

Bioequivalence of a biosimilar enoxaparin sodium to Clexane[®] after single 100 mg subcutaneous dose: results of a randomized, double-blind, crossover study in healthy volunteers

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Purpose: To demonstrate the pharmacokinetic/pharmacodynamic (PK/PD) equivalence of a biosimilar enoxaparin to the reference drug, and to assess its safety and tolerability in healthy volunteers.

Patients and methods: A randomized, double-blind, crossover, 2-sequence, single-dose study was conducted in healthy volunteers of both sexes. Participants were sequentially and randomly administered single subcutaneous injections of enoxaparin 100 mg manufactured by Rovi (test; Madrid, Spain) and Clexane[®] (enoxaparin 100 mg manufactured by Sanofi, reference) separated by a 1-week washout period. The primary PK/PD variables were maximum activity (A_{\max}) and area under the effect curve from time 0 to the last measured activity (T) ($AUEC_{0-T}$) and $AUEC_{0-\infty}$ of anti-FXa activity, and A_{\max} and $AUEC_{0-T}$ of anti-FIIa activity. Secondary variables were A_{\max} and $AUEC_{0-T}$, $AUEC_{0-\infty}$ of tissue factor pathway inhibitor, and the ratio of $AUEC_{0-T}$ anti-FXa to anti-FIIa activity. Biosimilarity would be shown when the 95% CI of the ratio of geometric least squares means (95% CI RGLSMs) of primary PK/PD parameters fell within the standard range of bioequivalence, ie, 80%–125%.

Results: The study sample consisted of 46 volunteers (33 males) aged 18–44 years and with body mass index ranging from 19.0 to 31.1 kg/m². Three subjects did not complete the study. The curves of anti-FXa, anti-FIIa and tissue factor pathway inhibitor activities corresponding to administration of the test and reference products were comparable. The 95% CI RGLSMs of A_{\max} , $AUEC_{0-T}$ and $AUEC_{0-\infty}$ for anti-FXa activity were 94.6%–105.9%, 99.8%–108.0% and 100.0%–108.6% respectively; A_{\max} and $AUEC_{0-T}$ for anti-FIIa activity were 94.7%–112.6% and 90.9%–117.9% respectively. In addition, the 95% CI RGLSMs of all secondary variables fell within the range 80%–125%. The incidence and types of adverse events after administration of the test and reference drugs were similar.

Conclusion: The results conclusively showed that the enoxaparin manufactured by Rovi is equivalent to the reference enoxaparin in all primary and secondary PK/PD parameters, as required by the European Medicines Agency to grant marketing authorization to a biosimilar low molecular-weight heparin.

Keywords: biosimilar, LMWH, pharmacodynamics, pharmacokinetics, anti-FXa, anti-FIIa, TFPI

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Introduction

Low molecular-weight heparins (LMWHs) are derived from unfractionated heparin (UFH) by chemical or enzymatic depolymerization. LMWHs have reduced inhibitory activity against thrombin (FIIa) compared to factor Xa (FXa), a more favorable

benefit–risk ratio than heparin, and superior pharmacokinetic (PK)/pharmacodynamic (PD) properties.¹ Enoxaparin is one of the most widely used LMWHs and is obtained by alkaline depolymerization of heparin benzyl ester derived from porcine intestinal mucosa. In contrast to UFH, enoxaparin has a higher ratio of anti-FXa to anti-FIIa activity, more consistent release of tissue factor pathway inhibitor (TFPI), weaker interactions with platelets, and less inhibition of bone formation; enoxaparin also has higher and more consistent bioavailability after subcutaneous (SC) administration compared with UFH and longer plasma half-life, and is less strongly bound to plasma proteins. These properties translate into a more reliable anticoagulant effect without the need for laboratory monitoring, and safety and efficacy have been well established in a wide range of both arterial and venous thromboembolic conditions.^{2,3}

The currently approved originator LMWHs differ in their PK/PD properties. Because of difficulties in physical detection of LMWH, conventional PK studies cannot be performed. LMWH absorption and elimination are instead studied using PD surrogate markers, most importantly anti-FXa and anti-FIIa activity. To compare the biosimilar/generic version to the reference LMWH, both the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) recommend measurement of these PD activities;^{4,5} moreover, measurement of the ratio of anti-FXa to anti-FIIa activity and the TFPI activity are also recommended by the EMA as secondary parameters.⁴ These PD properties should be investigated in a randomized, single-dose, 2-way crossover and preferably double-blind study in healthy volunteers using SC administration. Accordingly, this article reports the design and main results of a double-blind, randomized clinical trial comparing the PK/PD properties of a new biosimilar enoxaparin and its originator.

