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Generic and Branded Enoxaparin Bioequivalence: A Clinical and Experimental Study

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Abstract

Our aim is to compare a new generic version of enoxaparin (Enoxa®) with the parent brand (Lovenox®).

We included patients with acute coronary syndrome (ACS) for the clinical study and healthy volunteers for the experimental study. ACS patients were randomly assigned to receive a bolus of Enoxa® (n=86) or Lovenox® (n=83) and serum anti-Xa activity was measured 4 hours thereafter. For experimental study, blood from healthy volunteers was used to compare the effect of both formulations on thrombin generation in citrated platelet-poor plasma (PPP). We measured the half maximal inhibitory concentration of the drug (IC50) that is required to decrease 50% in-vitro thrombin generation parameters the mean rate index (MRI) and the endogenous thrombin potential (ETP). Both IC50 MRI and IC50 ETP were calculated in PPP.

In ACS patients, serum anti-Xa activity was found not different between Enoxa® and Lovenox®. Median anti Xa activity measured 4 hours after the initial bolus was 0.39 IU anti-Xa/ml [95 % CI 0.31-0.53] and 0.34 IU anti-Xa/ml [95% CI 0.27-0.53], for the Enoxa® group and Lovenox® group respectively. No difference in major cardiovascular events was observed during hospital stay. In healthy volunteers, IC50 MRI and IC50 ETP were similar between Lovenox® and Enoxa® [(2.5 µg/ml ± 0.2 µg/ml) versus (2.3 µg/ml ± 0.1µg/ml)] and [(4.8 µg/ml ± 0.8 µg/ml) versus (4.1 µg/ml ± 0.1 µg/ml) respectively for IC50 MRI and IC50 ETP; (p=0.2)]. With both formulations, anti-Xa activity and anti-Xa/anti-IIa ratio were similar.

The generic enoxaparin Enoxa® met the main regulatory criteria of bioequivalence with the branded product.

Keywords: Equivalence; Enoxaparin; Acute coronary syndrome; Generic

Introduction

Low molecular weight heparins (LMWHs) are a family of anticoagulants that are widely used in the treatment and the prophylaxis of venous and arterial thromboembolism. In the specific clinical setting of antithrombotic management of acute coronary syndrome (ACS), the LMWH enoxaparin has consistently shown its superiority in terms of efficacy when compared to unfractionated heparin (UFH) and other LMWHs [1-3]. In this clinical indication, enoxaparin is now the gold standard for anticoagulation and is extensively used in everyday practice [4,5]. In the last decade, patent rights and supplementary protection certificates of branded enoxaparin have expired raising the opportunity to develop copies of this innovator product. As a consequence, generic enoxaparin appeared on the market in many countries such as China, India and Brazil [6-8]. Similarly, a new generic enoxaparin was recently proposed in Tunisia. From an economic viewpoint, the use of generic enoxaparin results in a considerable savings of healthcare expenditures although some authors have been concerned about the introduction into the market of such copies [9]. Indeed, any generic of enoxaparin should have an equivalent activity and bioavailability as the original molecule. Otherwise, it is difficult to ensure that the benefit/risk ratio of enoxaparin and its copy are equivalent. This study was carried out to assess the bioequivalence of branded enoxaparin (Lovenox®; Sanofi US, Bridgewater, New Jersey) with a generic version of enoxaparin (Enoxa®; Medis Laboratory, Tunisia) in patients with ACS and in healthy volunteers using parameters of interest such as plasma anti-Xa and anti-IIa activities and thrombin generation (TG) tests.

Materials and Methods

This study combined clinical trial conducted in patients with ACS and an experimental study conducted in healthy volunteers.

The clinical study

This single-dose, randomized-sequence, open-label study was conducted at the emergency department (ED) of Fattouma Bourguiba University Hospital, Tunisia. It was performed in accordance with the revised declaration of Helsinki for Biomedical Research involving human subjects and the Guidelines for Good Clinical Practice. The protocol was approved by the ethics committee of our institution. This trial was carried out between December 2012 and April 2013. Prior to undergoing any screening procedures, all included patients provided written informed consent. Patients were included if they were older than 18 years and fulfilled the criteria of ACS within 48H of chest pain onset: signs suggestive of ACS without ST-segment elevation myocardial infarction, and electrocardiographic changes

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compatible with ischemia or elevated levels of cardiac biomarkers. Patients were ineligible if they had any of the following major exclusion criteria: cardiogenic shock, persistent ST-segment elevation, clinically significant hepatic disease, end-stage renal disease requiring dialysis, an increased risk of bleeding, platelet count of less than 50.000/mm^3 at the time of screening, a history of pathologic intracranial findings, the use of any heparin within the previous 72 hours, or history of allergy to this drug.

