A randomized trial of the safety, pharmacokinetics and pharmacodynamics of edoxaban, an oral factor Xa inhibitor, following a switch from warfarin

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Anticoagulant prophylaxis with vitamin K antagonists (VKAs) is recommended for the prevention of stroke and systemic embolic events in at-risk patients with atrial fibrillation and for treatment of acute deep vein thrombosis.
- VKAs are associated with several limitations including unpredictable pharmacokinetics and pharmacodynamics and a narrow therapeutic index.
- New oral anticoagulants, such as direct factor Xa (FXa) inhibitors, are becoming available and information is needed to guide switching patients from a VKA to these newer oral anticoagulants.

WHAT THIS STUDY ADDS

- This randomized, placebo-controlled study of the administration of a single 60 mg dose of the direct FXa inhibitor edoxaban 24 h after the cessation of warfarin therapy (international normalized ratio [INR] 2.0 to 3.0) appeared well tolerated and maintained continued anticoagulation without a prolonged risk of overcoagulation based on biomarkers in healthy subjects.
- Administration of edoxaban 24 h after the last dose of warfarin (INR 2.0 to 3.0) produced transient increases in INR, prothrombin time and other biomarkers compared with placebo, most notably at 2 h post-dose of edoxaban. The INR values in the placebo arm fell below the therapeutic range of warfarin (i.e. INR 2.0) at 48 h post last dose of warfarin.
- Initiating edoxaban treatment at least 24 h after the last dose of warfarin appeared to maintain anticoagulant effect while not presenting a prolonged risk of overcoagulation, but further evaluation of an optimal dosing window will be obtained from a phase 3 clinical study of patients with atrial fibrillation.

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Keywords

atrial fibrillation, edoxaban, pharmacodynamics, pharmacokinetics, safety, warfarin

Received

2 December 2011

Accepted

23 July 2012

Accepted Article

Published Online

26 August 2012

AIMS

To evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of edoxaban, an oral direct factor Xa inhibitor, in healthy subjects switching from warfarin.

METHODS

Seventy-two subjects were randomized to edoxaban 60 mg once daily ($n = 48$) or matching placebo ($n = 24$) for 5 days at 24 h after the last dose of warfarin treatment (INR 2.0 to 3.0). Safety/tolerability was the primary outcome measure. Pharmacokinetics, INR, aPTT, anti-FXa, thrombin generation and other coagulation assays were assessed.

RESULTS

Seventy-two subjects were randomized and 64 subjects received at least one dose of edoxaban ($n = 43$) or placebo ($n = 21$) after achieving a target INR of 2.0 to 3.0 on warfarin treatment. Edoxaban 60 mg administered 24 h post-warfarin appeared to be safe and well tolerated. Adverse events were similar across treatments. For bleeding-related adverse events, eight subjects tested positive for faecal occult blood, five subjects during warfarin treatment and three subjects during edoxaban treatment. The mean (SD) baseline (post-dose of warfarin) INR was 2.31 (0.193) which increased to 3.84 (0.744) over 2 h during the edoxaban treatment ($P < 0.0001$ vs. placebo), returning to post-warfarin baseline within 12 h. A similar time course of effects for the other coagulation assays was observed in accordance with the drugs' mechanisms of action.

CONCLUSION

In this study of healthy subjects, edoxaban administered 24 h after the last dose of warfarin was safe and well tolerated with transient increases across the various coagulation assays above post-warfarin baseline levels.

Introduction

Anticoagulant prophylaxis with dose-adjusted vitamin K antagonists (VKAs) is recommended for the prevention of stroke and systemic embolic events in at-risk patients with atrial fibrillation and for treatment of acute deep vein thrombosis (DVT) [1–3]. However, VKAs, such as warfarin, are associated with several limitations, including unpredictable pharmacokinetics (PK) and pharmacodynamics (PD), numerous drug and dietary interactions, genetic variability in metabolism and response and a narrow therapeutic index [4]. Therefore, frequent monitoring and dose adjustments are required for VKAs to ensure that a therapeutic level of anticoagulation is maintained. These drawbacks have prompted the development of new oral anticoagulants [5, 6].

Edoxaban is a novel oral direct factor Xa (FXa) inhibitor in development for the prevention and treatment of thromboembolic events. Edoxaban has demonstrated linear PK with an oral bioavailability of approximately 62% and a terminal elimination half-life ($t_{1/2}$) of 8 to 10 h [7, 8]. Edoxaban is being studied in two large ongoing phase 3 multinational studies, ENGAGE AF-TIMI 48 [9] and Hokusai-VTE [10]. The ENGAGE AF-TIMI 48 trial is evaluating the safety and efficacy of edoxaban in preventing stroke and systemic embolic events in patients with non-valvular atrial fibrillation. The Hokusai-VTE trial is assessing the safety and efficacy of edoxaban in the treatment and prevention of recurrent venous thromboembolism in patients with acute symptomatic DVT and/or pulmonary embolism.

This study was designed to assess the safety/tolerability, PK and PD of edoxaban in healthy subjects who achieved an international normalized ratio (INR) of 2.0 to 3.0 on warfarin and then switched to edoxaban 24 h after their last dose of warfarin. The intent of this study was to determine if patients could be safely transitioned from warfarin to edoxaban.

