Original Article

Comparison of Low-Molecular-Weight Heparins Prepared From Bovine Heparins With Enoxaparin

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Abstract

Heparin and its low-molecular-weight heparin (LMWH) derivatives are widely used clinical anticoagulants. These drugs are critical for the practice of medicine in applications including kidney dialysis, cardiopulmonary bypass, and in the management of venous thromboembolism. Currently, these drugs are derived from livestock, primarily porcine intestine. The worldwide dependence on a single animal species has made the supply chain for this critical drug quite fragile, leading to the search for other sources of these drugs, including bovine tissues such as bovine intestine or lung. A number of laboratories are currently examining the similarities and differences between heparins prepared from porcine and bovine tissues. The current study is designed to compare LMWH prepared from bovine heparins through chemical β-elimination, a process currently used to prepare the LMWH, enoxaparin, from porcine heparin. Using top-down, bottom-up, compositional analysis and bioassays, LMWHs, derived from bovine lung and intestine, are shown to closely resemble enoxaparin.

Keywords

bovine heparin, enoxaparin, chemical β-elimination, mass spectrometry, nuclear magnetic resonance spectroscopy, bioassay

Introduction

Heparin is a major clinical anticoagulant drug that is currently prepared from porcine intestinal mucosa in metric ton quantities.[1] Over half of the world's supply of heparin comes from China, and the demand for heparin continues to increase as modern medical procedures (ie, hemodialysis, open-heart surgery, treatment of thrombosis, and so on) requiring this critical drug have become more commonplace.[2] Moreover, heparin is the source material for the production of low-molecular-weight heparins (LMWHs) and anticoagulant/antithrombotic drugs commonly used in the management of venous thromboembolism,[3,4] further increasing the demand for heparin.

In 2007 to 2008, there was a contamination crisis in which crude porcine intestinal heparin produced in China was adulterated with oversulfated chondroitin sulfate.[2,5] This toxic, semisynthetic glycosaminoglycan led to a number of adverse effects including patient death through a hypotensive reaction driven by the release of bradykinin.[6] In response, the pharmacopoeial requirements for heparin and the level of regulatory oversight of heparin production have been enhanced,[7] putting further stress on the availability of heparin. More recently, there have been outbreaks of a number of diseases infecting swine, reducing the number of animals available for heparin production.[7,8] In July 2015, the US Food and Drug Administration urged heparin manufacturers to address the concerns of an unforeseen heparin supply by considering alternative sources of heparin, such as bovine tissues.[8] Bovine heparin had once been widely used but was voluntarily withdrawn from the

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US market in the 1990s as a result of the outbreak of bovine spongiform encephalopathy in Europe.[8]

Heparin and LMWHs are polypharmacological agents that pose a number of challenges in chemical, biological, and pharmacological analysis.[4,9] Over the past 25 years, there have been major technological advances in the chemical analysis of complex mixtures of polysaccharides, owing primarily to advances in nuclear magnetic resonance (NMR)[10-12] and mass spectrometry (MS).[13-17] These advances have led to bottom-up[14] and top-down analysis[15] of these complex mixtures of polysaccharides that allow the detailed comparison of heparins and LMWHs produced from different tissues, species, sources, and by different processes.[17-19]

In the current study, we examine an LMWH prepared from bovine intestinal and bovine lung heparins using a process commonly applied to produce the LMWH, enoxaparin (Lovenox), from porcine intestinal heparin. We compare Lovenox to the LMWHs prepared from both bovine intestinal and lung heparins.

Materials and Methods

Materials

The bovine heparins and bovine-derived LMWHs were obtained from Ronnsi (Jiangsu, China). Porcine intestinal heparin reference standard was obtained from the U.S. Pharmacopeial Convention (USP). Lovenox from Sanofi-Aventis (Bridgewater, New Jersey) was obtained from commercial suppliers. Unsaturated heparin disaccharide standards (0S: ΔUA (1 → 4) GlcNAc; NS: ΔUA (1 → 4) GlcNS; 6S: ΔUA (1 → 4) GlcNAc6S; 2S: ΔUA2S (1 → 4) GlcNAc; NS2S: ΔUA2S (1 → 4) GlcNS; NS6S: ΔUA (1 → 4) GlcNS6S; 2S6S: ΔUA2S (1 → 4) GlcNAc6S; and TriS: ΔUA2S (1 → 4) GlcNS6S, where ΔUA is 4-deoxy-β-L-*threo*-hex-4-enopyranosiduronic acid, GlcN is glucosamine, Ac is acetyl, and S is sulfo) were purchased from Iduron (Manchester, United Kingdom). Tributylamine was purchased from Sigma Chemical (St Louis, Missouri). Ammonium acetate, calcium chloride, acetic acid, water, and acetonitrile were of high performance liquid chromatography (HPLC) grade (Fisher Scientific, Springfield, New Jersey). Microcon YM-3 centrifugal filter units were obtained from Millipore (Bedford, Massachusetts). *Escherichia coli* expression and purification of recombinant *Flavobacterium heparinum* heparin lyase I, II, and III (Enzyme Commission #s 4.2.2.7, 4.2.2.X, and 4.2.2.8) were performed in our laboratory as described previously.[20] The LMWHs were desalted by dialysis using 1-kDa molecular weight cutoff dialysis tube (Spectrum Laboratories, California), lyophilized before NMR analysis, and redissolved in distilled water into stock solution (20 μg/μL) for liquid chromatography (LC) MS analysis.

Depolymerization of Bovine Heparins

The depolymerization of porcine and bovine heparins to prepare LMWHs has been described in detail previously.[3] Briefly, sodium heparin was converted into benzethonium salt of heparin, which was recovered by precipitation. This quaternary ammonium salt was dissolved in dichloromethane and benzylated with benzyl chloride. This heparin benzyl ester was recovered and treated with aqueous sodium hydroxide resulting in its alkaline depolymerization and debenzylation. Recovery by methanol precipitation and dialysis provided the LMWHs.