Materials and methods

Study design

This was a single-dose, randomized, double-blind, 2-period, 2-sequence crossover study conducted at the PRA Health Sciences' Clinical Research Unit, Zuidlaren, the Netherlands. The clinical study protocol and the informed consent forms were reviewed and approved by the national competent authority and the independent ethics committee of the Foundation "Beoordeling Ethiek Biomedisch Onderzoek" (Assen, the Netherlands). The study was carried out in accordance with the principles of the Declaration of Helsinki, the International Conference on Harmonisation E6 Guideline for

Good Clinical Practice, and the European Union Clinical Trial Directive 2001/20/EC.

All subjects provided written informed consent before study participation. They were screened up to 30 days before first administration of the study drug and were admitted to the clinic on Day 1 of Period 1 for baseline assessments. Subjects were subsequently randomized to one of the treatment sequences (test–reference or reference–test). The test drug was enoxaparin sodium 100 mg (10,000 IU anti-FXa/1.0 mL), manufactured by Rovi, Madrid, Spain; the reference drug was enoxaparin sodium 100 mg (Clexane® 10,000 IU anti-FXa/1.0 mL), manufactured by Sanofi, Maisson Alfort, France. Both drugs were similar in appearance and were supplied as pre-filled syringes containing a sterile, clear, colorless-to-pale yellow aqueous solution for SC injection. To ensure blinding, an unblinded pharmacist was responsible for dispensing the study drug, and a dedicated, unblinded team member administered the study drug.

On Day 1 of Period 1, subjects were given, in fasting conditions, a single SC injection of the test or the reference drug. Subjects returned to the clinic after a washout period of at least 7 days, and on Day 1 of Period 2, they were crossed over to receive a single SC dose of the reference or the test drug. Blood samples to assess PD parameters were collected in both study periods at the following time points: pre-dose, and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 20, 24, and 36 hours after dosing on Day 1. (EudraCT#2015-003489-10; [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03363477) NCT03363477).

Subjects

Healthy volunteers of both sexes aged 18–45 years were enrolled in the study after they had given informed consent. Subjects had no clinically significant abnormalities based on medical history, clinical laboratory results, vital sign measurements, 12-lead electrocardiogram (ECG) results, and physical examination findings. Exclusion criteria included body weight below 45 or 57 kg if female or male, respectively; calculated (Cockcroft & Gault formula) creatinine clearance <80 mL/minute; history of or positive test result for alcohol abuse or drug addiction; history of relevant drug and/or food allergies; any prescription drugs (with special attention to antiplatelet or anticoagulant drugs) or over-the-counter medications that could affect coagulation within 4 weeks of dosing; administration of any investigational drug within 60 days of the first dose of the study drug; a positive test for the human immunodeficiency virus (1 or 2) antibody, hepatitis B surface antigen, or hepatitis C virus antibody; a positive fecal occult blood test at screening; history and/or

current conditions of bleeding tendency; history of thrombocytopenia, including heparin-induced thrombocytopenia; known history of hypersensitivity to drugs with a chemical structure similar to enoxaparin sodium (eg, UFH, LMWH) or to pork products.

PK/PD assessments

Due to difficulties in measuring plasma LMWH levels, the following plasma PD surrogate parameters were calculated for anti-FXa and anti-FIIa activity, and baseline-adjusted TFPI levels using noncompartmental analysis: area under the effect curve (AUEC) from time 0 to infinity ($AUEC_{0-\infty}$); AUEC from time 0 to the last measured activity (T) ($AUEC_{0-T}$); maximum activity (A_{max}) observed for anti-FXa and anti-FIIa, and baseline-corrected TFPI levels; time to observed maximum measured plasma activity; ratio of $AUEC_{0-T}$ of anti-FXa to anti-FIIa (R_{AUEC}); apparent first-order terminal elimination half-life, apparent plasma clearance after extravascular administration (CL/F), and mean residence time (MRT), were also estimated (only if appropriate data permit it). PK/PD parameters were calculated using Phoenix Win-Nonlin Version 6.3 (Certara Inc., Princeton, NJ, USA).