Using a table-generated randomization schedule, eligible patients were assigned to receive an IV bolus of the branded enoxaparin (Lovenox®; Sanofi US, Bridgewater, New Jersey) or the generic one (Enoxa®; Medis Laboratory, Tunisia). We performed this study with at least two lots of Enoxa® and Lovenox®. Baseline demographics with clinical history data and results of routine laboratory tests including platelet count, thromboplastin time (PT) and activated partial thromboplatin time (aPTT) were recorded at ED admission. Sample collections were determined in conformity with the current international recommendations for haemostasis tests blood. Sampling was performed at inclusion and 4 hours after the first dose of the LMWH protocol products. Samples were centrifugated for 15 minutes at a speed of 2000-2500 g and at a temperature around 18°C. For the assessment of anti-Xa activity, the plasma was aliquoted and stored at -80°C. The anti-Xa activity was determined by chromogenic methods using the STA®-Liquid Anti-Xa reagent (Diagnostica Stago, Asnières sur seine, France). Precision studies indicated an intra and an inter-laboratory variation <5%. All the patients were admitted in the cardiology department and managed according to the current practice. A clinical follow-up was conducted for all the included patients until 30 days post-ED admission. All major cardio-vascular events (death, acute reperfusion, stroke) occurring during the follow-up period were recorded.

The healthy volunteers study

Samples preparation: Venous blood was obtained from 15 healthy volunteers not taking any medication interfering with haemostasis during the last 10 days. Blood was collected with atraumatic antecubital veinipuncture into siliconized vacutainer tubes (Becton Dickinson, Meylan, France) containing buffered trisodium citrate (0.129 mol/l, 9 parts of blood to 1 part of citrate solution). For each blood sample a platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared as follows: Whole blood was centrifuged for 10 min at 150 x g at 18°C, and the supernatant platelet-rich plasma (PRP) was removed. The remaining blood was further centrifugated for 15 min at 2000 × g at 18°C.

Anti-Xa and anti-IIa activity assessment: A volume of 1 ml of PPP was mixed with 5 µg of each studied product to obtain a final concentration of about 0.5 IU anti-Xa/ml. Subsequently, anti-Xa and anti-IIa activities were measured on STAR analyser using a validated commercial reagent (Diagnostica Stago, Asnieres Sur-seine France) and the anti-Xa /anti-IIa ratio was calculated. Anti-Xa activity was measured using the STA® Rotachrom® Heparin reagent (Diagnostica stago, Asnières sur seine, France). Anti-IIa activity was measured using S-2238 as a substrate.

Thrombin generation inhibition assessment: To study the effect of branded and generic enoxaparin on thrombin generation, PPP from 15 healthy volunteers was spiked with increasing concentrations (2, 4, 6, 8, 10 and 20 µg/ml) of Lovenox® or Enoxa®. Thrombin generation was studied *in-vitro* according to Hemker et al. [10] using the Calibrated Automated Thrombogram (CAT) assay (Thombinoscope b.v., Maastricht, The Neterlands). In each of a micro-plate, 80 µl of each PPP were mixed with 20 µl of PPP reagent® and 1/200 diluted Innovin® (from Siemens). Control samples of 80 µl of PPP spiked with 20 µl of thrombin calibrator (Biodis, Stago Laboratory, Asnières, France) were run in parallel with PPP. Thrombin generation was initiated by adding 20 µl of triggering solution containing $CaCl_2$ (16.7 mM final concentration) and fluorogenic substrate (Z-Gly-Gly-Arg-AMC, Bachem, Bubendorf, Switzerland, 417 µM final concentration). A plate reader fluorometer (Fluoroskan Ascent; Thermo Labsystems, Helsinki, Finland) and the appropriate software (Synapse, Maastricht, the Netherlands) were used for the assessment of thrombin generation. Thrombin generation was expressed by the mean rate index (MRI) and endogenous thrombin potential (ETP). MRI is defined as Peak/time to Peak – lag time. The effect of each enoxaparin on thrombin generation was expressed by the half maximal inhibitory concentration of the drug (IC50) that is required to inhibit 50% in-vitro thrombin generation (IC50 MRI and IC50 ETP). Both IC50 MRI and IC50 ETP were calculated in PPP.