Potency Evaluation

To determine the potency of each of these agents, all bovine heparins and enoxaparins were supplemented in pooled normal human plasma and tested in a concentration range of 0 to 10 μg/mL. The amidolytic anti-IIa and anti-Xa assays were run on the ACL ELITE (Instrumentation Laboratory, Lexington, Massachusetts) using bovine Xa and human thrombin from Enzyme Research Laboratories (South Bend, Indiana). Chromogenic substrates, Spectrozyme Xa and thrombin (TH), were obtained from American Diagnostica (Stamford, Connecticut). The potency of the bovine heparins was calculated using the USP standard for heparin (lot FOI 187). The potency of each of the heparins was calculated based on the calibration curve prepared with the USP heparin standard. The potency of the enoxaparins was calculated using the National Institute for Biological Standards and Control (01/608; Potters Bar, England) standard.

Molecular Weight and Activity Assays

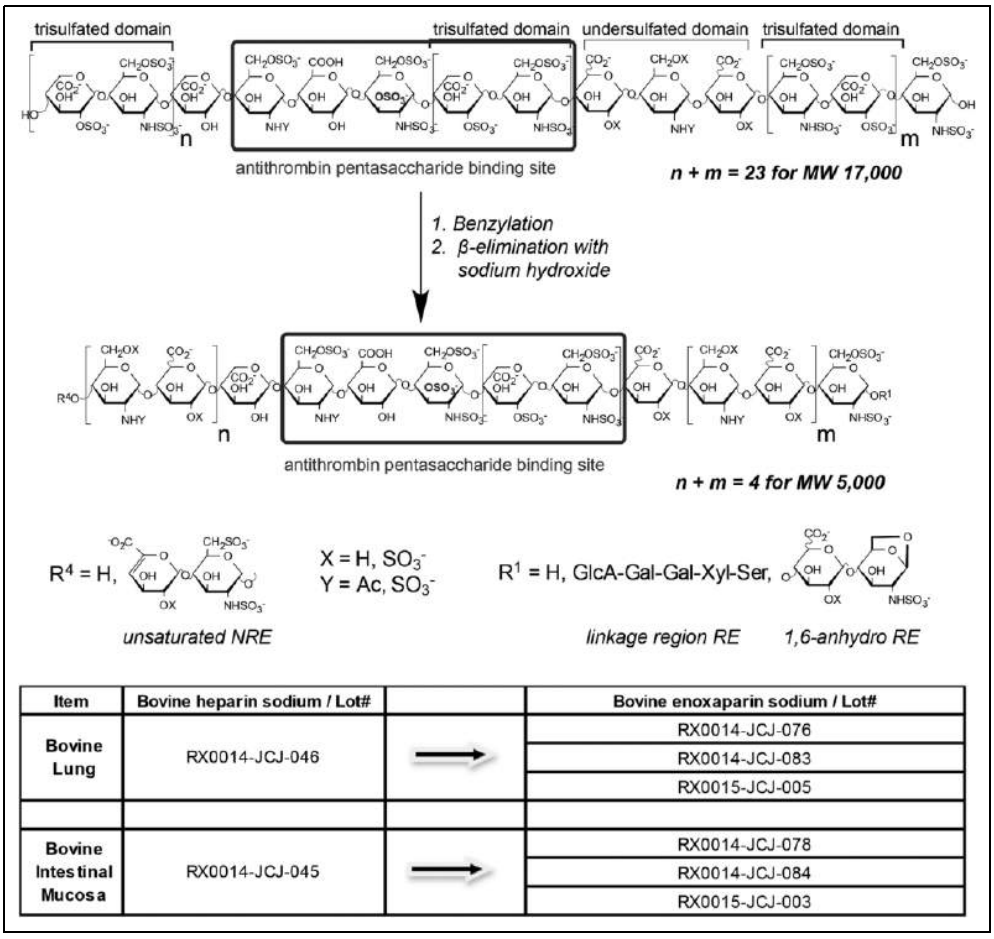
The molecular weight and activity assays for heparins and LMWHs followed USP methods.[21]

Disaccharide Analysis

Samples (100 μg) were added to 100 μL digestion buffer (50 mmol/L NH_4OAc, 2 mmol/L $CaCl_2$, pH 7.0). Heparin lyase I, II, and III (10 mU each in Tris–HCl buffer, pH 7.0) were added and mixed well. The samples were digested in 37°C water bath for 12 hours. The enzymatic digestion was terminated by removing the enzymes using a YM-3 centrifugal filter unit (Millipore, Billerica, MA). The filtrates were lyophilized and redissolved in distilled water at a concentration of 1 μg/μL. Reverse-phase, ion-pairing LC with online electrospray ion (ESI)-trap MS analysis was performed on an Agilent 1200 LC/MSD instrument (Agilent Technologies, Wilmington, Delaware) equipped with a 6300 ion-trap and a binary pump. Unsaturated disaccharide standard mixture solution was prepared (each at a concentration of 100 μg/μL) for relative quantification.

Bottom-Up Oligosaccharide Analysis of Heparin and LMWH Samples

Samples (100 μg) were added to 100 μL digestion buffer (50 mmol/L NH_4OAc, 2 mmol/L $CaCl_2$, pH 7.0). Heparin lyase II (10 mU in Tris–HCl buffer, pH 7.0) was added and mixed well. Samples were digested in 37°C water bath for 12 hours to produce fragments. Enzymatic digestion was terminated by



Item	Bovine heparin sodium / Lot#		Bovine enoxaparin sodium / Lot#
Bovine Lung	RX0014-JCJ-046	→	RX0014-JCJ-076
			RX0014-JCJ-083
			RX0015-JCJ-005
Bovine Intestinal Mucosa	RX0014-JCJ-045	→	RX0014-JCJ-078
			RX0014-JCJ-084
			RX0015-JCJ-003

Figure 1. Preparation of low-molecular-weight heparin analogue of enoxaparin from bovine tissue-sourced heparins.

removing the enzymes using a YM-3 centrifugal filter unit. The filtrates were lyophilized and redissolved in 100 μL of distilled water at a concentration of 1 μg/μL. Online hydrophilic interaction chromatography (HILIC) Fourier transform mass spectrometry (FTMS) was applied to analyze the oligosaccharide fragments.[14] A Luna HILIC column (2.0 × 50 mm^2, 200 Å; Phenomenex, Torrance, California) was directly connected online to the standard ESI source of LTQ-Orbitrap XL FTMS (Thermo Fisher Scientific, San Jose, California). Mass spectra were acquired at a resolution 60 000 with 200 to 1800 m/z range.

