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## ORIGINAL ARTICLE

# Comparison of the anticoagulant intensities of fondaparinux and enoxaparin in the organization to assess strategies in acute ischemic syndromes (OASIS)-5 trial

J. A. M. ANDERSON,*† J. HIRSH,* S. YUSUF,* M. JOHNSTON,‡ R. AFZAL,* S. R. MEHTA,*
K. A. A. FOX,† A. BUDAJ§ and J. W. EIKELBOOM*

*Divisions of Hematology and Thromboembolism, and Cardiology, Department of Medicine, Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada; †University and Royal Infirmary of Edinburgh, Edinburgh, UK; ‡Hemostasis Reference Laboratory, Hamilton, ON, Canada; and §Postgraduate Medical School, Grochowski Hospital, Warsaw, Poland

**Summary.** *Background:* In the OASIS-5 trial, fondaparinux reduced major bleeding with similar short-term efficacy as enoxaparin but lowered death and stroke during long-term follow-up. The mechanism of lower bleeding and improved efficacy with fondaparinux is uncertain. *Methods and Results:* We compared the anti-Xa concentration (reflecting drug levels), Xa clot time (reflecting anticoagulant effect) and endogenous thrombin potential (ETP; a global test of hemostatic function) in plasma samples collected 6, 24 and 72 h after the first dose of the study drug in 48 patients randomly assigned fondaparinux 2.5 mg day$^{-1}$ and 42 patients assigned enoxaparin 1 mg kg$^{-1}$ twice daily in the OASIS-5 trial. Patients assigned to fondaparinux compared with enoxaparin had a significantly lower mean anti-Xa level [0.52 IU mL$^{-1}$ (SD 0.22 IU mL$^{-1}$) vs. 1.2 IU mL$^{-1}$ (SD 0.45 IU mL$^{-1}$), $P < 0.0001$] and Xa clot time [64.9 s (SD 17.7 s) vs. 111.8 s (SD 29.6 s), $P < 0.0001$], and significantly higher ETP area under the curve (AUC) [386.7 mA (SD 51.5 mA) vs. 206.4 mA (SD 90.6 mA), $P < 0.001$] at 6 h, and these differences remained evident at 24 and 72 h. There was significantly less variability of the results of anti-Xa levels, Xa clot time and ETP AUC for fondaparinux compared with enoxaparin at 6 h ($P < 0.001$ for each comparison). *Conclusion:* Fondaparinux 2.5 mg day$^{-1}$ compared with enoxaparin 1 mg kg$^{-1}$ twice daily produces less variable anticoagulant effect and lower mean anticoagulant intensity. These results most likely explain the reduced risk of bleeding seen with fondaparinux compared with enoxaparin in

the OASIS-5 trial and suggest that a lower intensity of anticoagulation than used in the past may be sufficient to prevent recurrent ischemic events and death in patients with ACS who are concurrently treated with aspirin and clopidogrel.

## Introduction

Anticoagulants are effective in acute coronary syndromes (ACS) for the prevention of recurrent ischemic events and death. The major side effect of anticoagulant therapy is bleeding. Historically bleeding has been considered a reversible outcome, while thrombotic sequelae such as myocardial infarction and stroke are irreversible. Recently, evidence has accumulated that bleeding in patients with ACS is associated with an increased risk of later ischemic events and death [1–3], an observation that emphasizes the general principle of selecting the lowest effective anticoagulant dosage for each indication. In the Organization to Assess Strategies in Acute Ischemic Syndromes (OASIS)-5 trial [4,5], which compared fondaparinux with enoxaparin in patients with non-ST-elevation acute coronary syndromes (NSTE-ACS), fondaparinux resulted in (i) similar rates of early ischemic events, (ii) significantly lower rates of bleeding complications, and (iii) significantly lower rates of long-term mortality, compared with enoxaparin.

