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Pharmacodynamic Equivalence of Ovine Enoxaparin to Porcine Enoxaparin (Lovenox®) In Healthy Volunteers: A Randomized, Open-Label, 2-Way Cross-Over, Single Dose Study

Setiawati A*[1,2], Dharma R[3]

[1]Clinical Research Supporting Unit, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[2]Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[3]Department of Clinical Pathology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

***Corresponding Author:** Setiawati A, Clinical Research Supporting Unit, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya 6, Jakarta 10430, Indonesia. Tel: +62-813-1153-2528, Email: arinisetiawati@yahoo.com

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Abstract

Aim: To demonstrate the PK/PD equivalence of an ovine enoxaparin to the reference product, the originator porcine enoxaparin, Lovenox® from Sanofi, and to assess its safety and tolerability in healthy volunteers with s.c. administration.

Methods: A randomized, open-label, 2-way cross-over, single-dose study with 7 days wash-out period was conducted in healthy volunteers of both sexes. A single s.c. injection of 6,000 IU ovine enoxaparin from Metiska Farma (the test drug, T) or Lovenox® from Sanofi (the reference drug, R) was given randomly to each subject in fasting condition. The PD endpoints measured were anti-FXa and anti-FIIa activities in plasma, whereas the PD parameters determined for these endpoints were $AUEC_{0-t}$ (area under the effect curve from time 0 to the last measured activity (t)) and A_{max} (maximum activity). Bioequivalence (BE) is based on anti-FXa activity, the 90% CIs for GMR T/R (geometric means ratio of Test/Reference) of $AUEC_{0-t}$ and A_{max} must fall within the BE limits of 80.00 – 125.00%. The anti-FIIa data are in vivo supportive evidence only.

Results: A total of 23 healthy volunteers completed this study. The 90% CIs for GMR T/R of $AUEC_{0-t}$ and A_{max} for anti-FXa were 107.55 – 116.33% and 110.17 – 117.68%, respectively, while those for anti-FIIa were 100.93 – 122.56% and 105.19 – 124.44%, respectively. All parameters fell within the BE criteria of 80.00 – 125.00%. One AE (adverse event) occurred in one volunteer after s.c. injection of ovine enoxaparin, i.e. bruising which disappeared after a few days.

Conclusions: The ovine enoxaparin from Metiska Farma was bioequivalent to the reference porcine enoxaparin (Lovenox®) from Sanofi. Both enoxaparin products were shown to have high safety and tolerability after a single dose in healthy volunteers. This is the first study showing BE of a nonporcine enoxaparin to the reference porcine enoxaparin in Indonesia, a Moslem country

Keywords: Enoxaprin, LMWH, PK/PD, Anti-FXa, Anti-FIIa.

List of Abbreviations:

AE: Adverse Event.
A_{max}: Maximum Activity.
$AUEC_{0-t}$: Area under the efficacy curve from time 0 to last measured activity (t).
ANOVA: Analysis of Variance.
BE: Bioequivalence.
BPOM: National Regulatory Authority of Indonesia.
CTAD: Citrate, theophylline, adenosine, and dipyridamole.
CV: Coefficient of variation.
GMR: Geometric means ratio.
LLOQ: Lower limit of quantitation.
LMWHs: Low molecular-weight heparins.
OTC: Over the counter.
R: Reference drug.
T: Test drug.
UFH: Unfractionated heparin.
PK/PD: pharmacokinetic/pharmacodynamic.
anti-FXa: anti-factor Xa.
anti-FIIa: anti-factor IIa.

Introduction

Low molecular-weight heparins (LMWHs) are derived from unfractionated heparin (UFH) by chemical or enzymatic depolymerization. LMWHs have an average MW of 4,000 to 6,500 Daltons, while UFH has an average MW of 15,000 Daltons. Enoxaparin is one of the most widely used LMWHs and nowadays has become the treatment of choice for various thromboembolic diseases. It is obtained by alkaline depolymerization of heparin benzyl ester derived from porcine intestinal mucosa. In contrast to UFH, enoxaparin has higher and more consistent bioavailability after s.c. administration compared with UFH and has a longer plasma half-life [1].