Top-Down Oligosaccharide Analysis of LMWH Samples

Online HILIC-FTMS was applied to analyze the intact chains.[15] A Luna HILIC column (2.0 × 150 mm^2, 200 Å; Phenomenex) was used. Mass spectra were acquired at a resolution 60 000 with 200 to 2000 Da mass range.

Bioinformatics for Bottom-Up and Top-Down Analysis

Charge deconvolution was autoprocessed by DeconTools software (web source from PNNL at http://omics.pnl.gov/). The LMWH structural assignment was done by automatic processing using GlycReSoft 1.0 software developed at Boston University (http://code.google.com/p/glycresoft/downloads/list).[22] A theoretical database was generated by GlycReSoft 1.0 as described previously.[15] All of the relative quantitative data were normalized to the total identified oligosaccharides peak area (in the format of percentage).

Nuclear Magnetic Resonance Analysis

One-dimensional (1D) proton and carbon NMR spectra were obtained at 600 MHz n D_2O exchanged heparin (20 mg/mL in D_2O) using a Bruker Advance II 600 MHz spectrometer (Bruker Bio-Spin, Billerica, Massachusetts) with Topspin

Table 1. Molecular Weight and Anticoagulant Activity Properties of Bovine Lung Heparin Sodium and Bovine Intestinal Mucosa Heparin Sodium.

Item	Lot#/ Specification	M_W 15 000-19 000 Da	M_{24000} NMT 20%	$M_{8000\text{-}16\,000}/M_{16\,000\text{-}24\,000}$ NLT 1.0	Anticoagulant Activity, IU/mg >180	Anti-Xa, IU/mg >180	Anti-IIa, IU/mg >180	Anti-Xa/anti-IIa 0.9-1.1
Bovine lung heparin sodium	RX0014-JCJ-046	**13 588** Da	8.90%	2.7	**167**	**135**	**130.6**	1.04
Bovine intestinal mucosa heparin sodium	RX0014-JCJ-045	16,417 Da	16.60%	2.1	**152**	**123**	**160.7**	**0.77**

[a]Numbers shown in bold fall outside U.S. Pharmacopeial Convention (USP) specifications for porcine intestinal heparin.
Note. NMT, no more than; NLT, no less than.

Table 2. Molecular Weight and Anticoagulant Activity Properties of LMWHs Generated From Bovine Lung Heparin Sodium and Bovine Intestinal Mucosa Heparin Sodium.[a]

Species/Organs	Lot#/ Specification	M_W 3800-5000 Da	$M_{<2000Da}$ 12%-20%	$M_{2000\text{-}8000Da}$ 68%-82%	$M_{>8000Da}$ NMT18%	1,6-anhydro 15%-25%	Anti-Xa, IU/mg 90-125	Anti-IIa, IU/mg 20-35	Anti-Xa/Anti-IIa 2.5-5.5
Bovine lung	RX0014-JCJ-076	3932 Da	19.30%	73.20%	7.50%	21.60%	110	**38**	2.9
	RX0014-JCJ-083	4118 Da	18.10%	73.00%	8.90%	**26.30%**	100	**36**	2.7
	RX0015-JCJ-005	4279 Da	16.30%	73.70%	10.00%	21.60%	102	**37**	2.8
Bovine intestinal mucosa	RX0014-JCJ-078	4501 Da	16.30%	72.10%	11.60%	19.10%	93	28	3.3
	RX0014-JCJ-084	4171 Da	18.10%	72.40%	9.50%	20.40%	83	25	3.3
	RX0015-JCJ-003	4186 Da	18.20%	72.00%	9.80%	19.50%	103	35	2.9

[a]Numbers shown in bold fall outside U.S. Pharmacopeial Convention (USP) specifications for enoxaparin.
Note. NMT, no more than.

2.1.6 software (Bruker Bio-Spin) for signal integration. Two-dimensional HSQC-NMR spectra were obtained at 800 MHz on D_2O exchanged heparin (20 mg/mL in D_2O) using a Bruker Advance II 600 MHz spectrometer.[12] Detailed experimental parameters are presented in the supporting information.

Results and Discussion

Pharmaceutical heparin has been historically prepared from the organs of livestock including pig (intestine), beef cattle (lung and intestine), and sheep (intestine).[17-19] The structure and activity of these heparins vary considerably. Most heparins are prepared from porcine intestine, and the current USP monograph defines its required chemical and biological properties.[21] Until the 1990s, bovine lung heparin was available in the United States; bovine intestinal heparin continues to be marketed in South America and is used in certain countries that restrict the import of porcine products.[19] Currently, available LMWHs are made exclusively from porcine intestinal heparin and are marketed worldwide. While an LMWH prepared from bovine lung heparin using chemical β-elimination has been examined,[17] no such study has yet been performed on the more commonly available bovine intestinal heparin. Furthermore, no comparison of LMWHs prepared from bovine lung and intestinal tissues has been made.

