Propositional Debate on Biosimilar Enoxaparin in Brazil

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Abstract

Some patents of low-molecular-weight heparins (LMWHs) have expired and others are about to expire. Biosimilar versions of those drugs are available for clinical use in several countries. However, skepticism persists about the possibility of obtaining preparations similar to the original drug, because of the complexity of the process to generate LMWHs. In recent years, our laboratory has analyzed biosimilar samples of enoxaparin available for clinical use in Brazil (30 different batches and 70 finished products). Those preparations were assessed regarding their chemical structure, molecular weight distribution, *in vitro* anticoagulant activity, and pharmacological effects in animal models of thrombosis and bleeding. Our results have clearly shown that biosimilar preparations of enoxaparin are similar to the original drug. Our results have shown that those biosimilar versions of enoxaparin are a valid therapeutic alternative, which are, however, in need of appropriate regulation to ensure compliance with regulatory requirements.

Introduction

As LMWHs are a significant advance in the prevention and treatment of thromboembolic and cardiovascular diseases. Studies comparing those drugs with unfractionated heparins (UFHs) have revealed that LMWHs have the following characteristics: an inexpressive effect on the risk of bleeding; a longer half-life; a longer-lasting therapeutic effect; and subcutaneous administration. In addition, LMWHs can be prescribed in the outpatient context with no laboratory monitoring need.

Some patents of LMWHs have expired and others are about to expire. Biosimilar versions of those drugs are available for clinical use in several countries, such as Brazil and the United States. They allow for cost reduction in the treatment with LMWHs.

The introduction of those biosimilar LMWH products has raised the debate about their similarities with the original drug.

Keywords

Enoxaparin, heparin, heparin, low-molecular weight, fibrinolytic agents.

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In Brazil, that debate has been limited to a few public discussions and recent publications[1,2]. The international debate, however, has been much more challenging. For example, the involvement of cyclic sugars in the mechanism of action of the drug and their reduction in biosimilar enoxaparin have been proposed. Because of the lack of consistent publications confirming that hypothesis, the debate has cooled down.

Another aspect introduced was the impossibility of demonstrating similarity between preparations of heparin. Heparins do not have a precise molecular weight, because they are mixtures of products with several molecular weights and heterogeneous structures. Thus, would it be possible to ensure similarity between the heparin preparations?

That discussion has raised a relevant issue about the action of drugs. To demonstrate the similarity between two molecules of reduced molecular weight by using the current physicochemical methods is feasible and even easy. It is feasible even for a protein by using the current methods of amino acid sequencing in addition to determining the conformation through physicochemical methods and establishing the pattern of glycosylation.

Our laboratory has recently reported similarities between enoxaparin preparations[3] and dissimilarities between UFHs[4]. If we are able to ensure that a manufacturer obtains similar batches of enoxaparin, then why can we not ensure that, by using the same methodologies, a biosimilar preparation is similar to the original enoxaparin? Yes, this is the basic principle of the pharmacopeial concepts. Thus, the debate about the difficulty in ensuring similarity between the preparations of LMWHs has also cooled down.

Once that conceptual discussion carried out abroad has been overcome, the essential question is as follows: are the biosimilar enoxaparin products available in Brazil similar to the original drug?

The two articles recently published[1,2] in this journal have only provided information about the requirements for clinical trials with those drugs, but they have not provided data to answer the essential question of similarity between them. In fact, they are opinion articles.

A study about a certain drug, molecule or compound has very well defined steps that should be followed as formulated in some manuals aimed at analyzing biosimilar versions of LMWHs[5]. The sequence of steps involves the following: determination of the structure of the molecule; its biological effects *in vitro*; assays on pharmacological models (including experiments in animal models); pharmacodynamics and/or pharmacokinetics; and, finally, clinical tests.

Analyses of biosimilar enoxaparin products available in Brazil

Our laboratory has analyzed biosimilar samples of enoxaparin available for clinical use in Brazil (30 different batches and 70 finished products)[3]. Those compounds were initially analyzed by use of the methodologies described in American and European Pharmacopeias[6]. The results have shown a clear similarity between biosimilar enoxaparin available in Brazil and the original drug (fig. 1). For proton magnetic resonance spectroscopy (^{1}H-NMR), the most modern spectrometer commercially available was used. The coincidence between both samples can be seen (black *vs.* blue, Panel A). The quantitative values of the integrals of the ^{1}H-NMR spectra are shown in Table 1. Other values are shown in reference 3.

The analysis of the molecular weight distribution of the oligosaccharides found in enoxaparin preparations also evidences the similarity between biosimilar enoxaparins and the original drug, in both the 232-nm absorption assay (A_{232nm}) and the refraction index (black *vs.* blue in Panels B and C, respectively). The quantitative values of the molecular weight distribution are shown in Table 1. Finally, the anti-Xa and anti-IIa activities are similar for the samples of biosimilar enoxaparins and the original drug (Panels D and E; Table 1). Seventy batches of the finished products of biosimilar enoxaparins were also assessed, and showed 99% ± 4% (mean ± SD) and 102% ± 3% of the declared anti-Xa and anti-IIa activities, respectively.