Due to the heterogeneity of LMWHs in their physico-chemical characteristics, conventional PK studies cannot be performed. LMWH absorption and elimination are studied using PD endpoints, i.e., anti-FXa activity (factor which converts prothrombin to thrombin) and anti-FIIa activity (factor which converts fibrinogen to fibrin), which are central to enoxaparin action. Measurement of these PD endpoints is used to compare the biosimilar/generic products to the reference LMWH, as recommended by both EMA and US FDA [2,3].

The objective of this study was to demonstrate the PD equivalence of an ovine enoxaparin to the reference product, the originator porcine enoxaparin, Lovenox® from Sanofi, and to assess its safety and tolerability in healthy volunteers after s.c. administration. PD equivalence of a nonporcine enoxaparin to the reference porcine enoxaparin is important for a Moslem country like Indonesia.

Materials and Methods

Study Design

This was a randomized, open-label, 2-way cross-over, single dose study with at least 7 days wash-out period. The clinical study protocol and the informed consent form were reviewed and approved by the Ethical Committee of the Medical Faculty, Universitas Indonesia on 15 April 2019 and the National Regulatory Authority of Indonesia (BPOM) on 19 August 2019.

The study was carried out in accordance with the Principles of the Declaration of Helsinki 2013, the International Conference on Harmonization E6 Guideline for Good Clinical Practice, the EMEA Guideline on the Investigation of Bioequivalence 2010, and the Indonesian Guideline on Bioequivalence Test 2015 [4-7].

Subjects

Healthy volunteers of both sexes aged 18 – 45 years with BMI of 18-25 kg/m2 were enrolled in the study after signing the informed consent. Subjects had no clinically significant abnormalities based on medical history, clinical laboratory tests, vital sign measurements, 12-lead ECG results, and physical examination findings: were nonsmokers or past-smokers (at least 6 months before dosing). Exclusion criteria were females of < 45 kg or males of < 57 kg, creatinine clearance of < 80 mL/min (calculated with Cockroft & Gault formula), acute or chronic diseases which might influence the drug's safety, tolerability, absorption and/or pharmacokinetics, clinically significant illness within 4 weeks before the study drug dosing, including major surgery, serious mental disease or inability to cooperate with the clinical team, history of or positive test result for alcohol abuse or drug addiction, history of relevant drug and/or food allergies, any prescription drugs (especially antiplatelet or anticoagulant drugs) within 4 weeks before study drug dosing or over the counter (OTC) medications including herbal, supplement, etc. that could affect coagulation within 2 weeks before study drug dosing, administration of any investigational drug within 60 days before study drug dosing, taking an inducer or inhibitor of drug metabolizing enzymes within 60 days before study drug dosing, a positive test for HIV (1 or 2) Ab, HBsAg, or HepC Ab, a positive fecal occult blood test at screening, history and/or current conditions of bleeding tendency, history of thrombocytopenia, blood clotting disorders, known history of hypersensitivity to drugs with a chemical structure similar to enoxaparin (e.g., UFH, LMWH) or to pork or lamb products, if females, during menstruation period, pregnancy or lactation, or taking hormonal contraception (oral or injection), donation or loss of at least 300 mL of blood within 60 days before study drug dosing.

Study Drugs

The test drug was ovine enoxaparin sodium 60 mg (0.6 mL taken from 1.0 mL vial containing 100 mg = 10,000 IU anti-FXa), from Metiska Farma. The reference drug was enoxaparin sodium 60 mg (Lovenox® 0.6 mL prefilled syringe containing 60 mg = 6,000 IU anti-FXa) from Sanofi, France. An unblinded pharmacist prepared the study drug, and an unblinded medical doctor injected the study drug.

Study Procedure

This clinical study was conducted at Pharma Metric Laboratory.
After explanation about health protocol for COVID-19 and the study procedure, the subject was asked to sign the informed consent voluntarily.