Bovine lung and bovine intestinal heparins were used to prepare LMWHs through a β-elimination method based on using the Lovenox process (Figure 1). Bovine lung heparin had a lower molecular weight (M_W 13 588 Da) than bovine intestinal heparin (M_W 16 417), which fell within the USP molecular weight specifications for porcine intestinal heparin (Table 1). Both bovine heparin products fell outside the USP activity specifications for porcine heparin in various assays. The bovine intestinal heparin exhibited a much lower anti-factor Xa activity than either porcine intestinal or bovine lung heparins. However, in the anti-IIa assays, the bovine intestinal mucosal heparin showed much higher anti-IIa activity. Similar differences, between porcine intestinal, bovine lung, and bovine intestinal heparins, had been previously reported by our laboratory.[17,18] Three independent batches of LMWHs were prepared from a single lot of both bovine lung and bovine intestinal heparin using a chemical β-eliminative process modeled on that thought to be used for the preparation of Lovenox (Figure 1). The molecular weight and activity properties of these 6 batches of LMWH were examined (Table 2). Two of the batches of LMWH, prepared for bovine lung heparin, met all specifications for Lovenox, and 1 batch was slightly out of the USP specifications for 1,6-anhydro content (Table 2). All 3 batches of LMWH prepared from bovine intestinal heparin met the Lovenox molecular weight and 1,6-anhydro content USP specifications. All 3 batches of bovine lung enoxaparin met the USP specifications for the anti-Xa and anti-IIa activities. Two of the batches of bovine intestinal mucosal were within the

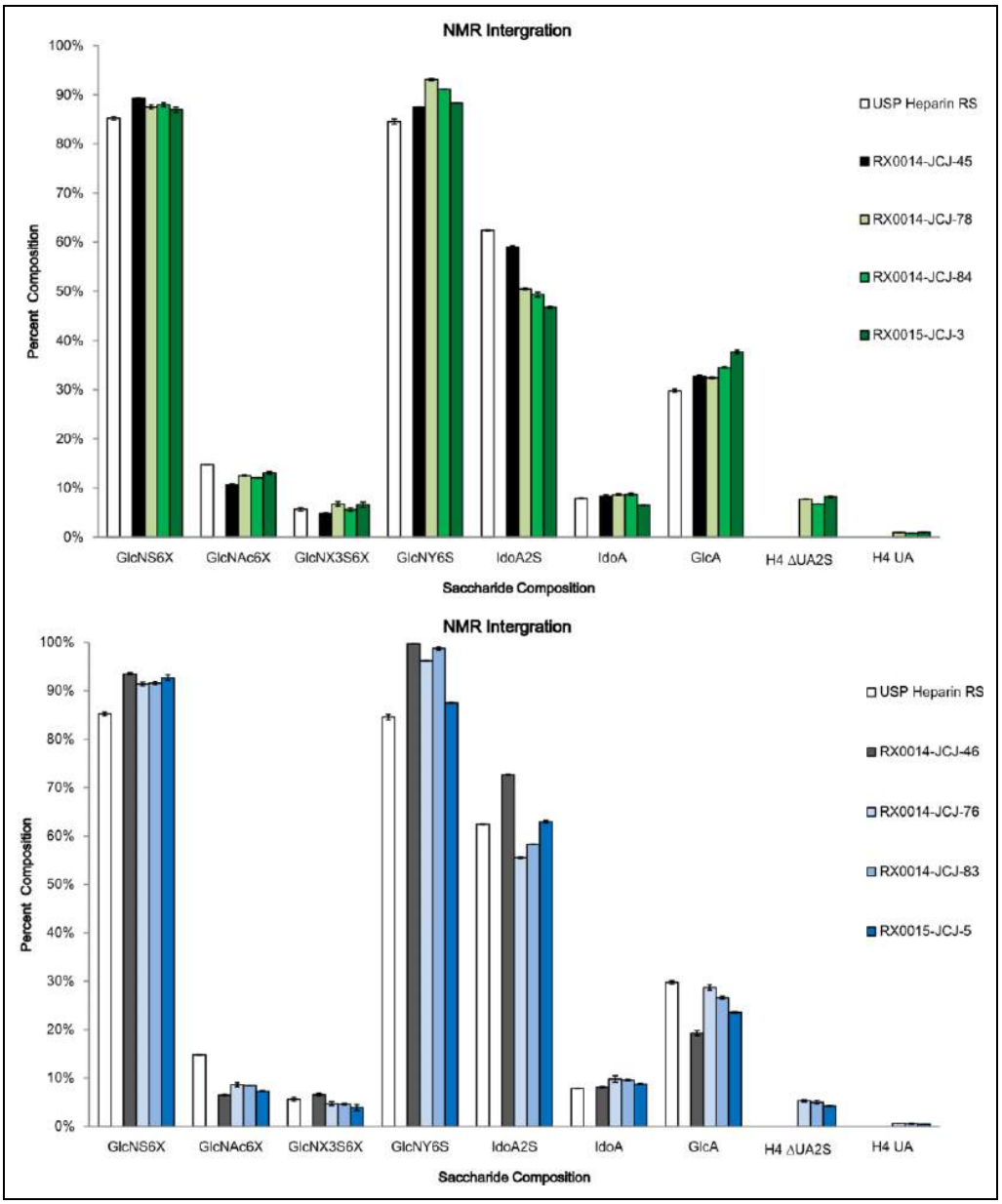


Figure 2. Composition by nuclear magnetic resonance (NMR) integration. The error bars indicate the analytical variability in the method.

USP specifications for the anti-Xa activity, while 1 was lower. A wide variation in the anti-IIa activity was noted among the 3 batches of bovine intestine mucosa enoxaparin. However, the anti-Xa/anti-IIa were also within the calculated range.

In the functional assays, the bovine lung heparin exhibited higher activity in the activated partial thromboplastin time (APTT) assay, whereas the bovine mucosal heparin showed lesser activity. Interestingly, the anti-Xa activities of the bovine lung and mucosal heparin followed a similar trend; however, in the anti-IIa assay, the bovine intestinal mucosal heparin showed a much higher activity, which may be due to the molecular composition of a higher proportion of the high-molecular-weight components. Regardless of these differences in the unfractionated heparin, the 3 batches of enoxaparin derived