Methods

Study design

This was a randomized, parallel, phase 1 study conducted in 72 healthy subjects. The primary objective was to assess the safety and tolerability of edoxaban when administered to healthy subjects 24 h after their last dose of warfarin (INR of 2.0 to 3.0). Other outcome measures included PK, PD and comparative effects of the two drugs on commonly performed tests of coagulation. Subjects were randomized 2:1 to receive either double-blind edoxaban 60 mg once daily (sequence 1) or matching placebo (sequence 2) for 5 days. All subjects received open label warfarin titrated to a target INR range of 2.0 to 3.0 (for 3 consecutive days) for a minimum of 6 and a maximum of 16 days. Subjects within the target INR range (post-warfarin baseline)

received edoxaban 60 mg once daily (sequence 1) or placebo (sequence 2) for 5 consecutive days, 24 h after the last warfarin dose.

The randomization schedule was blinded to all clinical and management personnel at the study centres. A pharmacist, otherwise uninvolved in study conduct, was unblinded to treatment, dispensed the assigned treatment and monitored on-site INRs. Local INR was measured once daily during both the open label warfarin titration phase and the double-blind edoxaban/placebo phase. If an INR exceeded the pre-established limits, the pharmacist did not dispense subsequent doses of warfarin. In the event of a medical emergency where the identity of the drug must be known to treat the subject properly, the randomization code for that subject could be broken and revealed by the statistician who prepared the code or by the pharmacist who prepared that subject's dose. In addition, the first local measurement post-dose of either edoxaban or placebo was on day 2 and the INR values for edoxaban and placebo were similar, so it would have been unlikely that blind would have been broken. The hourly INR samples on day 1 were analyzed at a separate laboratory and were not available until after study completion.

During warfarin therapy, treatment was discontinued if an INR >3.5 was observed with repeat assessment on the same day. For subjects with an INR 3.1 to 3.5, warfarin was temporarily interrupted and if the INR did not fall in range (i.e. <3.0) for 3 consecutive days, then the subject was discontinued. Subjects who did not meet the stopping rule were allowed to continue in the next phase (i.e. edoxaban or placebo treatments).

Subjects were provided a controlled diet that assisted in preventing a false positive reading for faecal occult blood (FOB). Consumption of foods and beverages containing vitamin K was prohibited 24 h before study and throughout the period of confinement. Subjects were followed for 4 days after completing double-blind treatment. Treatment compliance was evaluated by providing medication under supervision, and checking the hands and mouths of the subjects following each dosing. This study was conducted in accordance with principles originating in the Declaration of Helsinki and in compliance with Good Clinical Practice guidelines and local regulatory requirements. An institutional review board approved the study protocol and all subjects provided written informed consent before enrolment.

Subjects

Healthy males and females (non-child-bearing potential) aged 18 to 45 years, having a body mass index of 18 to 32 kg m⁻², with negative test for FOB and pregnancy, were eligible for the study. In addition, documentation of good health by the absence of significant deviations from normal, based on medical history, physical examination and clinical laboratory testing, was required.

Primary exclusion criteria included history of drug abuse, alcohol addiction, significant allergic response to any drug, psychiatric or emotional problems, clinically significant cardiac, hepatic, renal, pulmonary, endocrine, neurologic, infectious, gastrointestinal, haematologic or oncologic disease, coagulopathy or recurrent bleeding, estimated creatinine clearance (based on the Cockcroft–Gault equation) $<90 \text{ ml min}^{-1}$ and requirement for any concomitant medication.

Safety

The safety monitoring practices were selected to ensure the subjects' safety and to detect all treatment emergent adverse events (AEs). Subjects were confined from study day -1 to discharge on day 15. Safety assessments were conducted at the beginning of the trial, at regular intervals and at discharge, and could also be performed at any time at the investigators' discretion. Assessments included physical examinations, vital signs, continuous monitoring of AEs, clinical laboratory measurements (including prothrombin time [PT] and INR), FOB tests, vital signs, 12-lead electrocardiogram (ECG) and physical examinations.

Pharmacokinetics

Serial blood samples were collected to determine plasma concentrations of the R(+) and S(−) warfarin enantiomers and plasma concentrations of edoxaban.

Blood samples were collected for warfarin PK pre-dose (baseline) and on the last day of warfarin administration in subjects who were dosed to achieve an INR of 2.0 to 3.0 at pre-dose and at 0.5, 1, 2, 4, 6, 8, 12, 15, 24, 36, 48, 72 and 96 h post-dose. Plasma warfarin enantiomer concentrations of R(+) and S(−) were measured using a validated liquid chromatography-tandem mass spectrometry (LC/MS/MS) assay with a lower limit of quantitation (LLOQ) of 5 ng ml^{-1} for both enantiomers by PPD (Richmond, Virginia, USA).