After a run-in period of at least 1 week, on day-1 of period 1, subjects were given, in random order, a single s.c. injection of the test or the reference drug in fasting condition. Blood samples were collected at the following time points: pre-dose, and 1, 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 8, 10, 12, 16, 20, and 24 hours after dosing on Day-1 (16 sampling points). Six (6) mL of blood was drawn from cubital vein with a needle of 19 to 21 gauge with a maximum of 1 minute pressure with tourniquet, the first 3 mL of blood was collected in an empty tube (to be discarded), and the second 3 mL of blood was collected in a blood collection tube containing citrate, theophylline, adenosine, and dipyridamole (CTAD tube) for anti-FXa and anti-FIIa measurements. In case of failure in blood drawing, the phlebotomist should change the site of blood drawing and changed the needle used. Subjects returned to the clinic after a wash-out period of at least 7 days, and on day-1 of period 2, they were crossed over to receive a single s.c. dose of the alternate drug, and 16 blood samples were again collected. The blood samples in CTAD tubes were centrifuged within 2 hours after sample collection, at 2500 g for 15 minutes, using swing-out centrifuge, and plasma was decanted into a plastic tube. These plasma samples were used to measure the activities of anti-FXa and anti-FIIa. Anti-FXa activity was determined by a chromogenic method using a commercial

kit [STA-Liquid anti-FXa, and STA-Compact Max 2 (Diagnostica Stago S.A.S, France)] within 4 hours after sample collection at room temperature. Anti-FIIa activity was measured by a chromogenic method using a commercial kit [Biophen anti-FIIa (Hyphen Biomed, France), and STA Compact Max 2 (Diagnostica Stago S.A.S, France)] within 4 hours after sample collection at room temperature. These measurements were done at Prodia Clinical Laboratory.

Tolerability Assessments

Tolerability was assessed by questioning the subjects about symptoms of possible adverse events (AEs, e.g., bleeding, tiredness, headache). All AEs were to be recorded in the CRF.

Efficacy Assessments

Plasma PD parameters which were measured/calculated for anti-FXa and anti-FIIa activities were A_{max} (maximum activity) and $AUEC_{0-t}$ (area under the effect curve from time 0 to the last measured activity (t)), following EMEA Guideline on the Investigation of Bioequivalence 2010 [6] which mentions that the BE parameters used to determine BE after a single dose are AUC0-t and Cmax.

According to FDA Draft Guidance on Enoxaparin Sodium, 2011 [3], the PD endpoints measured are anti-FXa and anti-FIIa in plasma, whereas the PD parameters determined are $AUEC_{0-t}$ and A_{max}. Bioequivalence (BE) is based on anti-FXa, the 90% CIs for GMR T/R (geometric means ratio of Test/Reference) of both $AUEC_{0-t}$ and A_{max} must fall within the BE limits of 80.00-125.00% [3], while the anti-FIIa data are in vivo supportive evidence only [3].

For anti-FXa activity: the calibration curve ranged from 0.08-1.76 IU/mL, with a LLOQ (lower limit of quantitation) of 0.08 IU/mL. The inter-run accuracy and precision of 2 levels of quality control ranged between 94.5% – 114.1%, with CV of 2.73% – 3.01%. For anti-FIIa activity: the calibration curve ranged from 0.05-1.60 IU/mL, with a LLOQ of 0.05 IU/mL. The inter-run accuracy and precision of 2 levels of quality control ranged between 92.6%-104.9%, with CV of 5.56% – 5.75%.

Statistical Consideration

The criteria for bioequivalence (BE) between the test drug (T) and the reference drug (R) in this PD study is when the geometric means ratio (GMR) of T/R for 2 BE parameters, i.e. $AUEC_{0-t}$ (area under the efficacy/activity curve from time 0 to the last sampling time, 24 hours) and A_{max} (maximum activity), both are around 1 (100%) with 90% CI of 80.00-125.00% for anti-FXa and anti-FIIa, the primary and supporting PD endpoints for enoxaparin activity.