Pharmacological studies

The antithrombotic effect of biosimilar enoxaparin has been compared with that of the original enoxaparin and UFH in a classical model of experimental venous thrombosis (Panel F). Inhibition of thrombosis clearly requires a higher dose of enoxaparin than of UFH. However, most significantly, biosimilar enoxaparin and the original one have similar dose-response curves. Finally, the hemorrhagic tendency of those two LMWHs has been compared by use of an experimental model with rats. Their effects have also been similar, and both drugs have shown a significantly more reduced hemorrhagic tendency than UFH has (Panel G).

Clinical studies

In Brazil, the debate about the use of biosimilar versions of enoxaparin has emphasized neither physicochemical studies, nor *in vitro* tests, nor pharmacological assays. However, it has emphasized the clinical studies, which are expensive, longer lasting, and much more complex. The new version of the Brazilian Pharmacopeia from December 2010 lacks a monograph on LMWHs. The above-cited assays are easier, feasible, rapid and of lower cost. Inexplicably, they are not as appealing as they should be.

In 2009, a symposium was held in Boston, aimed at the discussion of biosimilar LMWHs[7]. During the discussions, requirements for the approval of biosimilar LMWHs, including those for clinical assays, were elected. During the symposium, Prof. JI Weitz (McMaster University, Canada) asked the following: "How many patients are required for a clinical study on LMWHs? Around 3,000 – 4,000 patients? Do you think that it is feasible that the companies that propose the commercialization of biosimilar LMWHs for the several types of diseases perform those studies? The manufacturers of the original drugs could fund those studies because they had the global monopoly for ten years; this is not feasible for the new manufacturers. Have you really considered what you are proposing?".

After that intervention, the debate cooled down. I believe that assessments such as that of Prof. JI Weitz are related to the recent decision of the FDA-USA to approve the commercialization of a biosimilar version of enoxaparin based on physicochemical and pharmacological studies, regardless of the lack of clinical assessment.

Thus, does anyone consider it feasible to propose to the five companies that commercialize enoxaparin to carry out clinical studies with 3,000-4,000 patients in Brazil, involving the different diseases requiring LMWHs? I make mine Prof. Weitz's words: have you really considered what you are proposing?

Propositions

The debate about the use of biosimilar LMWHs in Brazil has to necessarily move on to a phase in which propositions are made. It might be the opportunity to a closer association between researchers and regulating agencies, aiming at establishing feasible methodologies to provide physicians and patients with a highly effective drug, although still expensive, and unavailable for most of the Brazilian population.

We propose the following steps:

1) To incorporate one monograph about LMWHs into the Brazilian Pharmacopeia to normalize the analyses of the batches and finished products containing enoxaparin;

2) To establish a protocol of pharmacological studies to be performed by the companies that commercialize biosimilar LMWHs;

3) To create a committee of experts with recognized experience in the area to assess the results of the analyses of the biosimilar versions of LMWHs available in Brazil, and to propose further studies.

In conclusion, it is worth noting that demonstrating dissimilarity is easy in science. If, on the one hand, it suffices to show that two substances differ in a single structural detail or in a certain biological effect to state that they are distinct, on the other, demonstrating similarity/equality is an endless path. We can always argue that it lacks a new aspect to be investigated to state that there is no difference between them. One needs to be pragmatical. In addition, one should assess the feasibility of the data obtained and the impact of drug availability on the population in need of it, obviously considering the product's safety, efficacy, and cost. Those are the challenges we have to face with scientific rigor and prudence when analyzing the enoxaparin preparations available for clinical use in Brazil.

