



Full Length Article

Determination of edoxaban equivalent concentrations in human plasma by an automated anti-factor Xa chromogenic assay



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ABSTRACT

Introduction: This phase I, open-label, multiple-dose, two-treatment study assessed the relationship between edoxaban equivalent concentration derived from an anti-FXa assay with the summed concentration of edoxaban and its active metabolite, M-4, as assessed by liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS). This study also assessed the relationship between edoxaban plasma concentrations assessed by LC/MS/MS in sodium citrate and lithium heparin tubes.

Materials and methods: Healthy volunteers were randomized to receive once-daily edoxaban 60 mg or 90 mg for 5 days (15 participants per treatment group). Serial blood samples were collected for analysis by LC/MS/MS and by the anti-FXa assay. Edoxaban equivalent levels were assessed using a commercially available anti-FXa activity assay with an edoxaban-specific setup.

Results and conclusions: The day 5 concentration estimates were significantly correlated between the 2 assays ($P < 0.0001$ for both edoxaban doses). The geometric least squares mean (GLSM) ratio (90% confidence interval) for edoxaban equivalent concentrations vs edoxaban + M-4 concentrations was 114.3% (108.2–120.8) for edoxaban 60 mg ($P < 0.0001$) and 113.0% (107.1–119.2) for edoxaban 90 mg ($P = 0.0002$). The GLSM ratio for edoxaban concentrations in sodium citrate vs lithium heparin tubes for 60-mg and 90-mg edoxaban doses were 82.8% (78.5–87.3) and 83.9% (79.1–89.0), respectively. In this study, an anti-FXa chromogenic assay with edoxaban-specific calibrators and controls demonstrated good accuracy in estimating edoxaban concentrations across a wide range of concentrations relative to LC/MS/MS at steady state following the administration of once-daily edoxaban for 5 days.

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1. Introduction

Edoxaban is a direct, oral factor Xa (FXa) inhibitor with linear and predictable pharmacokinetics (PK) [1], indicated for the prevention of stroke in patients with nonvalvular atrial fibrillation (NVAF) and for the treatment of venous thromboembolism (VTE) [2]. Large phase III studies have demonstrated that direct oral anticoagulants (DOACs), including edoxaban, are at least as efficacious as warfarin and are associated with less major bleeding in patients with NVAF and VTE [3–10]. Unlike warfarin, DOACs typically do not require routine laboratory

monitoring [11]. Nevertheless, there are situations in which clinicians may want to be informed of DOAC drug concentrations, including before surgery or invasive procedures, when a patient is actively bleeding, following a DOAC overdose, on reoccurrence of thrombosis to determine if the drug is present in a therapeutic range, or if the patient develops renal failure [11,12].

Quantifying DOAC levels with liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) assays is specific, sensitive, and accurate [13,14]. However, LC/MS/MS assays are not readily available in routine clinical practice [15]. Clotting assays, such as prothrombin time (PT) and activated partial thromboplastin time (aPTT), are more widely available but are not quantitative and direct, oral FXa inhibitors have variable effects on these standard coagulation tests [11, 16–18]. Recent studies have suggested that some PT reagents could be useful to measure edoxaban, however, the impact of edoxaban on PT and aPTT depends on the reagents [19,20].

In a public workshop held by the US Food and Drug Administration (FDA) on in vitro diagnostic testing for DOACs, the FDA noted that appropriate assays should show accuracy and consistency at key medical

Abbreviations: AE, Adverse events; aPTT, activated partial thromboplastin time; ANOVA, Analysis of variance; CI, Confidence interval; DOACs, Direct oral anticoagulants; FDA, Food and Drug Administration; FXa, Factor Xa; GLSM, Geometric least squares means; LC/MS/MS, Liquid chromatography coupled with tandem mass spectrometry; LLOD, Lower limit of detection; PK, Pharmacokinetic; PT, prothrombin time; NVAF, Nonvalvular atrial fibrillation; VTE, Venous thromboembolism.

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decision levels [21]. Chromogenic anti-FXa assays, which are currently in clinical use for measuring the levels of low molecular weight heparin [22], offer the possibility to adequately measure direct anti-FXa drugs, including edoxaban in plasma [23,24]. To that end, specific anti-FXa chromogenic assays, which measure the inhibition of the activity of exogenously added FXa, have recently been tailored for use with direct oral anti-FXa inhibitors [16,25]. In these tests, the residual activity from an excess of exogenously added FXa is inversely proportional to the amount of direct, oral FXa inhibitors and any relevant active metabolites present in the plasma sample. To date, there is no universal anti-FXa test for measuring all of the direct, oral FXa inhibitors because these assays need to be specifically calibrated for each drug [16]. When used with appropriate calibrators and controls, anti-FXa assays can accurately quantify plasma concentrations for rivaroxaban and apixaban over a wide concentration range with good intra- and inter-laboratory consistency [17,18,25–29]. For measuring edoxaban levels, several studies have assessed the performance of chromogenic assays designed to measure heparins or anti-FXa chromogenic assays with non-edoxaban-specific calibrators and controls [19,20]. However, the performances of anti-FXa chromogenic assays with edoxaban-specific calibrators and controls have not been reported.