The analytical method for the PD assays was developed and validated (good laboratory practice [GLP] compliant) at Anapharm Europe (Barcelona, Spain). Blood samples were collected into blood collection tubes containing citrate, theophylline, adenosine, and dipyridamole to assess anti-FIIa and anti-FXa activity, or with sodium citrate (3.2%) for TFPI assessment. Anti-FXa activity was determined by a chromogenic method using a commercial kit (Biophen Heparin Anti-Xa, ref no A221010, Aniara, West Chester, OH, USA); the anti-FXa calibration curve ranged from 0.075 to 0.481 IU/mL, with a lower limit of quantitation (LLOQ) of 0.075 IU/mL. The inter-run accuracy and precision of 3 levels of quality controls ranged between 94.3%–99.8% and 8.3%–10.7%, respectively. Anti-FIIa activity was measured by a chromogenic method using a commercial kit (Biophen Heparin Anti-IIa, ref no A221025, Aniara); the anti-FIIa calibration curve ranged from 0.050 to 0.481 IU/mL, with an LLOQ of 0.050 IU/mL. The inter-run accuracy and precision of 3 levels of quality controls ranged between 96.3%–103.0% and 14.5%–17.3%, respectively. TFPI levels were measured using a commercial enzyme-linked immunosorbent assay (Human TFPI ELISA kit, ref no Ab108904, Abcam PLC, Cambridge, UK); the TFPI calibration curve ranged from 0.156 to 10.000 ng/mL, with an LLOQ of 0.156 ng/mL. The inter-run accuracy and precision of 3 levels of quality controls ranged between 91.2%–98.4% and 8.9%–14.1%, respectively.

Safety assessments

All adverse events (AEs) were coded using the MedDRA classification system, and frequency of treatment-emergent AEs (TEAEs) was listed by system organ class and preferred term. TEAEs, severity, and relationship of TEAEs to study drug, treatment-emergent serious AEs, and TEAEs leading to study drug discontinuation were summarized for each treatment group and overall.

Statistical methods

Assuming a within-subject coefficient of variation <18%,^{6,7} 40 subjects completing the study would provide at least 80% power to conclude biosimilarity, that is, PD equivalence, between the test and the reference enoxaparin compounds. To allow for dropouts, 46 subjects had to be enrolled.

The PD parameters $AUEC_{0-\infty}$, $AUEC_{0-T}$, and A_{max} for anti-FXa and baseline-adjusted TFPI levels as well as $AUEC_{0-T}$ and A_{max} for anti-FIIa activity, from the test treatment were compared with those from the reference treatment. An analysis of variance with fixed effects for sequence, period, and treatment, and random effect for subject nested within sequence was performed on the natural logarithms of $AUEC_{0-\infty}$, $AUEC_{0-T}$, and A_{max} for anti-FXa and baseline-adjusted TFPI levels, and the natural logarithms of $AUEC_{0-T}$ and A_{max} for anti-FIIa activity, to assess the differences between the test and reference treatments. The geometric mean ratio and 95% CI for $AUEC_{0-\infty}$, $AUEC_{0-T}$, and A_{max} for anti-FXa and TFPI, and for $AUEC_{0-T}$ and A_{max} for anti-FXa of both treatments were calculated by the antilog of the mean difference and 95% CI of the log-transformed values. Since TFPI levels can be physiologically detected in human blood, for PD assessments in each period, the value of the baseline TFPI measurement (on Day 1) was subtracted from the Day 1 pre-dose and all post-dose measurements of TFPI; these were the baseline-adjusted values used in the analysis, with the exception that any negative result was set to zero.

PD equivalence would be concluded if the 95% CI of the ratio of the geometric least squares means (95% CI RGLSMs) between the test and the reference treatments for $AUEC_{0-\infty}$, $AUEC_{0-T}$, and A_{max} of anti-FXa activity, and the $AUEC_{0-T}$ and A_{max} of anti-FIIa activity were completely within the standard 80.00%–125.00% interval.

In addition, $AUEC_{0-\infty}$, $AUEC_{0-T}$, and A_{max} of baseline-adjusted TFPI activity, as well as the ratio of the $AUEC_{0-T}$ of anti-FXa activity to anti-FIIa activity, were considered as supportive secondary PD parameters for the biosimilarity assessment. Therefore, it was also assessed whether the

95% CI RGLSMs between the test and the reference treatments fell within the 80.00%–125.00% interval.

Results

A total of 90 subjects were screened, of whom 46 were randomized and dosed in the study. Mean (SD) age was 26 (6) years (range, 18–44 years), and mean (SD) body mass index (BMI) was 23.8 (2.8) kg/m² (range, 19.0–31.1 kg/m²). Thirty-three (72%) subjects were male and 39 (85%) Caucasian. Forty-four subjects participated in both treatment periods (2 subjects received only 1 of the study drugs), 43 subjects completed the study according to the protocol and 3 subjects were withdrawn during the study: 1 withdrew consent, 1 because of a protocol deviation and 1 due to AEs (Figure 1). The latter one presented syncope, dizziness, headache, pharyngitis, and pyrexia the day before study drug administration of period 2, and all these AEs were considered by the investigator not to be related to the study drug. The PD analysis set consisted of 43 subjects.