It has been suggested that the results of the OASIS-5 trial are due to pharmacological differences between fondaparinux and enoxaparin. Fondaparinux is a selective inhibitor of activated factor X (FXa) while enoxaparin blocks both FXa and, to a lesser extent, thrombin (FIIa), and it is theoretically possible that the additional inhibition of FIIa increases the risk of bleeding without providing additional efficacy. This explanation is,

Correspondence: John W. Eikelboom, Division of Hematology and Thromboembolism, Department of Medicine, McMaster University, Hamilton, ON, Canada.
Tel.: +1 905 527 4322 ext. 44768; fax: +1 905 521 1551.
E-mail: eikelbj@mcmaster.ca

however, unlikely given other evidence that inhibition of FIIa prevents recurrent ischemic events in patients with ACS [6,7]. A more likely explanation is that reduced bleeding with fondaparinux is due to the lower anticoagulant intensity afforded by fondaparinux 2.5 mg day$^{-1}$ compared with enoxaparin 1 mg kg$^{-1}$ twice daily (or a reduced dose of 1 mg kg$^{-1}$ once daily in patients with a creatinine clearance $\leq$ 30 mL min$^{-1}$). Of interest and potential importance, the 2.5 mg day$^{-1}$ dose of fondaparinux provided sufficient antithrombotic activity, possibly because patients were receiving other antithrombotic medications (aspirin, clopidogrel) and therefore did not need more intense anticoagulant treatment.

We tested our hypothesis that fondaparinux given at a dose of 2.5 mg subcutaneously once daily, as used in the OASIS-5 trial, provides less intensive anticoagulation than enoxaparin at a dose of 1 mg kg$^{-1}$ twice daily, by comparing the anticoagulant intensities used in the OASIS-5 study. The comparison was performed by measuring the anti-Xa concentration and activity of the drugs, and by a global assay of hemostasis that measures thrombin generation, the endogenous thrombin potential (ETP) [8–10]. We measured the anti-Xa concentration, anti-Xa activity and the ETP in plasma samples collected 6, 24 and 72 h after the first dose of the study drug in 48 patients randomly assigned fondaparinux and 42 patients randomly assigned enoxaparin in the OASIS-5 trial.

## Methods

OASIS-5 was an international randomized double-blind trial evaluating the efficacy and safety of fondaparinux vs. enoxaparin in the acute treatment of NSTE-ACS. The primary aim of the study was to determine if fondaparinux is non-inferior to enoxaparin in the prevention of death, new myocardial infarction and refractory ischemia at 9 days (primary outcome) and at 30 days (secondary outcome) after randomization [4,5]. The study was approved by the Institutional Review Board at each participating centre and all patients provided written informed consent.

### Patients

The design of the OASIS-5 study has been published previously [5]. Briefly, patients were randomized as soon as possible, and no more than 24 h after presentation with signs and symptoms of unstable angina or non-ST elevation myocardial infarction. For inclusion into the study, patients were required to meet two out of the following three criteria: age $\geq$ 60 years, troponin T or I or CK-MB elevated greater than the upper limit of normal, and electrocardiogram changes compatible with ischemia. Patients with a contraindication to low-molecular-weight heparin (LMWH), such as renal insufficiency [defined as a serum creatinine of at least 3 mg dL$^{-1}$ (265 µmol L$^{-1}$)] or recent hemorrhagic stroke, and those with an indication for oral anticoagulation other than an acute coronary syndrome were excluded. In patients whose creatinine clearance was below 30 mL min$^{-1}$, the enoxaparin dosage was reduced (see below).

### Study treatments

If a patient had received anticoagulant therapy prior to entry into the trial, randomization was delayed by 6 h if LMWH was administered, and delayed by 2 h if unfractionated heparin (UFH) was administered. As soon as possible after randomization a first dose of enoxaparin/enoxaparin-placebo (1 mg kg$^{-1}$ twice daily or 1 mg kg$^{-1}$ once daily in those with a creatinine clearance < 30 mL min$^{-1}$) and a first dose of fondaparinux/fondaparinux-placebo (2.5 mg 0.5 mL$^{-1}$ once daily) were administered subcutaneously.

### Blood sample collection

Investigators at participating centres were encouraged to approach all patients participating in the OASSIS-5 study for consent to collect blood samples at baseline (prior to receiving the first doses of the study drug) and at 6, 24 and 72 h after randomization. Blood was drawn from an antecubital vein by clean venepuncture using a 21-gauge needle without a tourniquet into two 5 mL vacutainer tubes prefilled with 0.5 mL of 3.2% buffered sodium citrate (Becton Dickinson, Toronto, ON, Canada). The blood collection tubes were kept on ice for a maximum of 30 min before centrifugation at $1700 \times g$ for 15 min at room temperature. The resultant platelet-poor plasma was harvested and subjected to a second centrifugation at $1700 \times g$ for 5 min to ensure complete removal of platelets. Platelet-free plasma was harvested and frozen in aliquots at $-40°$ to 70 °C prior to shipment on dry ice or in liquid nitrogen to the central laboratory at Hamilton, Canada, where they were stored at minus 70 °C or below until analyzed.