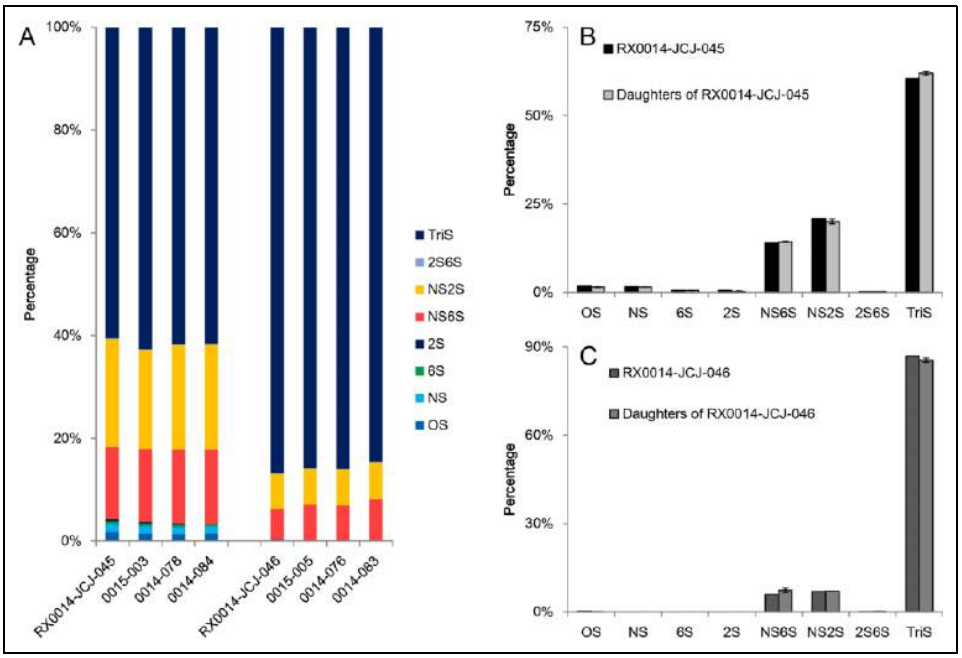


Figure 3. Disaccharide analysis by liquid chromatography mass spectrometry (LC-MS). A, Overall bar chart. B and C, Parent and daughter comparison bar chart.

from the bovine lung and mucosal heparins were comparable. Although the intestinal heparin-derived enoxaparins were slightly weaker, they still were in the range of the specifications.

The observed variation in one of the batches of bovine lung heparin for the anhydromanno content was only 1.3%, which may have been due to the process and can be controlled. The bovine intestinal mucosa preparations exhibited comparable anti-Xa and anti-IIa activities, with the exception of 1 batch showing relatively lower anti-Xa and anti-IIa activities. This may also be related to the process used.

Uronic Acid and Substituted Glucosamine Composition

Top-down NMR analysis provides information on composition at the monosaccharide level (Figure 2). Each parent heparin was compared with the USP heparin reference and their derived LMWHs. Quantitative analysis using 1D NMR[12] was used to determine the content of GlcA, IdoA, IdoA2S, ΔUA, and ΔUA2S of each enoxaparin product. The GlcNS6X peak was integrated from 3.26 to 2.99 ppm, the GlcNAc6X peak was integrated from 1.82 to 2.07 ppm, the GlcNX3S6X peak was integrated from 3.78 to 3.72 ppm, and the GlcNY6S peak was integrated from 66.72 to 65.6 ppm and 61.26 to 60.57 ppm on the carbon spectra. The IdoA2S peak was integrated from 5.21 to 5.01 ppm, the IdoA peak was integrated from 4.97 to 4.90 ppm, the GlcA peak was integrated from 3.26 to 3.10 ppm, the ΔUA2S peak was integrated from 6.32 to 6.19 ppm, and the ΔUA peak was integrated from 6.13 to 6.06 ppm. These peaks and this temperature (25°C) were selected to provide minimum spectral overlap and ensure the most accurate integration values. ΔUA and ΔUA2S were only present in the LMWHs. Parent RX0014-JCJ-46 showed lower GlcA and higher IdoA2S levels than USP heparin reference, which was not obvious on RX0014-JCJ-45 (Figure 2). The daughter LMWHs demonstrated lower GlcA and higher IdoA2S levels than the daughters of RX0014-JCJ-45. GlcNS6X in parent RX0014-JCJ-46 and its daughters were more than 90%, which is higher than other samples.

Disaccharide Analysis

By disaccharide analysis (Figure 3), 2 parent heparins have significantly different compositions. There are few monosulfated disaccharides in parent RX0014-JCJ-46 and its daughter LMWHs, which is consistent with the NMR results (higher GlcNS6X and higher IdoA2S; Figure 3A). In addition, parent heparins and their daughter LMWHs were highly correlated with disaccharide composition (Figure 3B and C). The comparison of bovine LMWHs and Lovenox disaccharide composition is shown in Figure S1; although the NS2S disaccharide is higher in LMWHs derived from RX0014-JCJ-045, the total 2-O-S substitution is about the same due to the low abundance of TriS disaccharide. The substitution results calculated from disaccharide analysis (Figure S1B) were consistent with the NMR analysis.