Following administration of a single SC dose of either the test or the reference enoxaparin compounds, mean peak anti-FXa and anti-FIIa activities were seen after ~4 and 5 hours respectively. Thereafter, mean plasma anti-FXa and anti-FIIa activities showed an initial rapid drop from peak

activity followed by a slower decline. Mean peak baseline-adjusted TFPI levels were reached within ~1 hour after both treatments. Mean plasma TFPI levels subsequently showed an initial rapid drop from peak levels, followed by a slower decline. The shapes of plasma anti-FXa and anti-FIIa activity, and the profiles of plasma TFPI levels were similar for both enoxaparin compounds (Figures 2 and 3).

When the test and reference drugs were compared, results showed that the 95% CI RGLSMs of anti-FXa activity for the PD parameters A_{\max} , $AUEC_{0-T}$, and $AUEC_{0-inf}$ were 94.6%–105.9%, 99.8%–108.0%, and 100.0%–108.6%, respectively, and were therefore well within the equivalence interval of 80.00%–125.00% (Table 1).

Similarly, the 95% CI RGLSMs of anti-FIIa activities for A_{\max} and $AUEC_{0-T}$ were 94.7%–112.6% and 90.9%–117.9%, respectively. Thus, they also met the acceptance criterion of 80.00%–125.00% (Table 2).

In addition, the 95% CI RGLSMs for the R_{AUEC} and of baseline-adjusted TFPI levels for A_{\max} , $AUEC_{0-T}$, and $AUEC_{0-inf}$ also fell within the aforementioned equivalence interval (Tables 3 and 4).

The overall incidence rates and types of TEAEs were similar between the test and the reference enoxaparin compounds. No overall trends or clinically relevant changes were seen in clinical laboratory parameters, vital signs, ECGs, or physical examinations.

Discussion

This clinical trial was specifically aimed at showing the PD bioequivalence of a new biosimilar LMWH, enoxaparin sodium manufactured by Rovi, to the reference medicinal product. It was designed in accordance to the relevant EMA guideline⁴ and consisted of a randomized, double-blind, single-dose, 2-way crossover study in healthy volunteers using SC administration. Anti-FXa and anti-FIIa activity was selected as the primary PD endpoint, and the ratio of anti-FXa to anti-FIIa activity, and TFPI activity were secondary endpoints. The FDA guidance on enoxaparin sodium⁵ was also taken into consideration for some aspects of the study protocol. The bioanalytical methods for quantitative measurement of anti-FXa, anti-FIIa, and TFPI activity in human plasma were duly validated and fully GLP compliant.

Our study has several strengths. Its double-blind design decreases potential bias during dosing, data collection, and evaluation of safety variables. The washout period of at least 7 days between study drug administrations in each period is an adequate margin to avoid any carry-over effect,

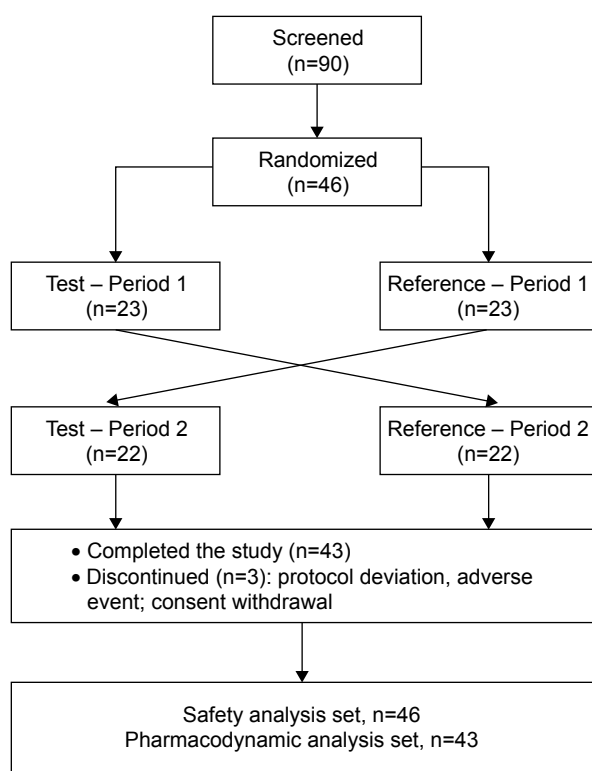


Figure 1 Study flow chart and subject disposition.