An analysis of variance (ANOVA) with fixed effects for sequence, period, and treatment, and random effect for subject nested within sequence was performed on the natural logarithms of $AUEC_{0-t}$ and A_{max} for anti-FXa and anti-FIIa activities, to assess the differences between the test and reference treatments, using WinNonlin software statistics for the calculation.

For sample size, based on data from literature, the within-subject coefficient of variation (CV) was about 18% for enoxaparin anti-FIIa activity (the more variable PD parameter) for both $AUEC_{0-t}$ and A_{max}. Then a sample size of 16 would be sufficient for a study power of 80% [7]. However, considering the probability of obtaining larger CVs, 20 subjects (+ 4 for estimation of dropouts) were randomized and dosed.

Results

From a total of 39 subjects screened, 24 subjects were randomized and dosed in the study. Mean (SD) age was 27.7 (7.8) years (range, 19-44 years), and mean (SD) BMI (body mass index) was 22.7 (1.8) kg/m2 (range, 18.6-24.9 kg/m2). Ten subjects were male. One male subject got accident just before attending period 2, and therefore only 23 subjects completed the study.

After administration of a single dose of either the test or the reference enoxaparin, mean maximum activity of anti-FXa was seen at around 4 hours, then declined rapidly until 10-12 hours, and slower afterwards (Fig. 1A). The maximum activity of anti-FIIa was at around 4.5 hours, declined rapidly until 10 hours, and slower afterwards (Fig. 1B).

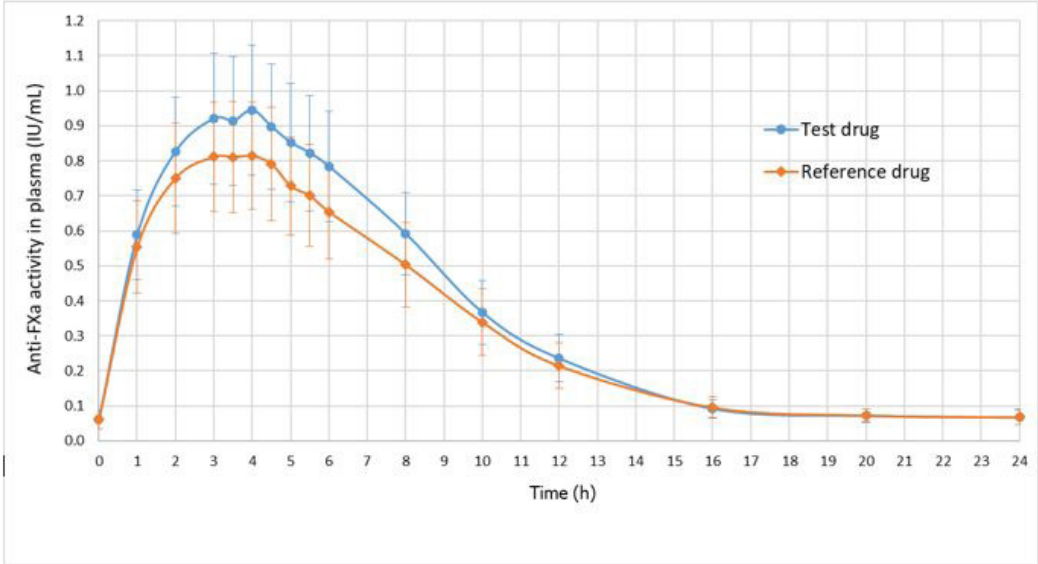

Figure 1A: Mean (SD) plasma anti-FXa activity versus time curves (linear scale) for 23 subjects after a single subcutaneous dose of the test drug or reference drug (in fasting condition).

