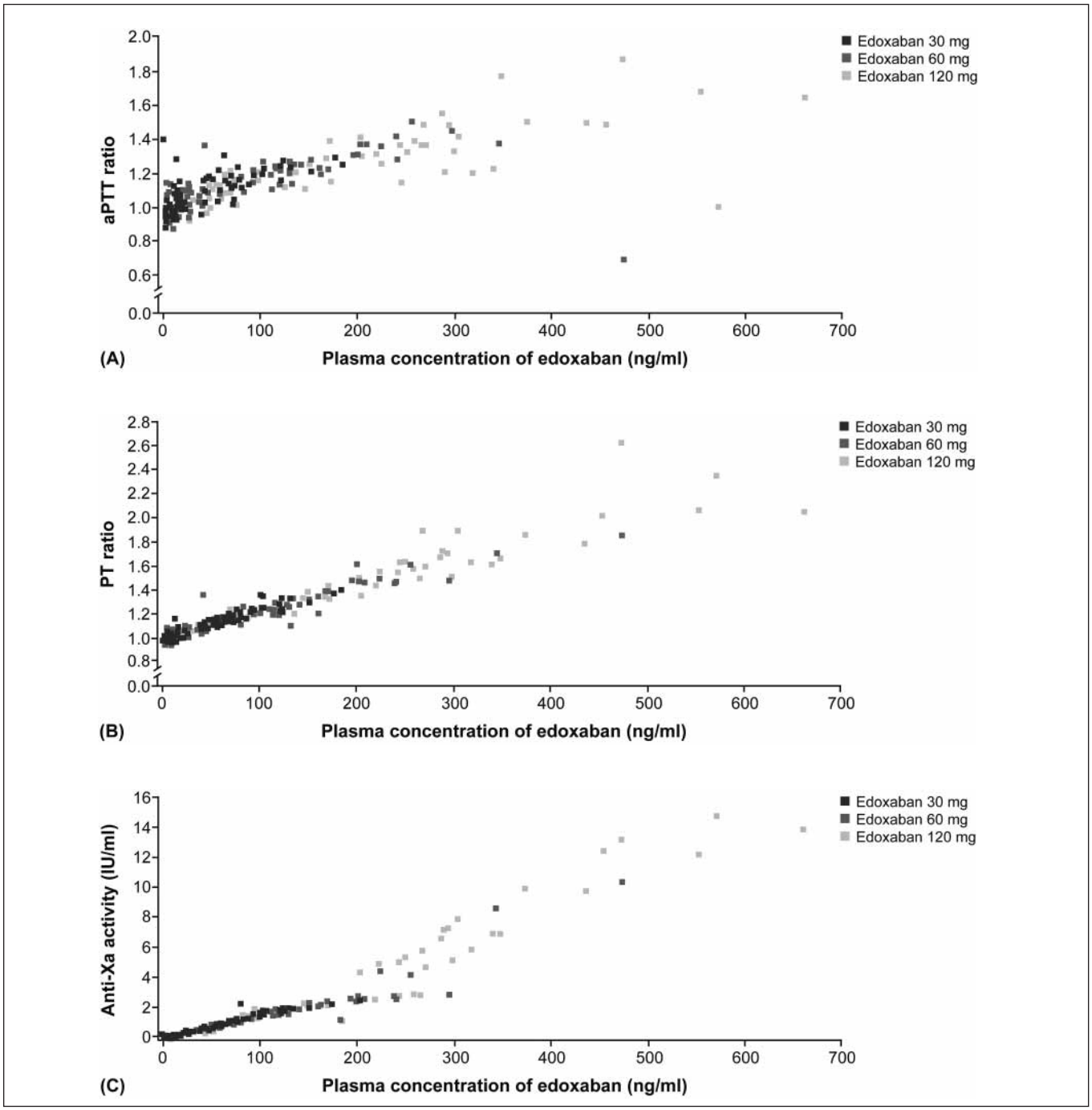

Figure 6: Correlation between plasma concentration of orally administered edoxaban and (A) aPTT, (B) PT, and (C) anti-Xa activity. aPTT = activated partial thromboplastin time; PT = prothrombin time.

< 0.001) and had no significant effect on PT. No prolongation of aPTT and PT was observed following placebo (data not shown).

Shed blood volume

Pre-dose shed blood volumes ranged from 192–284 µl (geometric means) for the five treatments. Following single oral doses of edoxaban 30, 60, and 120 mg, increases in shed blood volume were noted, with maximum increases of 41–57% relative to baseline at 12 h post-dose. Increases of 26% and 22% in shed blood volume were also observed at 12 h following placebo and subcutaneous fondaparinux, respectively. There were no significant differences between each edoxaban treatment and placebo at all time points, except at 24 h post-dose for the comparison of edoxaban 60 mg (arithmetic mean, 99 µl) with placebo (arithmetic mean, 5 µl; $p < 0.05$). Inasmuch as between-subject variability was high for

placebo and all treatments, the shed blood volume data should be interpreted with caution.