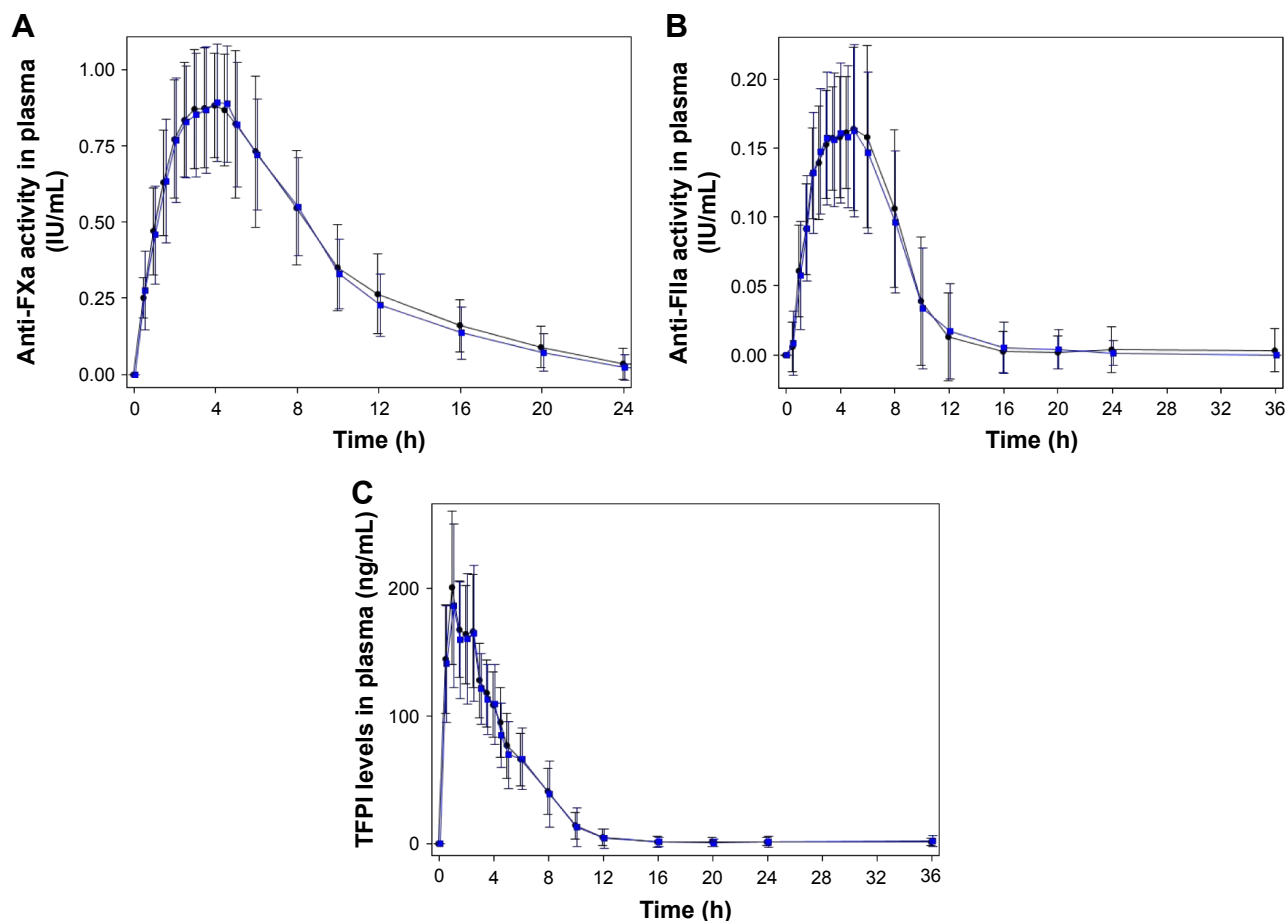


Figure 2 Mean (SD) plasma anti-FXa (A) and anti-FIIa (B) activity, and baseline-adjusted TFPI levels (C) versus time curves (linear scale) after fasting of single doses of the test or reference drug.

Notes: Test drug is enoxaparin sodium 100 mg (10,000 IU anti-FXa/1.0 mL) manufactured by Rovi, Madrid, Spain (●). Reference drug is enoxaparin sodium 100 mg (Clexane® 10,000 IU anti-FXa/1.0 mL) manufactured by Sanofi, Maison Alfort, France (■). Data shown from PK/PD analysis set (n=43).