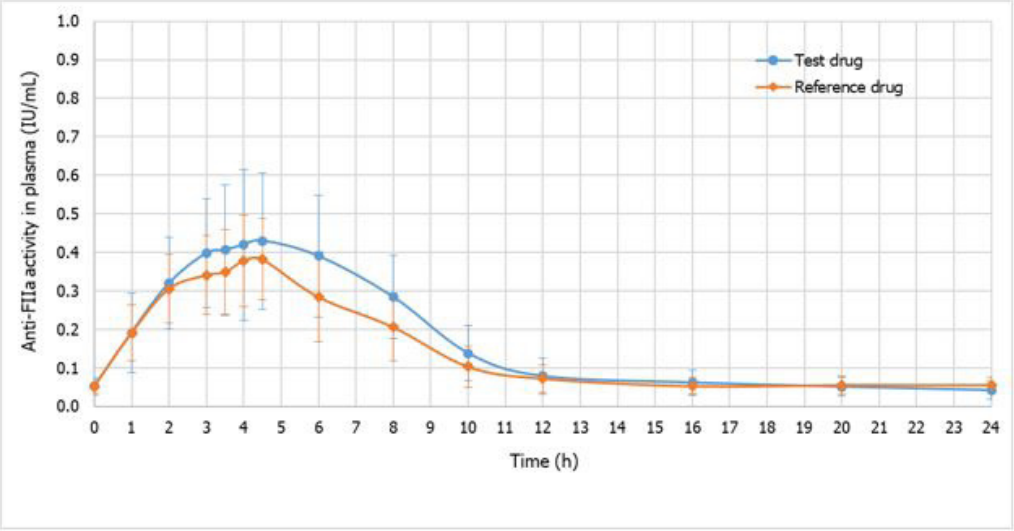

Figure 1B: Mean (SD) plasma anti-FIIa activity versus time curves (linear scale) excluding values at timepoints 5 and 5.5 hours
The test drug: ovine enoxaparin 60 mg (6,000 IU anti-FXa/0.6 mL), from Metiska Farma.
The reference drug: Lovenox® 60 mg (6,000 IU anti-FXa/0.6 mL) from Sanofi

Comparing the test drug with the reference drug, results for both anti-FXa and anti-FIIa activities are shown in Table 1. The table shows that for both anti-FXa and anti-FIIa activities, the 90% CI of GMR T/R for the PD parameters $AUEC_{0-t}$ and A_{max} were within the BE interval of 80.00-125.00%.

Parameter	N	Geometric Means		GMR T/R (%)	90% CI(%)	CV (%)
		Test	Reference			
Anti-FXa (16 timepoints)						
$AUEC_{0-t}$ (IU.h/mL)	23	8.70	7.79	111.86	107.55 – 116.33	7.74
A_{max} (IU/mL)	23	0.96	0.84	113.86	110.17 – 117.68	6.49
Anti-FIIa (14 timepoints, excluding 5 & 5.5 hours)						
$AUEC_{0-t}$ (IU.h/mL)	23	3.59	3.32	111.22	100.93 – 122.56	19.29
A_{max} (IU/mL)	23	0.48	0.40	114.41	105.19 – 124.44	16.66

Table 1: The results of $AUEC_{0-t}$ and A_{max} of anti-FXa and anti-FIIa for 23 subjects.

For anti-FIIa, values at timepoints 5 and 5.5 hours were excluded for both test drug and reference drug, because those values for Lovenox® were very low (especially for subjects' number 1, 3, 5, and 7), which could not be correct, considering their occurrences were around the peak values (at timepoint 4.5 hours). We did not know what happened or could possibly happen, because these values did not happen in other Lovenox® clinical trials [8,9]. These values could not be repeated, because: 1) the blood sample should be fresh (measured within 4 hours), 2) the study drug products had been expired this month (November 2020), and 3) the reagents were not available anymore. Therefore, all values at timepoints 5 and 5.5 hours from both Lovenox® and ovine enoxaparin should be excluded.

Bruising was found in one male subject at period one at the site of subcutaneous injection of the test drug (ovine enoxaparin) using 1 mL syringe. Start with a red dot after the time of injection, it widened to become a greenish spot of about 2 cm in diameter which emerged 24 hours after injection. One week later, at the time of the second injection (with Lovenox®), the colour of the spot has become bluish and fading, and disappeared a few days later. The subject felt mild pain when the coloured spot was pressed.