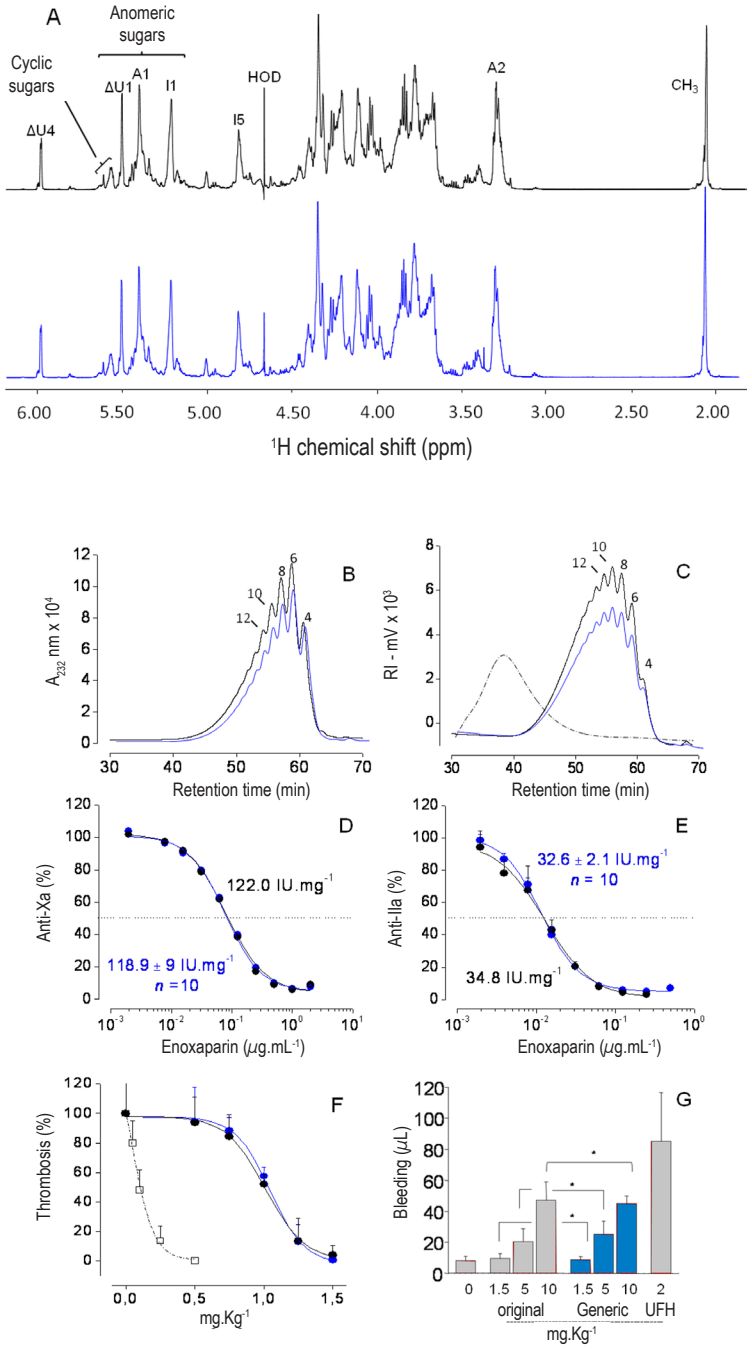

Figure 1 – *Comparative analysis between biosimilar versions of enoxaparin (in blue) and the original drug (in black). Panel A: approximately 20 mg of the samples were dissolved in 0.5 mL of 99.9% D_2O, and proton magnetic resonance spectroscopy (1D ^{1}H-NMR) at 800 MHz was recorded at 35°C. A, I and ΔU indicate residues of α-glucosamine N- and 6-disulfate, α-iduronic acid 2-sulfate, and 4,5-unsaturated hexuronic acid 2-sulfate at non-reducing ends, respectively. Panels B and C: gel-filtration chromatography with TSK G2000 and TSK G3000 columns, coupled to a HPLC system, and elution monitored by use of A_{232nm} (Panel B) and refraction index (RI) (Panel C). The numbers in the panels indicate elution of tetrasaccharide (4), hexasaccharide (6), octasaccharide (8), decasaccharide (10) and dodecasaccharide (12). In Panel C, the dotted line shows UFH elution in gel filtration. Panels D and E: determination of anti-Xa (Panel D) and anti-IIa (Panel E) activities by using the kinetic method. The numbers shown in the panels are mean ± SD for ten samples of biosimilar versions of enoxaparin compared with the original drug. Panel F: Antithrombotic activity in a venous model induced by hypercoagulant stimulus and vena cava stasis in rats. The values obtained for UFH are shown in the dotted line. Panel G: Assessment of the bleeding tendency induced by enoxaparin and UFH. The drugs were administered to rats through intravascular route, and, after five minutes, bleeding was assessed in the animals' tails based on extravasated blood. The results are expressed as mean ± SD. * indicates non-significant differences (p > 0.05) by use of T test. All methodologies used in those assays are described in references 3 and 4.*

Table 1 – Comparison between biosimilar preparations of enoxaparin and the original drug by use of ^{1}H-NMR, molecular weight distribution and in vitro* anticoagulant activity

		Original		Biosimilars	
		Mean ± SD	Number of batches	Mean ± SD	Number of batches
a) Integrals of the ^{1}H-NMR spectra (fig. 1A)	ΔU1	0.46 ± 0.02	3	0.48 ± 0.03	30
	ΔU4	0.25 ± 0.01	3	0.26 ± 0.02	30
	A1	1.00 ± 0.03	3	0.98 ± 0.03	30
b) Molecular weight distribution (% of the total) based on the refraction index (fig. 1C)	>8.000	12.4 ± 4.1 (<18)**	3	12.2 ± 3.7	19
	2.000-8.000	71.2 ± 3.8 (68-82)**	3	70.0 ± 3.3	19
	<2.000	16.3 ± 0.3 (12-20)**	3	17.7 ± 2.0	19
c) *in vitro* anticoagulant activity (fig. 1D and E)	Anti-Xa	122.0 ± 6.0	3	118.9 ± 9.6	10
	Anti-IIa	34.8 ± 1.5	3	32.6 ± 2.1	10

**Values are shown as mean ± SD. P > 0.05 for the comparison between biosimilar enoxaparin and original drug by using T test in items a, b and c.; **Values of the European and American Pharmacopeias (HTTP://www.usp.org/USPNF/notices/enoxaparinsodiuminjection.html.*

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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