Statistics

Data are presented as mean ± SD or median (25% interquartile range) as appropriate. Previous studies with enoxaparin sodium effects on anti Xa activity in healthy volunteers have shown an intra-individual variance of less than 10%. Thus, assuming a type I error of α=.05, a statistical power of 1 – β = .80, an equivalence acceptance intervals of 80% to 125%, and a mean ratio of test versus reference between 0.95 and 1.05, a sample size of n=80 in each arm has been calculated to be appropriate for the clinical study. To compare baseline demographic and clinical characteristics between groups, Student t test was used for continuous variables and Chi2 test for comparison of categorical variables. The unpaired student's t test was used to compare anti-Xa activity values between Lovenox® and Enoxa®. Anti Xa/anti IIa ratios, IC50 MRI, and IC50 ETP values in PPP of the generic and the branded enoxaparin were compared by paired Student's t test. P values <.05 were considered statistically significant. Statistical analysis was performed with SPSS software version 17.0 (Chicago, IL, USA).

Results

The clinical study

A total of 169 patients with ACS were enrolled and completed the study, 86 received Enoxa® and 83 received Lovenox®. Demographic characteristics and standard laboratory tests values are shown in Table 1; there were no significant statistical differences between Enoxa® and Lovenox® groups with regard to these variables. The mean enoxaparin dose was 7000 ± 100 IU in Enoxa® group as compared to 6900 ± 150 IU in Lovenox® group (p = 0.54). Median anti Xa activity measured 4 hours after the initial bolus was 0.39 IU anti-Xa/ml [95% CI 0.31-0.53] and 0.34 IU anti-Xa/ml [95% CI 0.27-0.53], for the Enoxa® group and Lovenox® group respectively; the difference was not statistically significant (p=0.82). All the patients were transferred to the cardiology department within 24 hours after their ED admission. No significant difference was observed between both groups with regard to major adverse cardiovascular events at 30 days post-ED admission (Table 2). There were no significant hemorrhagic complications in both groups during the follow-up.

The healthy volunteers study

The anti-Xa and anti-IIa activities per milligrams of the generic and the branded enoxaparin were similar as was the anti-Xa/anti-IIa ratio (Table2). When thrombin generation was assessed on PPP, both branded and generic enoxaparin showed comparable IC50 MRI (2.5

	Enoxa® (n=86)	Lovenox® (n=83)	p
Age (year) mean (SD)	61.5 (12.5)	63.7 (10.8)	0.249
Sex ratio (M/F)	2.0	1.6	0.465
Body mass index (kg.m^{-2}) mean (SD)	27.7 (4.2)	26.5 (3.5)	0.221
History of cardiovascular disease n (%)	37 (43)	44 (53)	0.18
Diabetes mellitus n (%)	39 (45)	33 (40)	0.41
Heart failure n (%)	10 (12)	8 (10)	0.71
Hemoglobin (g/dl)	12.4 ± 1.8	12.6 ± 2.1	0.52
Platelet count (1000 /mm^3)	230 ± 56	212 ± 50	0.29
Creatinine (µmol/l)	108.8 ± 43.0	106.5 ± 70.3	0.81
Glomerular filtration rate PT (%)	86.4 ± 21.2	86.9 ± 18.2	0.94
aPTT (sec)	30.3 ± 7.8	30.2 ± 9.4	0.77

Table 1: Demographic characteristics and biological assessment at baseline in patients with acute coronary syndrome.

	Enoxa® (n=86)	Lovenox® (n=83)	p
Clinical Study			
30 days major cardio-vascular events n (%)			
death	3 (3.4)	3 (3.5)	NS
acute reperfusion	1 (1.1)	0	
stroke	0	1 (1.2)	
combined events	4 (4.5)	4 (4.7)	
Anti-Xa activity median (95% CI) Baseline (anti-Xa IU/ml)	0	0	-
After 4hours (anti-Xa IU/ml)	0.39 (0.31-0.53)	0.34 (0.27-0.53)	NS
Healthy volunteers study			
Anti-Xa activity IU/µg	0.12	0.11	NS
Anti-IIa activity IU/µg	0.03	0.03	
Anti-Xa/Anti-IIa ratio	3.8	3.6	

NS : not significant

Table 2: Clinical outcome, plasma anti-Xa and anti-IIa activity with Lovenox® and Enoxa®

µg/ml ± 0.2 µg/ml) versus (2.3 µg/ml ± 0.1 µg/ml) respectively for Lovenox® and Enoxa® (p=0.2) and (4.8 µg/ml ± 0.8 µg/ml) versus (4.1 µg/ml ± 0.1 µg/ml) for IC50 ETP in Lovenox® and Enoxa® respectively (p=0.2) (Figure 1).