Blood samples were collected for edoxaban PK pre-dose at the post-warfarin baseline, after a single dose of edoxaban at pre-dose and at 0.5, 1, 2, 4, 8, 12, 15 and 24 h post-dose. Plasma edoxaban concentrations were measured using a validated LC/MS/MS method with a LLOQ of 1 ng ml^{-1} at BioDynamics Research Ltd (Northamptonshire, UK).

The PK parameters were calculated from the individual plasma concentration measurements by non-compartmental methods using WinNonlin Version 4.0 Professional software (Pharsight, Mountain View, California, USA). The PK parameters evaluated both for warfarin and edoxaban were $\text{AUC}(0,t)$ (the area under the plasma drug concentration–time curve to the last measurable concentration, calculated using linear trapezoidal rule), C_{max} , t_{max} , terminal elimination rate constant (λ_z) and $t_{1/2}$. The parameters measured exclusively for edoxaban were $\text{AUC}(0,\infty)$ (the area under the plasma drug concentration–time curve to infinity, calculated as $\text{AUC}(0,t) + \text{last measurable concentration}/\lambda_z$), CL/F and V_{area}/F . PK parameters of warfa-

rin were determined on the last day of warfarin administration in subjects achieving an INR of 2.0 to 3.0 and PK parameters of edoxaban were determined after the first dose of edoxaban.

Pharmacodynamics

Blood samples for analysis of biomarkers of anticoagulation were collected pre-dose and at 1, 2, 5, 8 and 12 h after both the last dose of warfarin and the first dose of edoxaban. Additional blood samples were collected before each subsequent edoxaban dose and 24 h after the last dose. Blood samples were sent to Biomnis, formerly Laboratoire LCL (Ivry-sur-Seine, France) for determination of INR, PT, activated partial thromboplastin time (aPTT), D-dimer, prothrombinase-induced clotting time (PiCT), calibrated automated thrombogram (CAT) thrombin generation (TG) test and anti-FXa activity. So as not to break the blinding, hourly INR samples on day 1 post-edoxaban or placebo were analyzed at a separate laboratory and were not available to the investigators until the study had been completed.

Briefly, human thromboplastin (Thromborel S, Dade Behring Marburg GmbH, Marburg, Germany) was used to measure the clotting time (PT) of the subjects' plasma, which was compared with that of a control sample. The INR was calculated according to the formula:

$\text{INR} = (\text{PT}/\text{MNPT})^{\text{ISI}}$, where MNPT is the mean normal PT and ISI is a known international sensitivity index. Measurements for aPTT were performed using the STA-R analyzer with STA PTT (Diagnostica Stago, Asnières-sur-Seine, France) and involved the recalcification of plasma in the presence of a standardized amount of cephalin (platelet substitute) prepared from rabbit cerebral tissue in a buffered medium with silica added as a particulate activator. D-dimer, fibrin degradation products, was measured by an enzyme-linked fluorescence assay method using an automated immunoanalyzer, VIDAS (bioMérieux SA, Marcy l'Etoile, France). The linear range was 45 to 7400 ng ml^{-1} . The anti-FXa activity was measured using the STA-Rotachrom Heparin assay with STA-R analyzer according to the manufacturer's recommendation with edoxaban as calibrator. The LLOQ was 19.0 ng ml^{-1} edoxaban. TG was measured by the CAT method using 5 pM of tissue factor and $4 \text{ }\mu\text{M}$ of phospholipids (normal PPP reagent, Stago, Gennevilliers, France). The parameters calculated included endogenous thrombin potential (ETP) and peak. PiCT was measured by the Pefakit PiCT (Pentapharm, Basel, Switzerland) assay using activated FXa, phospholipids, Russell's viper venom-V and calcium to form prothrombinase complex with time to clotting as the analytical endpoint.

Statistical analyses

Since the primary objective of this study was safety/tolerability, no inferential statistics were performed and, therefore, no formal sample size was estimated. Additional considerations for sample size were previous INR data for

subjects assigned to either edoxaban or placebo [11] and the potential number of subjects completing the warfarin titration phase in accordance with the prespecified criteria. Three data sets were to be analyzed: (i) safety analysis set, which included all subjects who received at least one dose of warfarin or the study drug, (ii) PK analysis set, which included all subjects who had at least one detectable warfarin or edoxaban measurement and (iii) PD analysis set, which included all subjects who received at least one dose of warfarin, edoxaban or placebo, had any measurement on any PD parameter and achieved a stable INR of 2.0 to 3.0 after the last dose of warfarin.

Safety parameters were summarized using descriptive statistics. AEs were coded using the Medical Dictionary for Regulatory Activities, Version 9.1. Descriptive statistics for safety endpoints included sample size (n), mean, standard deviation (SD), median, minimum and maximum. As part of the primary safety endpoint, INR and PT values were compared after repeated edoxaban treatments relative to the last warfarin dose. Other PD parameters were summarized by descriptive statistics, and comparisons between edoxaban and placebo treatment were made using an analysis of variance (ANOVA) model with treatment as a fixed effect. Plasma drug concentrations and PK parameters were summarized by treatment using descriptive statistics. Geometric mean and coefficient of variation were calculated for $AUC(0,\infty)$ and C_{max} . Relationships between plasma concentrations and coagulation parameters for both warfarin and edoxaban were explored graphically by scatterplots and evaluated using linear regression analyses.