### Laboratory analyses

The baseline plasma samples in the enoxaparin and fondaparinux groups were compared using a standard activated partial thromboplastin (aPTT) assay. The relative anticoagulant intensities of fondaparinux and enoxaparin at 6, 24 and 72 h were assessed by measurement of the anti-Xa effect using two assays, a chromogenic anti-Xa assay [11] and a quantitative assay of anti-Xa intensity, the 'Xa-clot time' [12], in addition to an assay of thrombin generation, the endogenous thrombin potential (ETP).

We based our primary analysis on the results obtained from the 6-h samples, which are most likely to reflect near peak anti-Xa effect for both fondaparinux and enoxaparin following first subcutaneous administration, despite their different half-lives and pharmacokinetic properties.

All investigators and laboratory personnel remained blinded to the treatment allocations and codes until completion of the laboratory assays.

*aPTT assay* Baseline aPTT assays were performed on the STA compact coagulometer using Dade Behring FSL aPTT reagent (Dade Behring, Marburg, Germany); 50 µL of citrated

plasma is incubated with 50 µL aPTT reagent for 3 min at 37 °C followed by 50 µL of 0.2 M CaCl$_2$. The time to clot formation is recorded in seconds.

*Anti-Xa assay*  The instrument used for anti-Xa activity was the Amax 190+ (Trinity Biotech, Bray Co. Wicklow, Ireland). The assay used was the Stachrom® Heparin (Diagnostica Stago, Asnières, France).

The assay is performed in the presence of excess FXa and exogenous antithrombin (AT) to compensate for any deficiency of AT in the plasma. The AT-drug complex neutralizes a given quantity of FXa. A substrate specific for FXa is added and the residual FXa cleaves the substrate, resulting in release of paranitroaniline (pNA). The reaction is read at an optical density of 405 nm and is inversely proportional to the amount of fondaparinux or enoxaparin present in the plasma. The plasma levels of fondaparinux and enoxaparin are determined by measuring their anti-Xa activities using a common calibration line established for both drugs. The common calibration line was established by making serial dilutions of a known concentration of enoxaparin (100 mg mL$^{-1}$) equivalent to 10 000 anti-Xa U mL$^{-1}$. After dilution 1/100 in normal pooled plasma to give a concentration of 100 U mL$^{-1}$, further dilutions (1, 0.75, 0.5, 0.25, 0.12 and 0.05 U mL$^{-1}$) were prepared. Similarly, serial dilutions were made of a known concentration of fondaparinux (lot 68 exp 05/2009), 2.5 mg 0.5 mL$^{-1}$ diluting to 50 mg L$^{-1}$, and then to 1 mg L$^{-1}$ in normal pooled plasma; 1 mg mL$^{-1}$ fondparinux is equivalent to 0.85 anti-Xa U mL$^{-1}$. The CV for this assay is 8.1% (low) and 3.0% (high).

*Xa-clot assay*  The instrument usedfor this assay was the Amax 200 (Trinity Biotech, Bray Co. Wicklow, Ireland). The assay used was the Hemostasis Reference laboratory in-house assay; components are (Xa 0.275 IU mL$^{-1}$), AT, 0.025 M CaCl$_2$, normal plasma, 0.85% NaCl.

Based on the modified Hepaclot assay [12], the assay is performed with exogenous AT. Purified AT is added to the test plasma, forming an AT-drug complex. Following the addition of excess purified FXa the clotting time of the recalcified plasma sample is measured. The CV for this assay is 8.0% (low) and 4.4% (high).

*Endogenous thrombin potential*  The instrument used for endogenous thrombin potential was the BCS (Siemens, Marburg, Germany).