Abbreviations: PD, pharmacodynamic; PK, pharmacokinetic; SC, subcutaneous; TFPI, tissue factor pathway inhibitor.

considering the known elimination half-life based on anti Xa activity and the “five half-lives” criterion. The 80%–125% interval is also appropriate because this is the most widely accepted interval for investigation of bioequivalence, not only for chemical medicines⁸ but also for enoxaparin.^{5–7,9,10} Moreover, bioequivalence had to be concluded considering the more stringent 95% CI RGLSMs between the test and reference treatments, as requested by the EMA.^{9,10} The 95% CI RGLSMs between the test and reference drugs for all PD parameters were within an even narrower interval, from 87.9% to 117.9%. Moreover, all 3 PD parameters of anti-FXa activity ($AUEC_{0-inf}$, $AUEC_{0-T}$, and A_{max}) fell within the tighter margin recommended for “narrow therapeutic index drugs”, that is, 90%–111.11%,⁸ which is very relevant because anti-FXa activity is the main factor that correlates with the expected efficacy and safety of enoxaparin. It should also be noted that bioequivalence was reached in both primary and secondary parameters. By contrast, in the pivotal PD

bioequivalence study of 2 new biosimilar medicinal products of enoxaparin recently authorized by the European authorities, the secondary endpoints AUC_{0-T} , AUC_{0-inf} , and A_{max} of TFPI did not meet the equivalence criteria,^{9,10} which were the same as established in our study. Similarly, in a previously reported bioequivalence study with a generic version of enoxaparin, the CI of the AUC_{0-T} (75%–140%) exceeded the limits at both ends of the accepted criterion of 80%–125%.⁷ TFPI indicates biological activity in the vascular endothelium, which is different for each LMWH, and this secondary parameter may therefore add information on the effect of the tested product, as differences in TFPI release/activity may result from structural differences in higher molecular weight saccharide chains.^{9,10} However, these differences do not seem to have an impact on the proposed dose regimen of those biosimilar LMWHs, probably because regulatory authorities consider more clinically relevant the anti-Xa and anti-IIa activity.