Results

Subjects

Seventy-two healthy subjects were randomized 2:1 to sequence 1 ($n = 48$) or sequence 2 ($n = 24$) (Figure 1) and were included in the safety analysis set ($n = 72$). Eight subjects withdrew and did not receive edoxaban or placebo: five from the edoxaban arm (INR >3.5 [$n = 3$], virus-related AE [$n = 1$] and personal reasons [$n = 1$]) and three from the placebo arm (INR >3.5 [$n = 2$] and positive FOB [$n = 1$]). Thus, the PK analysis set consisted of all subjects who completed the study ($n = 63$) plus one additional subject in the placebo group who withdrew early for personal reasons and had PK data ($n = 64$). The PD analysis set included all subjects who achieved a stable INR between 2.0 and 3.0 ($n = 55$). The randomized subjects had a mean age of 30.9 years, 98.6% (71/72) were male, 50% were Caucasian and 34.7% were African American.

Safety

Most vital signs and ECG results were within normal limits and none of the out of range values was considered clinically significant. There were no obvious differences between treatment arms. For both treatments, most indi-

vidual QT_c changes from baseline did not exceed 30 ms. Although change from baseline was occasionally between 30 and 60 ms for no more than two subjects (approximately 5% of all subjects) at each time point, QT intervals remained within normal limits when this occurred. No change from baseline exceeded 60 ms. Physical examination demonstrated minor abnormalities in some patients but none was considered clinically significant. Most haematology and serum chemistry values were within reference ranges. One subject who received warfarin followed by edoxaban had elevated alanine aminotransferase values (<2 times the upper limit of normal) outside the reference range, which was considered clinically significant but unlikely related to the treatment. No trends were observed in the shift tables ($>10\%$ change from baseline) across treatments.

AEs were reported in 33 of 72 (45.8%) subjects during warfarin treatment, in 15 of 43 (34.9%) subjects during edoxaban treatment and in three of 21 (14.3%) subjects during placebo treatment. Most (99%) AEs were mild and resolved with or without treatment. The most common AEs with edoxaban were pharyngolaryngeal pain, headache and positive FOB, all of which were mild and resolved with or without therapy (Table 1). Eight subjects tested positive for FOB in their stool. For three subjects who received warfarin followed by edoxaban, the positive FOB was observed during edoxaban treatment. Five subjects treated with warfarin followed by placebo also had positive FOB during at least 1 day on warfarin treatment and one of these subjects also was positive for FOB during placebo treatment. All FOB occurrences in both treatment groups were considered mild AEs. One subject reported a serious AE after study discontinuation following warfarin titration. The subject withdrew for personal reasons after completing 14 days of warfarin dosing. He was discharged 2 days after the last warfarin dose with an INR of 1.2, end of study assessments within normal limits, including a negative FOB, and having received a dose of vitamin K. The next day the subject experienced mild rectal bleeding and was apparently admitted to the local hospital for overnight observation. However, hospital records could not confirm his admission. The subject was assessed 7 days post last dose of warfarin and all assessments were within normal limits. Overall, more AEs were observed following warfarin administration compared with subjects receiving edoxaban and placebo.

Pharmacokinetics

The arithmetic mean $AUC(0,24\text{ h})$ values of the R(+) warfarin enantiomer were greater than those of S(-) warfarin after the last dose of warfarin in both treatments. Values (SD) for R(+) warfarin were 19 230 (7493) ng ml⁻¹ h and 18 014 (6688) ng ml⁻¹ h for sequence 1 and 2, respectively, vs. values for S(-) warfarin of 12 921 (5015) ng ml⁻¹ h and 11 628 (5787) ng ml⁻¹ h for sequence 1 and 2, respectively.

Figure 1
Study design and subject disposition of the safety analysis set. All subjects received warfarin monotherapy. Eight subjects did not receive either edoxaban or placebo; five subjects discontinued due to international normalized ratio (INR) >3.5 after warfarin treatment, two subjects discontinued due to adverse events and one subject withdrew consent for personal reasons

Table 1
Safety: frequent adverse events by treatment arm (≥5% of subjects overall)

Preferred term MedDRA v. 9.1	Warfarin followed by edoxabant		Warfarin followed by placebo†		Overall warfarin (n = 72)
	Warfarin (n = 48)	Edoxaban (n = 43)	Warfarin (n = 24)	Placebo (n = 21)	
Eye disorders	3 (6.3%)	1 (2.3%)	0 (0%)	0 (0%)	3 (4.2%)
Dizziness	3 (6.3%)	0 (0%)	0 (0%)	0 (0%)	3 (4.2%)
Headache	7 (14.6%)	3 (7.0%)	0 (0%)	1 (4.8%)	7 (9.7%)
Gingival bleeding	3 (6.3%)	3 (7.0%)	0 (0%)	0 (0%)	3 (4.2%)
Pharyngolaryngeal pain	4 (8.3%)	5 (11.6%)	0 (0%)	0 (0%)	4 (5.6%)
Cough	4 (8.3%)	1 (2.3%)	0 (0%)	0 (0%)	4 (5.6%)
Occult blood positive	0 (0%)	3 (7.0%)	5 (20.8%)	1 (4.8%)	5 (6.9%)

†Warfarin for a variable time followed by 60 mg edoxaban for 5 consecutive days. ‡Warfarin for a variable time followed by placebo for 5 consecutive days.