Current coagulation assays such as the prothrombin time (PT) and the aPTT measure the time at which fibrin first forms; however, at this stage only 1–2% of thrombin may have been formed. Thus, current coagulation tests do not take into account the kinetics of hemostatic pathways. The ETP assesses the overall coagulation status and takes into account procoagulant and anticoagulant factor activities. The ETP assay gives the opportunity to measure thrombin generation continuously by using a slow (low K$_m$ and K$_{cat}$) chromogenic substrate, with the parameters to calculate a thrombin gener-

ation curve. The ETP corresponds to the area under the thrombin generation curve (AUC) and represents the activity of thrombin multiplied by the time for which it remains active in plasma. ETP is measured by the conversion kinetics of a synthetic thrombin substrate, which measures the release of a chromophore into the plasma sample at a wavelength of 405 nm. Thrombin formation is activated by tissue factor pathway activation by the addition of a dilute PT reagent (Dade® Innovin®; Marburg, Germany) (0.6 µg) with calcium chloride. The substrate conversion kinetics are recorded by the 'BCS' system. A mathematical algorithm corrects for the activity of α$_2$-macroglobulin bound to thrombin. The corrected reaction curve corresponds to free thrombin kinetics, while the end level of the curve corresponds to the ETP value. The ETP value can also be derived from the AUC. The necessary calculations are performed automatically by the BCS system and the ETP calculations are analyzed using the Dade Behring *CURV*es software. The CV for this assay is 1.1% (normal) and 2.8% (abnormal).

*Statistical analyses*  Baseline characteristics and medications before and following randomization were compared between the groups using a chi-square test for categorical variables and a *t*-test for continuous variables. The baseline characteristics of patients included in this study were compared with the baseline characteristics of the remaining patients in the OASIS-5 study.

Baseline creatinine, aPTT and anti-Xa levels and 6-h anti-Xa, Xa clot time and ETP AUC levels were compared using *t*-tests. Variability of 6-h anti-Xa levels, 6-h Xa clot times and 6-h ETP AUC levels were compared by testing equality of variances. Correlation between age, creatinine levels and anti-Xa assays and ETP (AUC) was measured using a Pearson correlation coefficient.

We compared the effect of prerandomization treatment with UFH or LMWH on baseline aPTT and anti-Xa levels in the randomized treatment groups using a *t*-test. We performed a sensitivity analysis to explore the consistency of our results in patients who received prerandomization treatment with UFH or LMWH compared with those who did not receive either of these treatments.

A two-sided *P* value > 0.05 was considered statistically significant for all comparisons.

## Results

### Patient selection

The first 50 patients in each randomized treatment group in whom serial blood samples were collected were selected for inclusion in our analysis. Some of these patients did not have stored samples available at both baseline and 6 h, leaving 48 patients with samples in the fondaparinux arm and 42 patients with samples in the enoxaparin arm.

Three of the 90 patients had a creatinine clearance < 30 mL min$^{-1}$; two were randomized to receive enoxaparin and one to receive fondaparinux.

**Table 1** Baseline characteristics in the overall OASIS-5 study and in patients participating in the present study according to randomized treatment allocation

|  | OASIS 5 ($n$ = 20 078) | Enoxaparin ($n$ = 42) | Fondaparinux ($n$ = 48) | $P$-value |
|---|---|---|---|---|
| Age in years, mean (SD) | 66.6 (10.9) | 62.3 (13.2) | 64.6 (10.3) | 0.35 |
| *Creatinine, mean (SD) | 94.2 (29.6) | 71.6 (26.8) | 69.8 (17.9) | 0.70 |
| History of CV disease |  |  |  |  |
| MI, $n$ (%) | 5164 (25.7) | 15 (35.7) | 16 (33.3) | 0.81 |
| PCI, $n$ (%) | 2332 (11.6) | 13 (31.0) | 15 (31.3) | 0.98 |
| CABG, $n$ (%) | 1643 (8.2) | 11 (26.2) | 10 (20.8) | 0.55 |
| Any CAD, $n$ (%) | 6856 (34.1) | 11 (26.2) | 9 (18.8) | 0.40 |
| FH CAD, $n$ (%) | 4016 (20.0) | 9 (21.4) | 18 (37.5) | 0.10 |
| Stroke, $n$ (%) | 1244 (6.2) | 1 (2.4) | 0 | – |
| PAD | 1573 (7.8) | 5 (11.9) | 3 (6.3) | 0.35 |
| CV risk factors |  |  |  |  |
| Hypertension, $n$ (%) | 13498 (67.2) | 25 (59.5) | 37 (77.1) | 0.07 |
| Diabetes, $n$ (%) | 5078 (20.3) | 15 (35.7) | 15 (31.3) | 0.65 |
| History of cancer, $n$ (%) | 1184 (5.9) | 5 (11.9) | 10 (20.8) | 0.26 |