Discussion

Recently, several generic LMWHs have become available worldwide [11]. This study was carried out to compare the anticoagulant potency and pharmacodynamic responses of branded enoxaparin (Lovenox®) with a generic enoxaparin (Enoxa®). It is the first Tunisian report of a bioequivalence between original and generic versions of enoxaparin using validated parameters in a clinical trial conducted on ACS patients and experimental study performed in healthy volunteers.

Our study showed that bolus administration of equal doses of branded and generic enoxaparin in patients with ACS results to similar levels of plasma anti-Xa activity measured 4 hours after bolus administration. In addition, the *in-vitro* anti-Xa and anti-IIa activities as well as the anti-Xa/anti-IIa ratio were not significantly different between Lovenox® and Enoxa®. The effect of generic and branded enoxaparins on thrombin generation in PPP was similar as demonstrated by similar IC50 for both MRI and ETP.

In contrast to many other drugs, heparins cannot be reliably detected *in-vivo* as a chemical entity; therefore, pharmacodynamic surrogates are usually employed to describe their pharmacokinetic properties and their bioavailability [12]. According to the FDA bioequivalence requirements, pharmacodynamic surrogates of efficacy including anti-Xa activity, anti-IIa activity, and their ratios are established and validated bioequivalence criteria [13,14]. The strength of our study is that in addition to standard tests, we used the thrombin generation tests to assess bioequivalence between the enoxaparin innovator product and its generic version. The information obtained by these tests is particularly important and represents a significant improvement in the assessment of the different Xa inhibitors available [10]. In our study, we showed that the IC50 of Lovenox® and Enoxa® for MRI and ETP were similar in PPP. Taken together, our results are in accordance with those reported in the literature from previous studies where most of generic versions exhibited similarities with branded enoxaparin [8]. However, the present bioequivalence study had several limitations. First, the single-dose design in the clinical study including patients with ACS did not allow us to measure the area under the plasma concentration-time curve of anti-Xa activity which could be more informative than a single test [15,16]. However, our results in healthy volunteers indicated that similar anti-Xa/anti-IIa ratio was achieved by the original and the generic enoxaparin. Second, although this study did not specifically address a comparison between the two LMWHs on the basis of clinical outcome criteria, this condition for bioequivalence acceptance was not always required. On the other hand, we believe that compounds which have similar biological effects on the basis of an established criteria of sameness will likely have similar clinical effect. In one recent Tunisian study conducted in post-operative orthopedic patients, it was demonstrated that Enoxa® was as effective as Lovenox® in preventing deep venous thrombosis during 45 days follow-up [17]. Third, the assessment of adverse events was not sufficiently addressed in this study. Occurrence of heparin-induced thrombocytopenia (HIT) is an important safety criteria required for generic LMWH [18]. However, because HIT is reported at a rate of less than 1% of patients treated with LMWHs, it is not expected to be seen in a single-dose trial as in the present study. Nonetheless, available data did not indicate that the risk of HIT is superior with biosimilar versions of enoxaparin compared to original products [18].

Conclusion

In conclusion, the generic enoxaparin Enoxa® shares many similarities with originator product Lovenox® as demonstrated in our study on the basis of the commonly biologic and pharmacodynamic

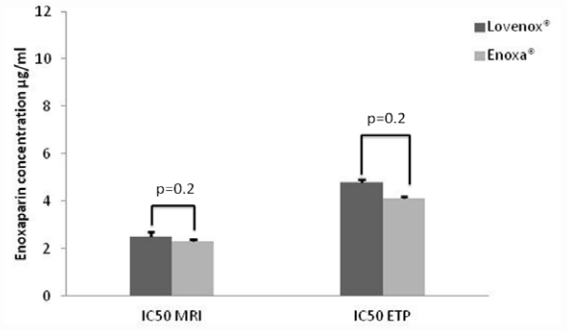

Figure 1: The Lovenox® and Enoxa® concentrations which reduced 50% of Mean Rate Index (IC50 MRI) and Endogenous Thrombin Potential (IC50 ETP) in platelet-poor plasma. Values are mean ± SD of 15 experiments.

criteria. Accordingly Enoxa®, as many enoxaparin generics would be an interesting alternative to patients especially those living in low income countries.

Acknowledgments

SN conceived the study, and designed the trial. SN, WB, RB, MHG, I E and HB supervised the conduct of the trial and data collection; GTG, MH, TC, and KB undertook recruitment of patients and managed the data, including quality control; SN and RR performed statistical analysis and analyzed the data; SN drafted the manuscript; TC and GTG revised the manuscript and all authors contributed substantially to its revision; SN took responsibility for the paper as a whole.

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Conflict of Interest

This work was supported by Medis Laboratory, Tunisia.

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New generic enoxaparin

Source of Support

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