Table 2

Pharmacokinetic parameters of edoxaban after a single 60 mg oral dose

PK parameter: arithmetic mean $\pm$ SD (n = 43)	
AUC(0,∞) (ng ml ⁻¹ h)	1901.10 $\pm$ 466.83
AUC(0,t) (ng ml ⁻¹ h)	1693.30 $\pm$ 466.61
C _{max} (ng ml ⁻¹)	232.83 $\pm$ 83.61
t _{max} (h)†	1.02 (0.48, 4.00)
t _{1/2} (h)‡	7.82 $\pm$ 3.46

†Median (min, max). ‡Sampled over 24 h. AUC, area under the curve; AUC(0,∞), area under the plasma drug concentration–time curve to infinity, calculated as AUC(0,t) + last measurable concentration/the terminal elimination rate constant; AUC(0,t), area under the plasma drug concentration–time curve to the last measurable concentration, calculated using linear trapezoidal rule; C_{max}, maximum observed plasma concentration; t_{1/2}, terminal elimination half-life; t_{max}, time to maximum plasma concentration.

However, mean exposure to both enantiomers of warfarin was similar for both treatment cohorts.

A single oral dose of edoxaban 60 mg was rapidly absorbed with mean (SD) peak plasma concentration (C_{max}) of 232.83 (83.61) ng ml⁻¹ reached in a median of approximately 1 h, and then, following bi-exponential elimination, demonstrated a mean (SD) t_{1/2} of 7.82 (3.46) h estimated over a 24 h collection period (Table 2).

Pharmacodynamics

International normalized ratio The mean INR values were within 2.0 to 3.0 at the time of the last warfarin dose (post-warfarin baseline). The mean (SD) INR values at baseline 24 h post warfarin dose (post-warfarin baseline) were 2.31 (0.193) prior to edoxaban treatment and 2.30 (0.199) prior to placebo treatment cohorts (Table 3). The mean INR values increased to a peak of 3.84 (0.744) at 2 h post-dose of the single, 60 mg dose of edoxaban vs. 2.23 (0.223) for placebo ($P < 0.0001$) and returned to the post-warfarin baseline by 12 h after edoxaban administration. In contrast, mean INR values declined after administration of placebo. The INR values in the edoxaban treatment group were greater than those in the placebo treatment group at all measured time points between 1 and 12 h post-dose (Table 3). However, INR values were similar between edoxaban and placebo groups at 24 h post-dose. In one subject, INR values of 6.1 and 6.0 were reported at 1 and 2 h, respectively, after first edoxaban dose administered at post-warfarin baseline, and decreased to a value of 2.0 by 24 h. However, during study conduct INR measured locally at the clinic did not exceed 2.7 and no AEs were reported for this subject except FOB (mild in severity) on the evening of the third edoxaban dose.

Prothrombin time During the last day of warfarin treatment, mean (SD) PT values ranged from 26.8 (1.89) s to 28.7 (2.89) s. The mean (SD) PT values at post-warfarin baseline were 27.0 (2.21) s and 27.1 (2.71) s for edoxaban and placebo treatments, respectively (Table 3). Following the

edoxaban dose, PT increased consistent with the increase in INR. The mean (SD) PT increased to 43.9 (8.36) s at 2 h post-edoxaban dosing, corresponding approximately to maximum plasma edoxaban concentrations, vs. 26.4 (2.87) s for placebo ($P < 0.0001$). The mean (SD) PT values then returned to near post-warfarin baseline levels of 26.3 (4.01) s at 12 h post-dose and then decreased further to 21.4 (2.18) s by 24 h in the edoxaban group. Mean (SD) PT did not increase over the 12 h period after placebo administration [range 27.7 (6.19) to 24.0 (4.30) s] but declined to 21.2 (2.00) s by 24 h. PT values in the edoxaban group were greater than those in the placebo group at all measured time points between 1 and 12 h on day 1 (Table 3).

Activated partial thromboplastin time The mean (SD) aPTT increased from 35.6 (4.20) to 50.8 (8.81) s post-warfarin baseline. After edoxaban treatment, mean aPTT increased further to 67.4 (18.0) s at 1 h post-dose and then started to decrease, reaching baseline levels by 12 h post-dose (Figure 2). In comparison, mean aPTT did not change for 5 h after placebo and then slowly decreased to pre-warfarin values over the next 4 days.

Prothrombinase-induced clotting time The mean (SD) PiCT values remained relatively constant with warfarin, and then increased to 37.1 (5.96) and 37.2 (5.32) s at 1 and 2 h after edoxaban dosing, before declining (Figure 3). However, mean PiCT remained above baseline during the 5 day edoxaban administration period. In comparison, mean PiCT levels remained essentially unchanged following placebo.