CABG, coronary artery bypass graft; CAD, coronary artery disease; CV, cardiovascular; FH CAD, family history of coronary artery disease; MI, myocardial infarction; PAD, peripheral arterial disease; PCI, percutaneous intervention.
*Creatinine units are $\mu$mol L$^{-1}$.

Twenty-six patients receiving enoxaparin and 28 patients receiving fondaparinux were pretreated prior to randomization with either UFH or LMWH. Five patients receiving enoxaparin and 11 patients receiving fondaparinux underwent percutaneous coronary intervention (PCI) within 6 h of randomization.

### Baseline characteristics

Baseline characteristics, including age and creatinine, of the patients in the fondaparinux and enoxaparin group were comparable and were similar to baseline characteristics of patients in the overall study (Table 1). The mean baseline aPTT in patients randomized to receive fondaparinux was 38.9 s and in those randomized to receive enoxaparin was 37.8 s. The mean baseline anti-Xa level in patients randomized to receive fondaparinux was 0.63 IU mL$^{-1}$ and in those randomized to receive enoxaparin was 0.69 IU mL$^{-1}$.

### Concomitant therapies

There were no significant differences between the fondaparinux and enoxaparin groups regarding the anticoagulant and antiplatelet medications prior to, and following, randomization. Prerandomization treatment with UFH or LMWH did not affect baseline aPTT or anti-Xa levels (data not presented) (Table 2).

### Laboratory results

*Anti-Xa levels:* Mean anti-Xa levels at 6 h were about 50% lower in patients assigned to fondaparinux compared with those assigned to receive enoxaparin [0.52 IU mL$^{-1}$ (SD 0.22 IU mL$^{-1}$) vs. 1.2 IU mL$^{-1}$ (SD 0.45 IU mL$^{-1}$), $P <$ 0.001]. There was significantly less variability of the anti-Xa

**Table 2** Pre- and postrandomization antithrombotic drug use

| Overall ($n$ = 20078) | Enoxaparin ($n$ = 42) | Fondaparinux ($n$ = 48) | $P$-value |
|---|---|---|---|
| Aspirin pre, $n$ (%) | 39 (92.9) | 42 (87.5) | 0.40 |
| Aspirin post, $n$ (%) | 40 (95.2) | 48 (100) | 0.13 |
| Clopidogrel pre, $n$ (%) | 15 (35.7) | 18 (37.5) | 0.86 |
| Clopidogrel post, $n$ (%) | 35 (83.3) | 40 (83.3) | 1.0 |
| GPIIb/IIIa pre, $n$ (%) | 9 (21.4) | 18 (37.5) | 0.10 |
| GPIIb/IIIa post, $n$ (%) | 22 (52.4) | 32 (66.7) | 0.17 |
| UFH pre, $n$ (%) | 2 (4.8) | 4 (8.3) | 0.50 |
| UFH post, $n$ (%) | 8 (19.0) | 17 (35.4) | 0.08 |
| LMWH pre, $n$ (%) | 24 (57.1) | 25 (52.1) | 0.63 |
| PCI < 6 h | 5 (11.9%) | 11 (22.9) | 0.11 |

GP IIb/IIIa, glycoprotein IIb/IIIa inhibitor; UFH, unfractionated heparin; LMWH, low-molecular-weight heparin.

inhibitory effect of fondaparinux compared with enoxaparin (Table 3, Fig. 1; $P <$ 0.001).

*Xa-clot assay results:* Patients in the fondaparinux arm had a significantly lower mean 6-h Xa clot time compared with patients in the enoxaparin arm [64.9 s (SD 17.7 s) vs. 111.8 s (SD 29.6 s), $P <$ 0.0001]. There was significantly less variability of the Xa clot time of fondaparinux compared with enoxaparin (Table 3, Fig. 1; $P <$ 0.001).