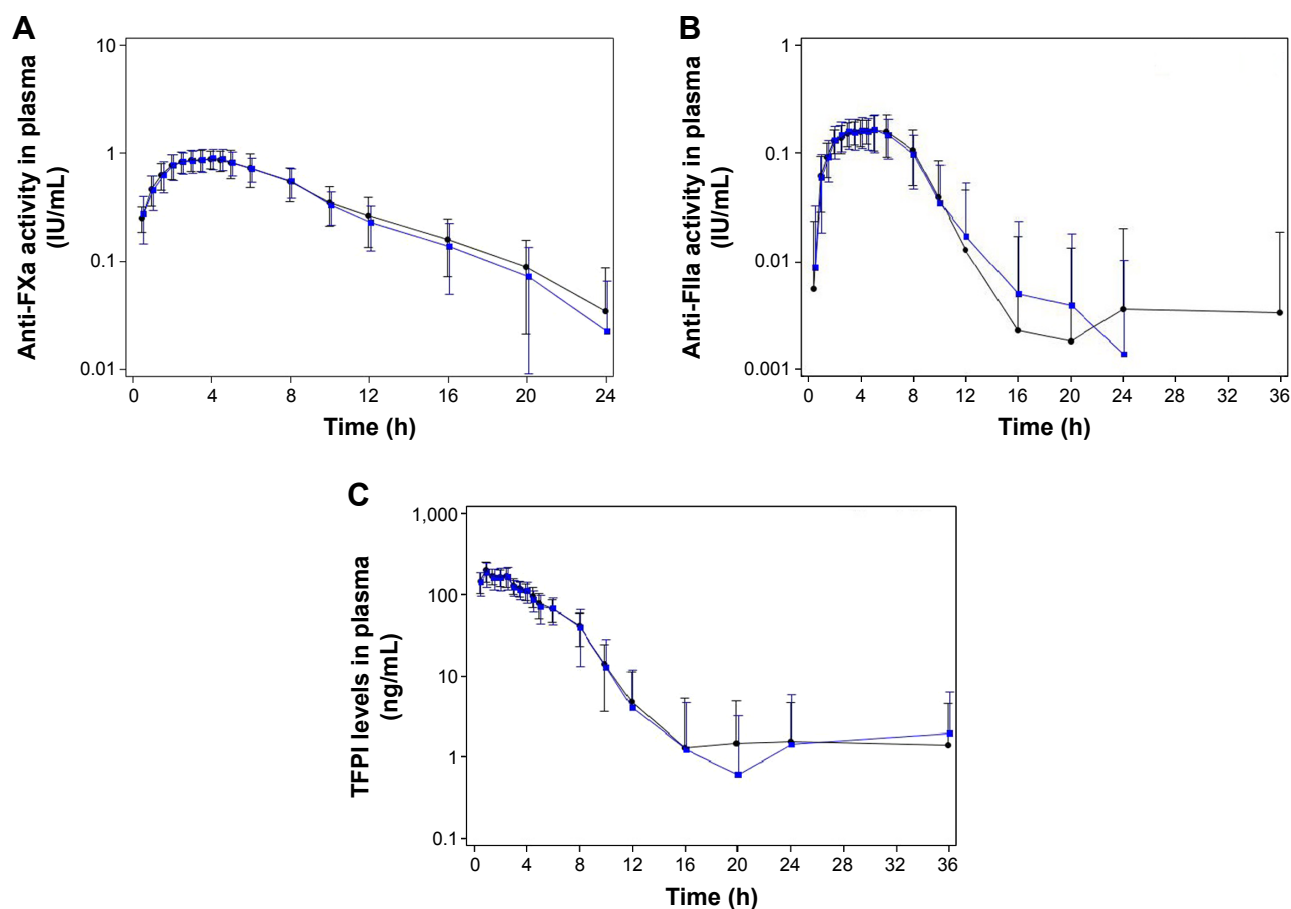


Figure 3 Mean (SD) plasma anti-FXa (A) and anti-FIIa (B) activity, and baseline-adjusted TFPI levels (C) versus time curves (semi-logarithmic scale) after fasting SC administration of single doses of the test or reference drug.

Notes: Test drug is enoxaparin sodium 100 mg (10,000 IU anti-FXa/1.0 mL) manufactured by Rovi, Madrid, Spain (●). Reference drug is enoxaparin sodium 100 mg (Clexane® 10,000 IU anti-FXa/1.0 mL) manufactured by Sanofi, Maison Alfort, France (■). Data shown from PK/PD analysis set (n=43).

Abbreviations: PD, pharmacodynamic; PK, pharmacokinetic; SC, subcutaneous; TFPI, tissue factor pathway inhibitor.

On the other hand, our study may have some limitations. Several previously reported bioequivalence studies of generic/biosimilar enoxaparin compounds used lower dose strengths.^{6,7,9,10} However, a 100 mg dose of enoxaparin has been found to have effects independent of changes in body weight^{5,11} and provides a significant number of measurable plasma samples above the LLOQ, which allows for accurate

characterization of the terminal elimination characteristics of the molecules; therefore, the dose selected was in the sensitive part of the dose-response curve, as stated in European guidelines.⁴ Besides, the 100 mg strength is the enoxaparin dose recommended by the FDA for PD bioequivalence purposes.⁵ In addition, the intravenous route was not tested, despite the fact that enoxaparin may be used to treat acute

Table 1 Statistical analysis of plasma PK/PD parameters for anti-FXa activity (PD set)

Parameter	Treatment	Geometric LS means	Treatment comparison	Estimate	95% CI (lower–upper)	CV (%)
A_{max} (IU/mL)	Test	0.974	Test/reference	100.1	94.6–105.9	13.0
	Reference	0.973				
AUEC _{0–T} (h×IU/mL)	Test	8.05	Test/reference	103.8	99.8–108.0	9.1
	Reference	7.75				
AUEC _{0–inf} (h×IU/mL)	Test	8.91	Test/reference	104.2	100.0–108.6	8.8
	Reference	8.55				

Note: A linear mixed effects model was applied to the natural log-transformed PD parameters with sequence, period, and treatment as fixed effects, and subject nested within sequence as a random effect.

Abbreviations: A_{max} , maximum activity observed; AUEC_{0–T}, area under the effect curve from time 0 to the last measured activity (T); AUEC_{0–inf}, AUEC from time 0 to infinity; CV, coefficient of variation; LS, least squares; PK, pharmacokinetic; PD, pharmacodynamic.

Table 2 Statistical analysis of plasma PK/PD parameters for anti-FIIa activity (PD set)

Parameter	Treatment	Geometric LS means	Treatment comparison	Estimate	95% CI (lower-upper)	CV (%)
A_{\max} (IU/mL)	Test	0.181	Test/reference	103.3	94.7–112.6	20.2
	Reference	0.175				
AUEC _{0-T} (h×IU/mL)	Test	1.08	Test/reference	103.5	90.9–117.9	30.5
	Reference	1.04				

Note: A linear mixed effects model was applied to the natural log-transformed PD parameters with sequence, period, and treatment as fixed effects and subject nested within sequence as a random effect.

Abbreviations: A_{\max} , maximum activity observed; AUEC_{0-T}, area under the effect curve from time 0 to the last measured activity (T); CV, coefficient of variation; LS, least squares; PK, pharmacokinetic; PD, pharmacodynamic.