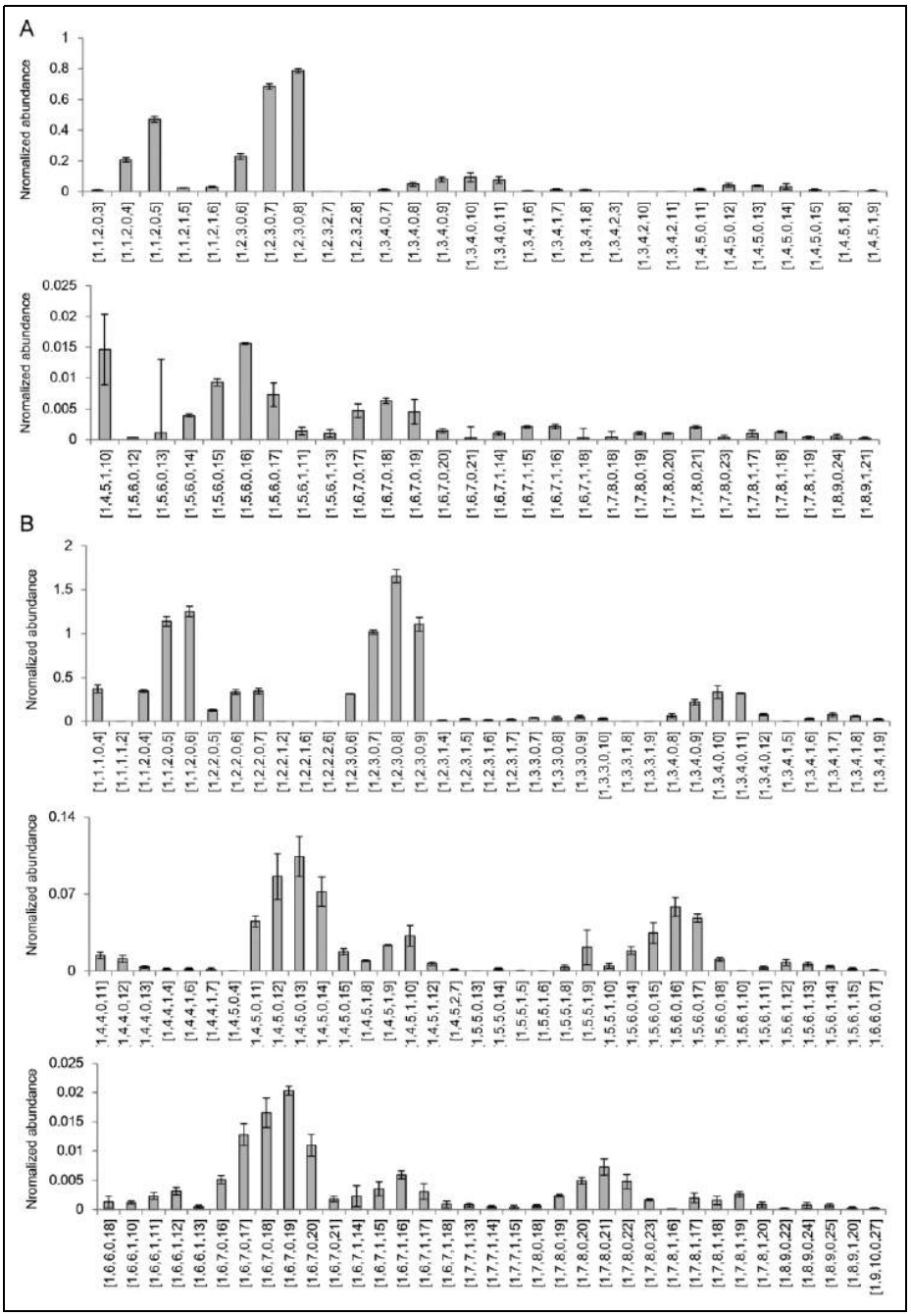


Figure 4. Top-down analysis of low-molecular-weight heparins (LMWHs). For the 3 daughters of RX0014-JCJ-045, intact chains detected in top-down analysis whose (A) reducing ends are 1,6-andyro, (B**)** nonreducing ends are unsaturated, and (C) nonreducing ends are saturated. The error bars indicate sample variability.

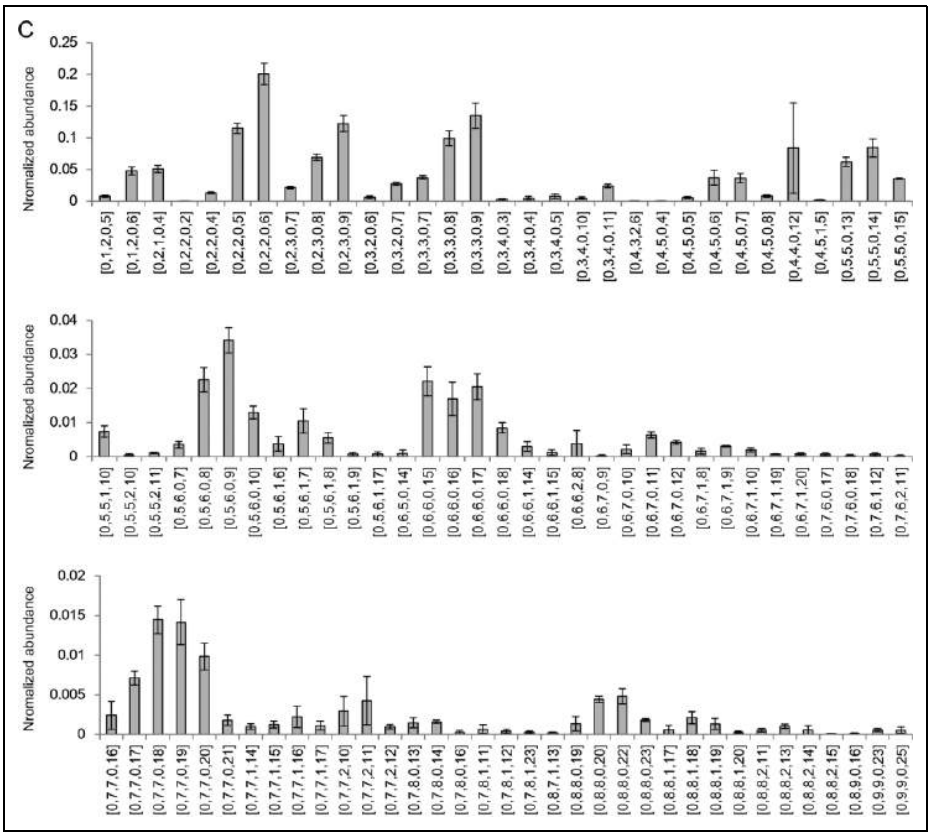


Figure 4. (continued).