*Endogenous thrombin potential (ETP)* In the fondaparinux arm, analysis of the AUC at the 6-h sampling time provided a mean result of 386.7 mA (SD 51.5 mA). In the enoxaparin arm, the mean result was 206.4 mA (SD 90.6 mA, $P <$ 0.0001). There was significantly less variability of the ETP AUC of fondaparinux compared with enoxaparin (Table 3, Fig. 1; $P <$ 0.001).

*Results at 24 and 72 h* Anti-Xa concentrations, Xa clotting times, and areas under the curve for thrombin generation at 24 and 72 h are presented in Table 3. The contrast between the

two randomized treatment groups at 6 h remained evident at 24 and 72 h, although anti-Xa concentrations and Xa clotting times were lower, and areas under the curve for thrombin generation were higher. The latter finding is expected because the 6-h samples reflect peak drug levels, whereas the 24- and 72-h levels are more likely to be trough levels.

### Correlation between assays

There was a strong positive correlation between the anti-Xa level and Xa clot time ($r = 0.952$; $P < 0.0001$) and a strong negative correlation between anti-Xa levels and the ETP AUC ($r = -0.846$, $P < 0.0001$) at 6 h after the first dose of the study drug.

### Sensitivity analyses

Results at 6 h were consistent irrespective of whether patients received prerandomization treatment with UFH or LMWH [fondaparinux vs. enoxaparin: anti-Xa 0.49 IU mL$^{-1}$ (SD 0.24 IU mL$^{-1}$) vs. 1.22 IU mL$^{-1}$ (SD 0.50 IU mL$^{-1}$); $P < 0.0001$] or they did not receive any prerandomization anticoagulant treatment [fondaparinux vs. enoxaparin: anti-Xa 0.55 IU mL$^{-1}$ (SD 0.22 IU mL$^{-1}$) vs. 1.19 IU mL$^{-1}$ (SD 0.39 IU mL$^{-1}$); $P < 0.0001$].

### Discussion

Our results demonstrate that patients in the OASIS-5 trial receiving 2.5 mg fondaparinux subcutaneously once daily compared with enoxaparin 1 mg kg$^{-1}$ subcutaneously twice dailyhad approximately 50% of the peak anti-Xa concentration and Xa clotting time and significantly less inhibition of thrombin generation at 6 h after the first dose of the study drug. This difference remained evident at 24 and 74 h although absolute anti-Xa concentrations and Xa clotting times were lower and areas under the curve for thrombin generation were higher at later time points because the 6-h samples reflect peak levels whereas the 24- and 72-h samples reflect trough levels. Our results indicate that patients randomized to enoxaparin

compared with fondaparinux in the OASIS-5 trial received more intensive anticoagulation and provide an explanation for the increased bleeding observed in patients who received enoxaparin. Greater variability of the anticoagulant effect of enoxaparin compared with fondaparinux might also contribute to the increased risk of bleeding with enoxaparin because higher peaks are likely to be associated with more bleeding.

Fondaparinux, a synthetic pentasaccharide, acts by the selective AT-dependent inhibition of FXa; structurally, the pentasaccharide analogue is too short to bridge AT to thrombin (FIIa), and thus has no activity against FIIa. Enoxaparin, a LMWH produced by chemical depolymerization of UFH, also acts by AT-dependent inhibition of FXa, but in contrast to fondaparinux, some of the LMWH molecules have sufficient chain length to bridge AT to thrombin, thereby causing an anti-IIa effect [13]. The higher intensity of anticoagulation produced by the anti-Xa and anti-IIa effect of enoxaparin compared with the anti-Xa effect of fondaparinux is reflected by substantially greater suppression of ETP.

The more predictable anticoagulant effect of fondaparinux compared with enoxaparin may reflect the differing propensities of fondaparinux and enoxaparin to bind non-specifically to plasma proteins [14]. Non-specific protein binding of UFH molecules to plasma proteins accounts for its unpredictable anticoagulant effect. Although, compared with UFH, the shorter chain lengths of LMWH are associated with reduced non-specific binding and a more predictable anticoagulant response, non-specific binding of LMWH still occurs, accounting for some variability in anticoagulant effect. In contrast, fondaparinux binds selectively to AT, providing a highly predictable bioavailability, and our findings demonstrate that this is associated with a more predictable anticoagulant effect than LMWH.