ST-segment elevation myocardial infarction. However, SC administration covers both LMWH absorption and elimination, and SC PD data are therefore considered to be more sensitive. This is the reason why both European and US health authorities do not require additional pharmacological studies for intravenous or intra-arterial use.^{4,5,9,10}

Another interesting point to be discussed here is the clinical and regulatory implications of this study. Both European and US health authorities currently support the demonstration of biosimilarity/bioequivalence of a biosimilar/generic LMWH based on a strong and convincing physicochemical and functional data package and on comparable PD profiles. A dedicated comparative efficacy trial is therefore not considered necessary.^{4,12} Indeed, efficacy trials do not seem to have enough sensitivity or statistical power to detect differences in clinical endpoints, as they have never been able to detect differences between different LMWHs with evident differences in PK/PD and anti-FXa activity.¹³ This PD/PK study has therefore been used as pivotal clinical evidence for requesting marketing authorization in Europe of the biosimilar enoxaparin manufactured by Rovi through a decentralized procedure, which has recently ended with a positive outcome.¹⁴

Finally, it is worth commenting about the current debate on switching from an original biological medicine to its biosimilar. The FDA has approved several generic versions of enoxaparin under the abbreviated new drug application (ANDA) pathway, which implies that the interchangeability of the branded enoxaparin with its generic version is fully

allowed in the USA.¹⁵ Conversely, EMA considers LMWHs as biological medicines and does not regulate interchangeability, switching, and substitution of a reference medicine by its biosimilar, leaving this decision at the national level.¹⁶ Up to now, several national regulatory authorities, including the Dutch Medicines Evaluation Board, the Finnish Medicines Agency, Healthcare Improvement Scotland, the Irish Health Products Regulatory Authority, and Paul Ehrlich Institute in Germany, have already taken national positions to endorse the interchangeability of biosimilars under the supervision of the prescriber.¹⁷ Indeed, a recent questionnaire-based survey, conducted between November 2016 and January 2017 among experts from several Central and Eastern European countries, showed that substitution and interchangeability of original biologic drugs and their corresponding biosimilars were generally allowed, although in most countries that decision was taken at the discretion of the physician after a clinical assessment and the biosimilars were usually in the same homogeneous group, and internal reference pricing was usually employed.¹⁸

Conclusion

The results of this clinical trial conclusively showed that the enoxaparin manufactured by Rovi is equivalent to the reference enoxaparin in all primary (anti-FXa and anti-FIIa activity) and secondary (ratio of anti-FXa to anti-FIIa and TFPI activity) PK/PD parameters, as required by the EMA to authorize commercialization of a biosimilar LMWH. The overall safety and tolerability profiles of both medicinal products were similar.

Table 3 Statistical analysis of plasma PK/PD parameter R_{AUECs} for anti-FXa/anti-FIIa activity (PD set)

Parameter	Treatment	Geometric LS means	Treatment comparison	Estimate	95% CI (lower-upper)	CV (%)
R_{AUEC}	Test	7.46	Test/reference	100.3	87.9–114.5	31.1
	Reference	7.44				

Note: A linear mixed effects model was applied to the natural log-transformed PD parameters with sequence, period, and treatment as fixed effects and subject nested within sequence as a random effect.

Abbreviations: AUEC, area under the effect curve; CV, coefficient of variation; LS, least squares; PK, pharmacokinetic; PD, pharmacodynamic; R_{AUEC} , ratio of AUEC_{0-T} of anti-FXa activity to anti-FIIa activity.

Table 4 Statistical analysis of plasma PK/PD parameters for baseline-adjusted TFPI levels (PD set)

Parameter	Treatment	Geometric LS means	Treatment comparison	Estimate	95% CI (lower-upper)	CV (%)
A_{\max} (ng/mL)	Test	207	Test/reference	104.1	95.6–113.4	19.9
	Reference	199				
AUEC _{0-T} (h×ng/mL)	Test	913	Test/reference	105.9	99.1–113.1	15.2
	Reference	863				
AUEC _{0-inf} (h×ng/mL)	Test	910	Test/reference	108.4	102.1–115.2	9.0
	Reference	839				

Note: A linear mixed effects model was applied to the natural log-transformed PK/PD parameters with sequence, period, and treatment as fixed effects and subject nested within sequence as a random effect.

Abbreviations: A_{\max} , maximum activity observed; AUEC_{0-T}, area under the effect curve from time 0 to the last measured activity (T); AUEC_{0-inf}, AUEC from time 0 to infinity; CV, coefficient of variation; LS, least squares; PK, pharmacokinetic; PD, pharmacodynamic; TFPI, tissue factor pathway inhibitor.

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Disclosure

All authors are employees of Laboratorios Farmacéuticos Rovi, S.A. The authors report no other conflicts of interest in this work.

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