Discussion

This study followed FDA Guidance on Enoxaparin, 2011 [3]. This was a pharmacodynamic (PD) study in normal healthy volunteers using single dose enoxaparin 1 mg/kg (60 mg) as s.c. injection in fasting condition. After 1 week wash-out period, the subjects were crossed to receive the alternate product. PD endpoints measured were anti-FXa and anti-FIIa activities in plasma, and the PD parameters determined were $AUEC_{0-t}$ and A_{max}. Bioequivalence is based on the 90% CIs of GMR T/R for $AUEC_{0-t}$ and A_{max} for anti-FXa which must fall within the BE limits of 80.00 – 125.00%. The anti-FIIa data are supportive evidence only. Also, for monitoring of LMWH administration, only anti-FXa activity is measured at 4 hours after injection.

Table 1 showed that for both anti-FXa and anti-FIIa, the 90% CI of GMR T/R (ovine enoxaparin vs Lovenox®) for both $AUEC_{0-24\ hrs}$ and A_{max} are within the BE criteria of 80.00 – 125.00%. Since the anti-FXa and anti-FIIa are the most important PD endpoints for enoxaparin activity and taken as the primary and supporting endpoints, and their parameters showed BE, then it was concluded that the test drug, ovine enoxaparin, was bioequivalent to Lovenox®, the reference drug. The within-subject coefficient of variation (CV) for anti-FIIa (the more variable PD endpoint) found in the present study was around 20% (Table 1), then the sample size required was 20 for a study power of 80%, according to the Indonesian Guideline on Bioequivalence Test, 2015 [7]. Since 23 subjects participated in the present study, this would mean that the study had > 80% power to conclude biosimilarity, i.e., PD equivalence, between the test and the reference enoxaparin products.

It is already known that anti-FXa has more consistent values, while the values of anti-FIIa are more variable, and these were confirmed in the present study. These were evident from the relatively small CV values of anti-FXa (7.74% and 6.49%) compared to those of anti-FIIa (19.29% and 16.66%) (Table 1). Mismatch between the reagent (Biophen) and the apparatus (Stago) used to measure anti-FIIa activity, although method validation had been done, might also cause the higher variation in the measurements of this PD activity even higher. Method validation for measurement of anti-FIIa was only cross reaction using Biophen reagent with Stago apparatus. Cross validation between reagents was not performed because Stago reagent was not available.

The activity-time profiles of anti-FXa and anti-FIIa were consistent with the respective half-lives of both PD activities, i.e., 3.8 hours for anti-FXa and 1-2 hours for anti-FIIa [12]. After a single dose s.c. administration of enoxaparin, the anti-FXa has been eliminated after 16 – 20 hours (flat curve after 5 times its half-life = 5x 3.8 hours), while the curve of anti-FIIa became flat after 10 – 12 hours (5x 2 hours) (Figures 1A and 1B).

One interesting point to be discussed here is that LMWHs are considered as chemicals (drugs) by FDA, but as biologicals by EMA [10]. Consequently, the copy products are generics according to FDA, and biosimilars according to EMA. As a biosimilar, enoxaparin requires clinical trials (efficacy trials) to show the therapeutic equivalence to its reference product. According to EMA, equivalence in terms of efficacy and safety could be shown by one clinical trial, which should be performed in the most sensitive clinical setting. However, efficacy trials do not seem to have enough sensitivity or statistical power to detect differences in clinical endpoints, since they have never been able to detect differences between different LMWHs with evident differences in PK/PD and anti-FXa activity. If clinical endpoint is not able to distinguish between different LMWHs with different PK/PD properties, then it cannot be expected to be able to detect differences between the reference product and the biosimilar product with equivalent PK/PD properties [10]. Therefore, it is not justified to require such a therapeutic equivalence trial. With the existing knowledge, the most sensitive study is a PD study based on anti-FXa and anti-FIIa activities [10].