The dose of fondaparinux used in the OASIS-5 trial was selected on the basis of previous dose-finding studies [15–17], which showed an optimal efficacy-safety profile at a relatively low dose. Similar dose-finding studies have not been performed for any of the LMWH preparations currently used in the management of ACS. An even higher dose of enoxaparin than is currently used was tested in the phase II Thrombolysis in

**Table 3** Anti-Xa levels, Xa clot times, and endogenous thrombin potential 6, 24 and 72 h after initial study drug administration

| | Enoxaparin ($n = 42$) | | Fondaparinux ($n = 48$) | | |
| --- | --- | --- | --- | --- | --- |
| | Mean | SD | Mean | SD | *P*-value |
| 6-h anti-Xa (IU mL$^{-1}$) | 1.2 | 0.45* | 0.5 | 0.2* | < 0.0001 |
| 6-h Xa-clot (seconds) | 111.8 | 29.6* | 64.9 | 17.7* | < 0.001 |
| 6-h ETP AUC (mA) | 206.4 | 90.6* | 386.7 | 51.5* | < 0.001 |
| 24-h anti-Xa (IU mL$^{-1}$) | 0.8 | 0.3 | 0.2 | 0.2 | < 0.0001 |
| 24-h Xa-clot (seconds) | 79.6 | 20.3 | 46.2 | 10.6 | < 0.0001 |
| 24-h ETP AUC (mA) | 264.4 | 138.8 | 422.9 | 63.1 | < 0.0001 |
| 72-h anti-Xa (IU mL$^{-1}$) | 0.6 | 0.4 | 0.3 | 0.1 | 0.002 |
| 72-h Xa-clot (seconds) | 65.5 | 27.8 | 48.1 | 9.0 | 0.006 |
| 72-h ETP AUC (mA) | 310.9 | 132.2 | 425.7 | 45.7 | < 0.001 |

ETP AUC, endogenous thrombin potential area under the curve.
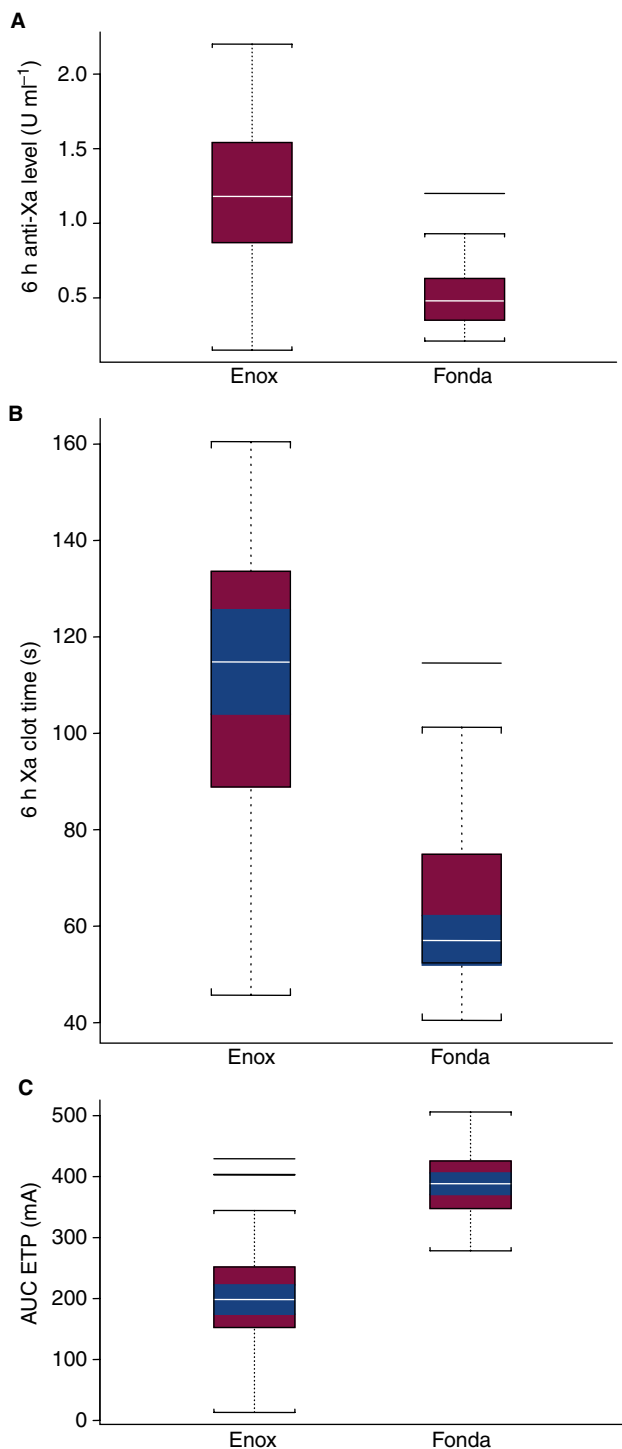*Test for equality of variances: $P < 0.001$ for each comparison.