Calibrated automated thrombogram assays TG ([ETP], which represents AUC of TG) declined during warfarin dosing by approximately 70%, indicative of reduced endogenous TG potential. ETP decreased by a further 67% (from 511 to 168 nmol min) after a single 60 mg dose of edoxaban and returned to pre-dose levels by 24 h, whereas ETP did not change in the 24 h period after placebo dosing (Figures 4A and 4B). The mean TG peak values declined from baseline, 346 (89.9) nmol to 105 (31.2) nmol at post-warfarin baseline. Following edoxaban, CAT peak further declined to 15.4 (17.2) nmol at 2 h post-edoxaban dose, the maximum observed change from baseline and returned to 95.1 (28.6) nmol at 24 h post-edoxaban dose (Figures 4C and 4D). The mean TG peak declined only slightly in the placebo group.

Anti-FXa activity Anti-FXa activity was mostly undetectable during warfarin treatment (<0.10 IU ml⁻¹), but increased following edoxaban, reaching peak concentrations of 2.68 IU ml⁻¹ at 1 to 2 h post-dose and then declining to approximately baseline concentrations by 24 h post-dose. The increases in anti-FXa activity were not evident in the placebo group, except for one subject who at 1 h post-dose showed a value of 3.07 IU ml⁻¹, which returned to

Table 3Mean ($\pm$ SD) INR and PT values by treatments for each time point

Time post-warfarin baseline	Mean INR Edoxaban 60 mg (n = 36)	Mean INR Placebo (n = 19)	P value
0 h, pre-dose†	2.31 $\pm$ 0.193	2.30 $\pm$ 0.199	0.5412
1 h‡	3.83 $\pm$ 0.757	2.36 $\pm$ 0.577	<0.0001
2 h‡	3.84 $\pm$ 0.744	2.23 $\pm$ 0.223	<0.0001
5 h‡	3.04 $\pm$ 0.557	2.10 $\pm$ 0.186	<0.0001
8 h‡	2.61 $\pm$ 0.371	2.05 $\pm$ 0.209	<0.0001
12 h‡	2.25 $\pm$ 0.339	2.03 $\pm$ 0.338	0.0034
Day 2, pre-dose§	1.81 $\pm$ 0.200	1.77 $\pm$ 0.156	0.2395
Day 3, pre-dose§	1.41 $\pm$ 0.133	1.37 $\pm$ 0.106	0.4544
Day 4, pre-dose§	1.23 $\pm$ 0.141	1.16 $\pm$ 0.068	0.1960
Day 5, pre-dose§	1.14 $\pm$ 0.091	1.08 $\pm$ 0.054	0.2311
Day 5, 24 h§	1.18 $\pm$ 0.626	1.05 $\pm$ 0.061	0.3814
	Mean PT (s) Edoxaban 60 mg (n = 36)	Mean PT (s) Placebo (n = 19)	P value
0 h, pre-dose†	27.0 $\pm$ 2.21	27.1 $\pm$ 2.71	0.3513
1 h‡	43.8 $\pm$ 8.67	27.7 $\pm$ 6.19	<0.0001
2 h‡	43.9 $\pm$ 8.36	26.4 $\pm$ 2.87	<0.0001
5 h‡	35.2 $\pm$ 6.38	25.0 $\pm$ 2.43	<0.0001
8 h‡	30.4 $\pm$ 4.35	24.5 $\pm$ 2.87	<0.0001
12 h‡	26.3 $\pm$ 4.01	24.0 $\pm$ 4.30	0.0027
Day 2, pre-dose§	21.4 $\pm$ 2.18	21.2 $\pm$ 2.00	0.1807
Day 3, pre-dose§	16.8 $\pm$ 1.40	16.6 $\pm$ 1.44	0.4245
Day 4, pre-dose§	14.8 $\pm$ 1.75	14.2 $\pm$ 0.89	0.1904
Day 5, pre-dose§	13.7 $\pm$ 0.89	13.2 $\pm$ 0.72	0.1579
Day 5, 24 h§	14.4 $\pm$ 7.48	13.0 $\pm$ 0.92	0.2961

†0 h = first day of edoxaban or placebo dose administration approximately 24 h after the last warfarin dose. Comparisons of interest are as follows: ‡(Respective time point of edoxaban minus respective time point of warfarin treatment) vs. (Respective time point of placebo minus respective time point of warfarin treatment). §(Respective pre-dose of edoxaban minus pre-dose on the last day of warfarin treatment) vs. (Respective pre-dose of placebo minus pre-dose on the last day of warfarin treatment). INR, international normalized ratio; PT, prothrombin time.

below detectable limits (<0.10 IU ml^{-1}) at the next time point (2 h post-dose). Comparison with other assays performed on this subject's plasma at this time point indicated that this outlier resulted from mislabelling of a sample from an edoxaban recipient as placebo. A linear relationship was apparent between anti-FXa activity and plasma edoxaban concentrations (correlation coefficient >0.95) (Figure 5).

D-dimer Most D-dimer concentrations were below the pre-determined cutoff level (i.e. <500 ng ml^{-1}) during treatment with warfarin and the subsequent treatment with edoxaban and placebo.