Pharmacodynamic and pharmacokinetic relationship

There was a direct linear correlation between the observed range of plasma concentrations of edoxaban during the dosing interval and the blood coagulation parameters aPTT and PT, with correlation coefficients of 0.738 and 0.957, respectively (▶Fig. 6A and B). Despite the increased variability observed in anti-Xa at edoxaban plasma concentrations greater than 200 ng/ml, a direct linear correlation was observed between plasma edoxaban and anti-Xa activity, with a correlation coefficient of 0.957 (▶Fig. 6C).

Safety

The incidence of AEs was low and ranged from 5–20% for each treatment group, with the majority of events being mild in severity and resolving spontaneously. Single, isolated bleeding-related AEs considered possibly related to edoxaban were reported by two subjects at the edoxaban 30-mg dose (haematochezia and haematoma) and one subject at the 120-mg dose (epistaxis). For all three subjects, the bleeding-related events were mild in severity and resolved without treatment.

Discussion

In this study, orally administered edoxaban at doses of 30, 60, and 120 mg produced significant, prolonged suppression of thrombin generation markers F_{1+2} and TAT and platelet activation marker β-TG in the shed blood model of a procoagulant, pathophysiologic state. The effect of edoxaban on β-TG is most likely an indirect effect, due to inhibition of thrombin-mediated platelet activation observed during endothelial injury; the reduction in thrombin generation led to reduced platelet activation. The effect of direct FXa inhibition with edoxaban on these thrombin generation and platelet activation markers was consistently greater than the effect of indirect FXa inhibition with subcutaneous fondaparinux 2.5 mg, the standard dose used for VTE prophylaxis.

The shed blood model is a model of microvascular injury and tissue factor-induced blood coagulation (17) that has been used previously to examine the effects on thrombin generation and platelet activation of antithrombotic agents acting by a variety of mechanisms, including aspirin, LMWH, and the direct thrombin inhibitor, ximelagatran (17–20, 25). It provides an assessment of the coagulation system and drug effects under activated conditions at the site of plug formation, as opposed to *in vitro* activation in clotting tests (such as aPTT, thrombin generation). The resemblance to thromboembolism or arterial ischaemic events is not known. However, patients requiring anticoagulants frequently have comorbid cardiovascular disease, which predisposes them to chronic endothelial injury. This model may offer insight into the pharmacology of edoxaban at the site of local injury. Comparison of the magnitude of inhibition of thrombin generation and platelet activation by various anticoagulants in the shed blood model may reflect drug mechanism and doses to be used in patients for the prevention of thromboembolic disorders. The predictability of the shed blood model is uncertain at this time.

The baseline levels for measures of thrombin generation for the shed blood samples were approximately 10-fold compared with the venous blood samples. The higher levels indicate the greater levels of thrombin in samples collected post-incision. In addition, greater absolute changes from baseline values were observed in the shed blood versus venous blood samples for edoxaban. However, mean change from baseline values of F_{1+2} and TAT in the placebo control group remained consistent or increased.

An explanation for the discrepancy between the significant effect of edoxaban on thrombin generation parameters in shed blood but not in venous blood may lie in the differences in the activation state of the clotting system. The exposure of shed blood to subendothelial tissues that are rich in tissue factor results in immediate activation of coagulation, thrombin generation, and feedback amplification of the coagulation mechanism (16). By interfering with this activation and amplification of the clotting system, direct FXa inhibition appeared to be more effective in relative terms on inhibition of thrombin generation. In contrast, in venous blood the coagulation system is in a resting state, feedback systems are less amplified, smaller amounts of activated thrombin are generated, and the effect of direct FXa inhibition appears lessened. Results of previous studies with LMWH in shed blood models have been consistent in showing more marked effects on thrombin generation parameters in shed versus venous blood (18).

The observation of a significantly greater effect of direct FXa inhibition with oral edoxaban than of indirect FXa inhibition with subcutaneous fondaparinux on thrombin generation parameters likely reflects the difference in mechanism of action. Since its mechanism is mediated by antithrombin, fondaparinux can only inhibit free FXa and does not affect the prothrombinase complex. Direct inhibition with edoxaban targets both free FXa and FXa in the prothrombinase complex, leading to the greater effect on reduction of thrombin generation as manifested by lower amounts of F_{1+2} and TAT (2).