**Fig. 1.** Box and whisker plots showing (A) anti-Xa levels (IU mL$^{-1}$); (B) Xa clot times (s); (C) AUC ETP (mA) for enoxaparin and fondaparinux. The boxes represent the first to third quartile and the whiskers represent the upper and lower extremes of the results. Outliers are represented by horizontal lines above or below the upper and lower extremes of the results. The horizontal line in the box represents the median.

Myocardial Infarction (TIMI)-11A Study but the higher dosing regimen was abandoned because of excessive bleeding [18].

Preservation of efficacy during treatment with less intensive anticoagulant therapy with fondaparinux compared with enoxaparin raises the possibility that lower doses of enoxaparin might produce less bleeding without compromising antithrombotic efficacy. Consistent with this hypothesis, a recent retrospective case series of 1400 patients with ACS treated with aspirin and clopidogrel reported that enoxaparin given at a dose of 0.5 mg kg$^{-1}$ every 12 h prior to PCI and 0.3–0.5 mg kg$^{-1}$ thereafter was safe and efficacious [19]. However, the efficacy and safety of reduced intensity enoxaparin therapy compared with presently approved dosing regimens remains unproven.

Our study has several potential limitations. First, we studied a small subset of patients enrolled in the OASIS-5 trial and were unable to include patients who did not have a blood sample collected at 6 h. However, the baseline characteristics of patients included in our study were comparable with those of the overall OASIS-5 study population and the differences that we observed were very large. It is therefore extremely unlikely that minor differences in baseline characteristics could explain our results. Second, our analyses included patients who underwent PCI prior to the 6-h blood collection. However, this would not have affected our conclusions because the OASIS-5 protocol recommended the administration of additional fondaparinux in patients who underwent PCI within 6 h of administration of fondaparinux (except in those receiving a glycoprotein IIb/IIIa inhibitor) but did not recommend any additional enoxaparin in patients who underwent PCI within 6 h of enoxaparin. A subsequent protocol amendment enabled investigators to add open label heparin at the time of PCI but a higher proportion of patients randomized to fondaparinux compared with enoxaparin received additional UFH. Both of the aforementioned strategies would reduce the contrast between the two treatment groups. A further limitation of our study is that some patients received UFH or LMWH prior to randomization but a sensitivity analysis confirmed that our results were consistent irrespective of whether patients received, or did not receive, prerandomization treatment with UFH or LMWH. Finally, although our results provide a plausible explanation for differences in the safety and efficacy of fondaparinux compared with enoxaparin they do not prove that lower intensity and more predictable anticoagulant effects of fondaparinux compared with enoxaparin account for the findings of the OASIS-5 trial.

In conclusion, our results suggest that the lower rates of bleeding and improved efficacy of fondaparinux compared with enoxaparin in the OASIS-5 trial may be explained by a less intense and more predictable anticoagulant effect of fondaparinux. Lower doses of anticoagulant than used in the past may be sufficient to prevent recurrent ischemic events and death in patients with ACS who are concurrently treated with aspirin and clopidogrel. Determining the optimal balance for anticoagulant intensity, whereby antithrombotic efficacy is maximized and bleeding is minimized, is important because both reduced ischemic events and bleeding translate into improved long-term outcomes.

## Addendum

## Acknowledgement

## Disclosure of Conflict of Interests

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