Another point to be discussed is about interchangeability, switching, and substitution of a reference product by its biosimilar. In the US, products approved as generics (including enoxaparin) by FDA are directly interchangeable with the reference products, and these generics are interchangeable between them [10]. On the contrary, EMA considers LMWHs as biologicals, the interchangeability of the biosimilars is not discussed during the assessment or for the approval because this is a national issue. EMA only deals with the approvability or prescribability of the products [10]. Several national regulatory authorities, including the Dutch, Finnish, Scotland, Irish, Germany have already taken national positions to endorse the interchangeability of biosimilars under supervision of the prescriber [10, 11]. Biosimilars licensed in the EU are interchangeable, if the patient is clinically monitored, will receive the necessary information, and, if needed, training on the administration of the new product [11].

The only one drug-related adverse event, bruising with mild pain on pressure, which lasted for a few days, found in one subject in the present study, indicated the high safety and tolerability of enoxaparin after single doses in healthy volunteers.

For drug administration of ovine enoxaparin, that should be drawn 0.6 mL from a vial of 1 mL using a syringe of 1 mL, no matter how careful the medical doctor in charge has been, the precise amount is still very difficult to obtain (manual), and therefore prefilled syringe will be best in order to obtain the precise amount (machine).

Bruising which occurred in one subject after s.c. injection of the ovine enoxaparin may be caused by the injection of the 1 mL syringe used to deliver the ovine enoxaparin. The needle of the 1 mL syringe may pierce the blood vessel in the subcutaneous tissue and caused blood to leak out and colour the skin. If this was correct, then the bruising was due to the needle of the syringe and not due to the drug. This would mean that both enoxaparin products did not cause any adverse event in this study.

Finally, the strengths and limitations of this study have to be discussed. The strengths of this study are: 1) the cross-over design conducted in the same subject removes intersubject biological variations (because every subject becomes his/her own control), 2) this design makes the number of subjects required very small, i.e., only 20 subjects, although 24 subjects were randomized in

this study (see sample size above), 3) random drug administration at period one makes sequence effect and period effect balanced between the 2 groups of the cross-over design, 4) wash-out period of at least 7 days between 2 periods of study drug administration is sufficient (more than 5 times half-lives of both PD activities, i.e., 3.8 hours for anti-FXa and 1-2 hours for anti-FIIa). 5) the low within subject CVs of both AUEC0-t and Amax for anti-FXa (the primary endpoint) indicate the low variability in obtaining those values in this study, and 6) the final aim of this study is reached, i.e., BE of a nonporcine enoxaparin to the reference porcine enoxaparin in Indonesia, a Moslem country.

Meanwhile, the limitations of this study are: 1) use of 1 ml syringe to draw 0.6 ml ovine enoxaparin from 1 ml vial makes the precise amount is difficult to obtain (see Discussion, above), and 2) use of 1 ml syringe for subcutaneous injection may cause the adverse event bruising (see Discussion, above).

Conclusions

The results of this pharmacodynamic BE study showed that the ovine enoxaparin from Metiska Farma was bioequivalent to the reference porcine enoxaparin (Lovenox®) from Sanofi based on the anti-FXa and anti-FIIA activities as the primary and supporting PD endpoints for enoxaparin activity, according to FDA Guidance on enoxaparin sodium, 2011 [3]. Both enoxaparin products were shown to have high safety and tolerability after a single dose in healthy volunteers.

Recommendation

In order to obtain precise amount of drug to be injected, it is recommended for enoxaparin from Metiska Farma to be presented as single doses in prefilled syringes.

One other valid reason to recommend prefilled syringe is because prefilled syringe has a very fine needle, while ordinary syringe has larger and longer needle that may pierce the blood vessel in the subcutaneous tissue and cause blood to leak out and colour the skin (cause bruising).

Acknowledgments

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This study is registered at ClinicalTrials.gov with registration identification number NCT04402762
This study was organized by CRSU (Clinical Research Supporting Unit), which is one of the research clusters of IMERI (Indonesia Medical Education and Research Institute), Faculty of Medicine, Universitas Indonesia.

Disclosure

Both authors have no conflicts of interest in this study.

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