The effects of direct thrombin inhibition were previously evaluated in the shed blood model in a study of ximelagatran 60 mg, and r-hirudin versus the LMWH enoxaparin; direct thrombin inhibition was not found to have a greater effect on thrombin generation parameters than the antithrombin-mediated action of enoxaparin (17).

Anti-Xa activity in this study was much lower with fondaparinux than with edoxaban. However, it is also possible that the anti-Xa assay, developed for responsiveness to heparins with LMWH as the calibrator, is more sensitive to the activity of direct FXa inhibitors. The higher levels of anti-Xa, almost 12-fold for the 120-mg edoxaban dose relative to fondaparinux at peak, do not

necessarily reflect the difference in anticoagulant activity between the two compounds. This difference could be related to the different mechanism of action, i.e. indirect catalytic and irreversible versus direct and reversible. We used a standard commercially available anti-Xa assay in this study for pragmatic reasons, because it is a generally applicable test and laboratories will likely not have access to tests designed specifically to measure the effects of these new FXa inhibitors. The measurement of fondaparinux was performed with the same method and kit, but with a calibration curve made of fondaparinux rather than the reference preparation of LMWH. The anti-Xa levels for subcutaneous fondaparinux 2.5 mg, the prophylactic dose, were consistent with those observed in other studies (10, 26).

A rapid chromogenic assay, specific for direct FXa inhibitors, and without interference of plasma factors concentration and of indirect polysaccharide type inhibitors, has recently been developed. It is specific and highly sensitive and could be more appropriate to measure the effects of direct FXa inhibitors (27), including edoxaban.

This study showed a prolonged effect of oral edoxaban on thrombin generation parameters in the shed blood model up to 24 h, whereas the plasma edoxaban profile demonstrated a terminal elimination half-life of approximately 8–10 h. The exact relevance of the shed blood model to local activity in the endothelium is unknown. However, in this study, the duration of anticoagulant activity for edoxaban appears longer based on levels of the coagulation markers in shed blood samples compared with venous blood samples. Since the shed blood model represents activated coagulation, one could postulate that the inhibitory effects of edoxaban have a longer duration of effect under activated coagulation, as experienced during local endothelial injury, than would be predicted by the time course of coagulation markers measured in venous blood. The suppression of thrombin generation parameters by edoxaban was rapid and dose related. Maximum suppression was at 1.5 h, consistent with the time to reach C_{max} (T_{max}) of 1–3 h. The anticoagulant effect of edoxaban was also dose related. In addition, the predictable dose response of edoxaban was supported by the correlations of pharmacodynamic parameters of venous aPTT, PT, and anti-Xa activity with plasma concentrations. The findings of predictability and consistency of effect across the dose range are in agreement with findings of other studies of edoxaban pharmacokinetics and pharmacodynamics (12).

Single doses of edoxaban were safe and well tolerated in this study, and comparable to findings with daily administration of edoxaban 30 and 60 mg. Three subjects had bleeding-related events, which were mild in severity and resolved without treatment.

A limitation of the shed blood model is that despite its mimicking a state of pathophysiologic procoagulant activity, the shear rates in the incision vessels are low, less than 20 s^{-1} after incision, compared with >1,000 s^{-1} in intact arterioles (16). Furthermore, microcirculation of the skin may be different from other vascular beds. Since the shed blood model is triggered by exposure of tissue factor and intravascular thrombin generation may be different, bleeding risk cannot be derived from this model.

In conclusion, orally administered edoxaban at single doses up to 120 mg caused rapid, sustained, dose-related inhibition of coagulation up to 24 h as demonstrated by decreased F_{1+2}, TAT, and β-TG in the shed blood model, which approximates a procoagulant pathophysiologic state.

What is known about this topic?

- Edoxaban, an oral direct factor Xa inhibitor, is in phase III clinical development for stroke prevention in atrial fibrillation and treatment of venous thromboembolism.
- The shed blood model is a well-validated model of microvascular injury and tissue factor-induced blood coagulation and has been previously used to examine the effect of anticoagulants and other agents on thrombin generation and platelet activation under physiologic conditions.

What does this paper add?

- Edoxaban caused rapid, sustained, and dose-related inhibition of coagulation up to 24 hours in the shed blood model.
- Comparison of the magnitude of inhibition of thrombin generation and platelet activation by various anticoagulants in the shed blood model may provide insight into drug mechanism and effective doses to be used in patients for the prevention of thromboembolic disorders.

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Conflict of interest

This study was supported by Daiichi Sankyo. M. Wolzt, S. Kapiotis and M. M. Samama report no conflict of interest. K. Ogata, J. Mendell and S. Kunitada are employees of Daiichi Sankyo.

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