Discussion

This phase 1 study was conducted in healthy subjects to assess the safety and tolerability, the primary outcome measure, and biomarker effects of edoxaban 60 mg administered 24 h post-dose of warfarin (INR 2.0 to 3.0). In this healthy volunteer population, the treatments were well tolerated. Most AEs were mild and typical of phase 1

studies, with the exception of positive FOB reported in eight subjects (i.e. five during warfarin treatment, and three during edoxaban treatment). Clinical laboratory and other assessments did not demonstrate any trends by treatment. One serious AE of mild rectal bleeding after warfarin titration was reported by a subject which may have resulted in a period of observation at a local hospital, but neither this AE nor the hospital admission could be confirmed.

For subjects switched from warfarin to edoxaban 60 mg once daily, INR values increased transiently above 3.0, the upper limit of the therapeutic range for warfarin, for approximately 5 h post-dose. In previous phase 1 studies of edoxaban 60 mg, the mean INR values observed for a single 60 mg dose ranged from 1.1 to 2.0 post-dose [8]. The INR values observed in this study appear to represent an approximately additive effect of the residual warfarin and edoxaban anticoagulation. The edoxaban INR effects (differences between placebo and edoxaban treatment arms) ranged from 0.94 to 1.61 over the initial 5 h post-dose of edoxaban.

Edoxaban was rapidly absorbed following oral administration, reaching C_{max} in a median time of 1 h and

Figure 2

Mean ($\pm$ SD) aPTT by time of treatment. Pre* denotes the pre-dose value on study day 1. Breaks in the x-axis represent the 24 h washout period following the last warfarin dose within the INR target range

demonstrated a mean $t_{1/2}$ of 8 h when samples were collected over a 24 h period. The disposition of edoxaban administered 24 h post-dose of warfarin was consistent with observations in previous studies in which no warfarin had been administered. In a single ascending dose study, edoxaban demonstrated linear PK with similar exposure at the 60 mg dose with C_{max} observed at 1 to 2 h post-dose and $t_{1/2}$ from 5.8 to 10.7 h [8]. Edoxaban, as a direct FXa inhibitor, demonstrates a rapid onset of anticoagulant activity corresponding to peak plasma concentrations. The mean INR and PT values increased to peaks of 3.84 and 43.9 s, respectively, within 2 h post-warfarin baseline.

Seventy-nine percent of subjects titrated with warfarin achieved INR levels within 2.0 to 3.0 during the 16 day period. Five subjects had INR values >3.5 . The lack of ease in titrating healthy subjects who are confined to the clinic with restricted diets and concomitant medications attests to the reported difficulty of achieving optimal INR ranges with warfarin in outpatient populations [12].

FXa inhibitors and VKAs (e.g. warfarin) inhibit the formation of thrombin through different mechanisms, and

therefore, these classes of drugs have different relative effects on the various coagulation assays. VKAs inhibit thrombin formation by preventing carboxylation of vitamin K-dependent coagulation factors (prothrombin and FVII, FIX and FX) whereas edoxaban directly inhibits FXa to prevent the prothrombinase complex from activating FII to thrombin [5]. The results of the changes in the coagulation assays reflect the different mechanisms of action of edoxaban and warfarin. Both drugs produced increases in PT, INR and CAT parameters while warfarin showed minimal to no effect on PiCT, aPTT and anti-FXa activity. PiCT, a clotting assay that is sensitive to FXa inhibitors and thrombin inhibitors, is based on adding FXa and snake venom FV activator to platelet-poor plasma [13, 14]. This assay has been shown previously to be effective for measuring PD properties of rivaroxaban, a direct FXa inhibitor [15]. The commercially available chromogenic anti-FXa assay correlated linearly with edoxaban plasma concentrations over the 60 mg dose range. However, it is important to emphasize that edoxaban has a greater effect than LMWH on anti-FXa levels. Thus, clinicians should be

Figure 3

Mean prothrombinase-induced clotting time (PICT) ($\pm$ SD). Pre* denotes the pre-dose value on study day 1. Treatment sequence 1 is warfarin for a variable time followed by 60 mg edoxaban for 5 consecutive days (A). Treatment sequence 2 is warfarin for a variable time followed by placebo for 5 consecutive days (B).

mindful that until assays are developed to measure specifically edoxaban activity using an edoxaban calibrator and expressed in edoxaban units, therapeutic concentrations of edoxaban may be reported above the anti-FXa range that is used to monitor treatment with LMWH. As expected, warfarin had no effect on anti-FXa, but exposure levels of novel FXa inhibitors, such as rivaroxaban, appear to be well characterized by anti-FXa.

CAT is an exploratory assay that measures the amount of thrombin generated over time, represented by thrombograms that can be described by several parameters. The decrease in area under the thrombogram, ETP, during warfarin treatment was similar to changes observed in other studies [16]. Chen *et al.* have shown that the mean ETP in patients on warfarin with INR 2.0 to 3.0 was 604.47 nmol min in patients [16]. This current study showed post-warfarin baseline values as 511 (127) nmol min. The administration of edoxaban 60 mg further

decreased ETP to 142 (203) nmol min at 2 h post-dose of edoxaban. A similar trend was observed for CAT peak with a decrease from baseline of 346 (89.9) nmol to 105 (31.2) nmol at post-warfarin baseline with a further decrease to 15.4 (17.2) nmol at 2 h post-dose of edoxaban, indicating almost complete inhibition of observed thrombin generation.