Top-Down Analysis

In the top-down analysis of LMWHs, daughters of RX0014-JCJ-045 intact chain composition (261 species shown in Figure 4) and daughters of RX0014-JCJ-046 intact chain composition (137 species shown in Figure S2) were significantly different. A species was considered identified only when all 3 lots showed a peak corresponding to that species. The bovine lung heparin-derived LMWH contained a number of very minor species not observed in the other 2 samples. Since only the identified components present in all 2 different LMWHs could be compared, the LMWHs derived from porcine intestine and bovine intestine contained approximately the same number of species, while LMWH derived from bovine lung heparin contained fewer species. Compared to Lovenox (283 species shown in Figure S3), daughters of RX0014-JCJ-045 had similar intact chain composition showing high complexity. The comparison of Lovenox and the daughters of RX0014-JCJ-045 is shown in Figure 5. For this comparison, components in low abundance were neglected; abundant components present in Lovenox and both of the bovine LMWHs comprise 157 species in total, which means there is a similarity between bovine and porcine LMWHs. Meanwhile, the normalized abundance of highly sulfated oligosaccharides ([1,4,5,0,11]-[1,4,5,0,15], where the saccharide structure is listed as [ΔHexA, HexA, HexN, Ac, SO_3^-]), was higher in the bovine LMWHs, while the normalized abundance of highly acetylated oligosaccharides ([1,4,5,1,10]-[1,4,5,1,12] and [1,6,7,1,15]-[1,6,7,1,18]) are higher in Lovenox. The information shown in intact chain composition cannot be seen clearly by disaccharide analysis and NMR monosaccharide analysis, since comparisons using top-down analysis were more specific for oligosaccharides identical on substitution condition.

Terminal Structures

Enoxaparin terminal structures were complicated due to the alkaline β-eliminate cleavage, where 1,6-anhydro hexosamine reducing ends (REs; about 20%) and 4,5-unsaturated uronic acid nonreducing ends (NREs; 100%) are generated.[23,24] The original RE and NRE structures, which were linkage region oligosaccharides (-GlcA/IdoA-GlcNAc-GlcA-Gal-Gal-Xyl-O-Serine) and saturated uronic acid, respectively, from parent heparins, still remain. Comparison of the terminal structures is shown in Figure 6. The normalized abundance of linkage

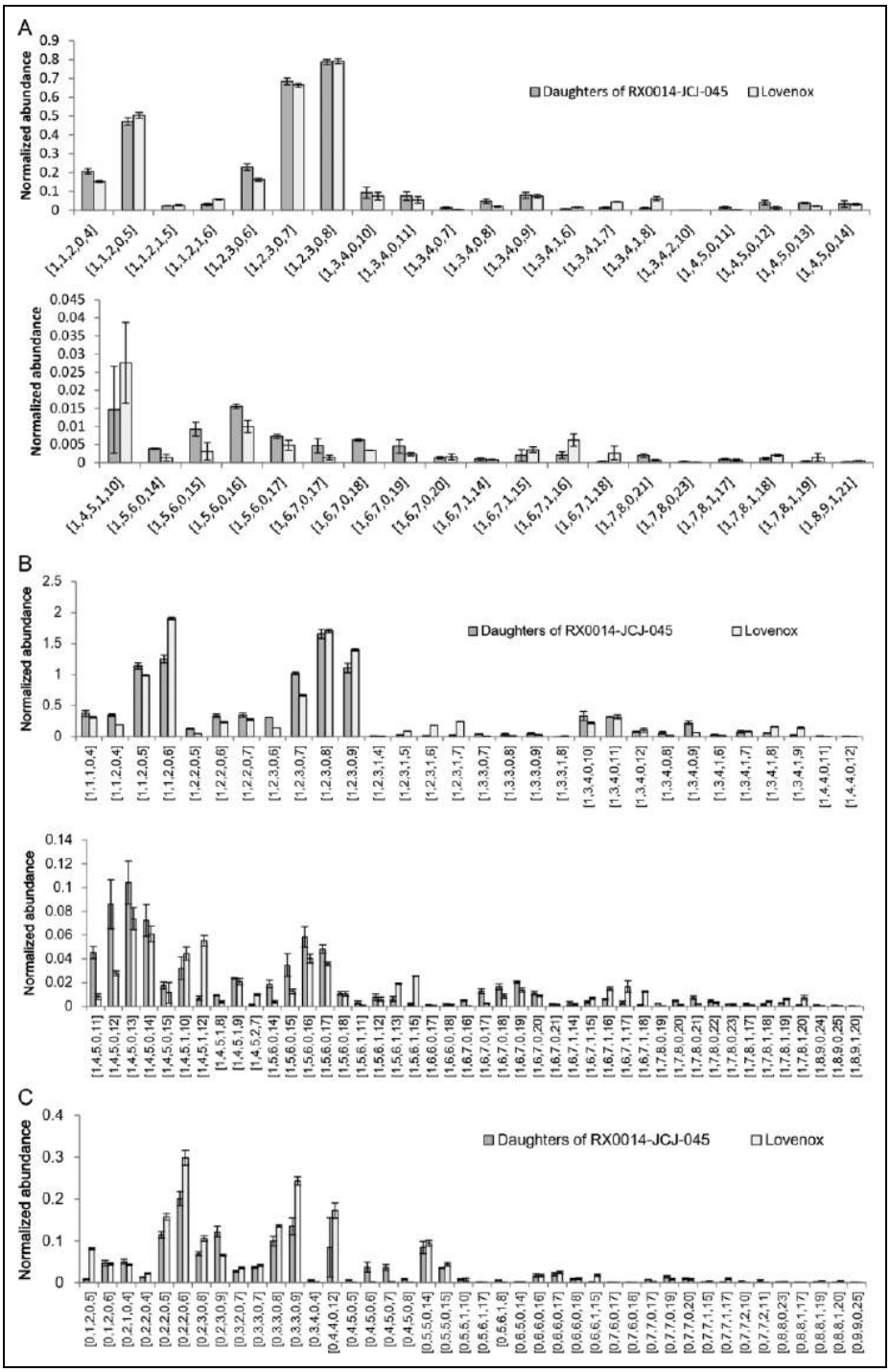


Figure 5. Top-down analysis of low-molecular-weight heparins (LMWHs). Comparison of daughters of RX0014-JCJ-045 and Lovenox about intact chains detected in top-down analysis whose (A) reducing ends are 1,6-anhydro, (B) nonreducing ends are unsaturated, and (C) nonreducing ends are saturated.