The aim of the present study was to assess the relationship between edoxaban equivalent concentration derived from an anti-FXa assay, using edoxaban-specific calibrators and controls, with the summed concentration of edoxaban and its most abundant, active metabolite, M-4, as assessed by LC/MS/MS. The anti-FXa assay measures the activity of all active molecular moieties, therefore, in this context, edoxaban equivalent concentration refers to the level of edoxaban and all its active metabolites. The predominant active metabolite of edoxaban is M-4, and it reaches <10% of the exposure of edoxaban in healthy individuals [1,2,30]. Therefore, summing edoxaban and M-4 concentrations should closely approximate the edoxaban equivalent concentration.

Blood samples for the anti-FXa assays are typically collected in sodium citrate tubes while blood samples for LC/MS/MS PK analysis in the clinical development of edoxaban were collected in lithium heparin tubes. Therefore, this study also assessed the relationship between plasma concentrations of edoxaban and M-4 as assessed by LC/MS/MS in 0.109 M sodium citrate and in lithium heparin tubes. The sodium citrate tubes contained pre-added buffer equal to 10% of the maximum blood collection volume, while the lithium heparin tubes had no buffer (spray-coated).

2. Material and methods

2.1. Study design

This phase I, open-label, multiple-dose, two-treatment study was conducted in accordance with the International Conference on Harmonisation Harmonised Tripartite Guideline on Good Clinical Practice. The study was conducted in healthy adult male and female participants at one site in the US (Medpace Clinical Pharmacology Unit; Cincinnati, Ohio). All participants gave written informed consent prior to participating in the study.

At check-in (day –1), participants were screened for inclusion/exclusion criteria, underwent a physical examination, provided blood and urine samples for clinical laboratory testing, and had vital signs checked. Participants remained in the unit through day 7. Participants were randomly assigned to receive once-daily edoxaban 60 mg or 90 mg on days 1 to 5. All treatments were administered with 240 mL of water in the morning following an overnight fast of 10 h. On days 1 to 4, participants continued to fast for 2 h after dosing; on day 5 participants continued to fast for 4 h postdose. Adverse events (AEs) were continuously monitored throughout.

2.2. Study participants

Healthy males and females were eligible to enroll if they were between 18 and 55 years of age with a body mass index between 18 and 30 kg/m² at screening and check-in. Exclusion criteria included a history of any clinically significant disorder that might prevent the successful completion of the study or a clinically significant illness; stomach or intestinal surgery or resection that might alter absorption and/or excretion of orally administered drugs; a history of major bleeding, major trauma, or major surgical procedure of any type within 6 months of the first dose or a history of peptic ulcer, gastrointestinal bleeding, or dysfunctional uterine bleeding; a history of eye surgeries or trauma to the head or eye within 14 days of the first dose; a history or presence of an abnormal electrocardiogram or a Fridericia's corrected QT ≥ 450 msec for males and ≥ 470 msec for females at screening; and a previous edoxaban study within 6 months prior to the first dose. Female participants could not be pregnant or lactating. In addition, all participants who used an anticoagulant, coagulant, or antiplatelet medications within 30 days prior to the first dose or aspirin within 10 days prior to the first dose were excluded.

2.3. Sample collection

Serial blood samples were collected for analysis by LC/MS/MS (in both lithium heparin and 0.109 M sodium citrate tubes) and by the anti-FXa assay (in 0.109 M sodium citrate tubes) on days 2, 3, and 4 prior to dosing (trough) and on day 5 predose and postdose (0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 14, 22, 24, 28, 32, 36, and 48 h).

The lithium heparin sample tubes containing blood for plasma preparation were gently inverted 8 to 10 times to ensure thorough mixing of anticoagulants (spray-coated lithium heparin) and blood. Within 30 min of blood draw, the samples were centrifuged for approximately 10 min under 1500 $\times g$ and 4 °C. The harvested plasma samples were stored in a –20 °C freezer.

For blood samples collected in sodium citrate tubes, the volume of pre-existing 0.109 M sodium citrate buffer in the collection tube was equal to 10% of the specified total sample volume of the tube (eg, 0.3 mL citrate solution plus 2.7 mL of blood). The sample processing procedures were similar to those used for the lithium tubes, except that the centrifugation conditions were 2500 $\times g$ at room temperature, and the resulting plasma were stored in a –70 °C or below freezer. The blood samples for the anti-FXa assay were collected and processed according to the Clinical and Laboratory Standards Institute guidelines [31].

2.4. Assay methods

2.4.1. Anti-FXa

Edoxaban equivalent levels were assessed by Medpace Research Laboratory (Cincinnati, OH) using a commercially available anti-FXa activity assay (STA®-Liquid Anti-Xa; Diagnostica Stago, Asnières sur Seine, France) with an edoxaban-specific setup using the STA®-Edoxaban Calibrator and STA®-Edoxaban Control on the STA®-R analyzer (Diagnostica Stago, Asnières sur Seine, France). The assay comprises 4 levels of calibrators and 2 levels of controls. The nominal concentrations for calibrators are 0 (blank) and approximately 30, 90, 140 ng/mL and for controls approximately 37 and 100 ng/mL, with lot-specific concentration values assigned based on independent LC/MS/MS measurements. The edoxaban concentration levels of each lot of calibrators and controls were determined by high-performance liquid chromatography-mass spectrometry. All calibrator and control reagents were reconstituted in distilled water per the kit instructions. The assay was performed according to a detailed standardized protocol provided by the manufacturer. The lower limit of detection (LLOD) for this assay was 15 ng/mL. The upper limit of quantification for this assay was 450 ng/mL. The assay includes an automated redilution of plasma samples containing an edoxaban equivalent concentration higher than

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