Despite the lack of a unique coagulation assay to measure both warfarin and edoxaban anticoagulant effects, the totality of these coagulation assay data indicates a transient and moderate increase in anticoagulation for edoxaban 60 mg administered 24 h post-dose of warfarin INR 2.0 to 3.0. While PT/INR are routinely used to monitor VKAs to ensure that doses are in the therapeutic range of 2.0 to 3.0, this range is not applicable to edoxaban or other direct FXa inhibitors since these compounds have a reduced effect on this coagulation assay. With edoxaban, INR returned to post-warfarin baseline values 12 h after

Figure 4

Calibrated automated thrombogram (CAT) thrombin generation (endogenous thrombin potential, ETP) (A, B), and CAT thrombin generation (peak) (C, D) vs. time by treatment. Pre* denotes the pre-dose value on study day 1. Treatment sequence 1 is warfarin for a variable time followed by 60 mg edoxaban for 5 consecutive days (A, C). Treatment sequence 2 is warfarin for a variable time followed by placebo for 5 consecutive days (B, D)

Figure 4

Continued

Figure 5

Relationship between edoxaban concentration and anti-factor Xa (FXa) activity

administration and further decreased to warfarin pre-dose levels at approximately 24 h. The relatively low sensitivity of PT/INR to direct FXa inhibitors is further complicated by differing effects of the various commercially available thromboplastins used in these assays and the INR correction factor [17–19].

Similarly, aPTT, which has traditionally been used to measure heparin effects, is also not ideal to measure the anticoagulant effects of the oral direct FXa inhibitors due to low sensitivity. Edoxaban has a much greater effect on anti-FXa activity than warfarin. Therefore, the other exploratory coagulation assays such as CAT were also used to characterize the effects of edoxaban and residual warfarin and may perhaps represent a more global coagulation assay, albeit not one routinely used in clinical practice. Consistent with INR changes, these assays also demon-

strated an approximately additive effect of the residual warfarin and edoxaban anticoagulant effects. However, the absolute value of the inhibition of CAT has not been correlated with clinical bleeding or validated in large clinical trials, and therefore, these values cannot be used to predict bleeding potential.

The optimal time window for switching from warfarin to edoxaban depends on several factors, including a patient's INR value prior to discontinuation of warfarin, and would be best characterized by the data from a phase 3 study. The 24 h interval selected for this study was based on the once daily dosing regimens of warfarin and edoxaban and the assumption that the anticoagulant effects of warfarin should have diminished, but maintain therapeutic effect (i.e. INR 2.0 to 3.0). The mean predose INR baseline value for these healthy subjects (post-warfarin titration)

was 2.3. The mean INR value for the placebo group fell below 2.0 at 48 h, the therapeutic range for sufficient anticoagulation with warfarin. For the phase 3 ENGAGE AF-TIMI 48 trial, subjects on prior warfarin therapy were required to have an INR <2.5 prior to randomization. Further identification of the optimal window for switching from warfarin to edoxaban in patients with atrial fibrillation will be provided from this phase 3 study [20].

This study has several limitations that prevent extrapolation of these results to clinical practice. In this phase 1 study, subjects were healthy, younger adults who remained in clinic throughout the observation period, followed a controlled diet and did not receive any concomitant medications. Typical patients with atrial fibrillation or venous thromboembolism tend to be elderly and frequently have comorbid disease. Warfarin response tends to be more variable in these elderly patients, particularly in the outpatient setting due to varied diet, potential compliance issues and concomitant medications. In this study, the healthy subjects had optimal conditions for titration with daily INR measures to confirm that warfarin response was maintained within the therapeutic range. Another limitation of this study was the lack of a specifically stated hypothesis or predefined sample size based on statistical power to evaluate either safety or coagulation assay results. Therefore, this study was not sized to fully characterize the safety and tolerability of edoxaban administered 24 h post-warfarin (INR 2.0 to 3.0). While biomarkers were used to assess the potential effects of edoxaban and warfarin, no unique biomarker was sufficiently indicative of bleeding potential for both compounds. The results of this study served to provide guidance for the design of phase 3 studies.

The results suggest that initiating edoxaban treatment at least 24 h after the last dose of warfarin (e.g. mean INR value of 2.3) was well tolerated and the biomarker results suggest a transient risk of overcoagulation, most notably at 2 h post-dose of edoxaban.

Conflict of Interest

J. Mendell and M. Shi are employees of Daiichi Sankyo. R.J. Noveck has been a consultant for Daiichi Sankyo and Eisai Inc. This study was funded by Daiichi Sankyo Pharma Development, Edison, NJ, USA.

The authors would like to acknowledge writing assistance provided by Sameena Azmi, PhD and editorial assistance by Evince Communications, Norwalk, CT, USA, which was funded by Daiichi Sankyo.

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