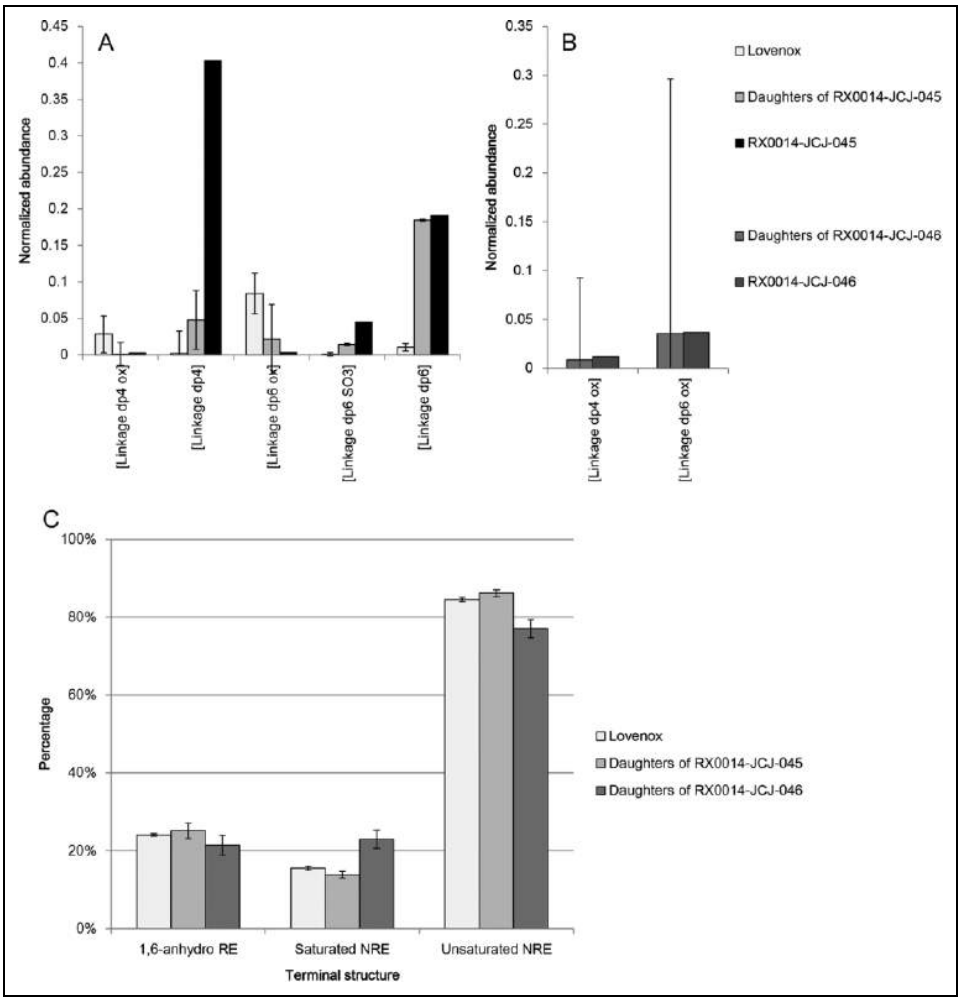


Figure 6. A and B, Comparison of the oligosaccharides generated from heparin–protein linkage region (bottom-up analysis). C, Comparison of the daughters and Lovenox on terminal structure composition (top-down analysis). Error bars on Lovenox indicate the analytical error, and error bars on the daughters indicate sample variability (Linkage dp4 ox: ΔUA-Gal-Gal-Xyl-O-Ser$_{OX}$; Linkage dp6 ox: ΔUA-GlcNAc-GlcA-Gal-Gal-Xyl-O-Ser$_{OX}$; Linkage dp4: ΔUA-Gal-Gal-Xyl-O-Ser; Linkage dp6: ΔUA-GlcNAc-GlcA-Gal-Gal-Xyl-O-Ser; and Linkage dp6: ΔUA-GlcNAc (S) -GlcA-Gal-Gal-Xyl-O-Ser).

region oligosaccharides was obtained by bottom-up analysis (whole comparison shown in Figures S4 and S5). For RX0014-JCJ-045 and its daughter LMWHs, a comparison with Lovenox was performed (Figure 6A). During the manufacture of LMWHs, the serine could be oxidized into -CH_2COO^- as was demonstrated previously.[14,25,26] The amount of linkage dp4 without oxidization is less in the daughter LMWHs than in their parents, while under the same conditions, levels of dp6 are correlated for parent and daughter samples. For heparin RX0014-JCJ-046 and its daughters (Figure 6B), only 2 types of linkage region oligosaccharides were detected, and the variability between the daughters was large. Comparison of the 3 major terminal structures detected in the top-down analysis is shown in Figure 6C; daughters of RX0014-JCJ-045 are similar with Lovenox, which is consistent with the top-down analysis results. The percentage of saturated NRE oligosaccharides is high in daughters of RX0014-JCJ-046, which may be due to the reduced chain length of the parent (heparin RX0014-JCJ-046), leading to more saturated uronic acids remaining during the LMWH processing.

Conclusion

Enoxaparin represents the most widely used LMWH, which is derived by the depolymerization of porcine mucosal heparin. Compositional differences among the heparins from various

sources are known, however, in the biologically cross-referenced potency against a reference; the potency-adjusted heparins exhibit similar biologic activities. Microchemical differences such as the one reported in this article may contribute to the pharmacologic profile of heparins; however, the depolymerized product, such as enoxaparin obtained from bovine tissues, were found to be comparable. Thus, while bovine unfractionated heparins in comparison to porcine heparin may exhibit differential functional and structural profiles, such differences are minimal in the depolymerized products. Therefore, LMWHs produced by various manufacturing processes may result in comparable products when bovine or porcine unfractionated heparin is used.

The studies reported in this article suggest that depolymerized LMWHs such as enoxaparin can be derived from bovine lung and intestinal mucosal heparins. Although the potency for both the bovine lung and mucosal heparins is lower than the porcine mucosal heparin, the enoxaparin derived from these sources exhibits similar potency in the anti-Xa and anti-IIa assays in comparison to the commercially available porcine enoxaparin preparations. These studies underscore the hypothesis that depolymerized LMWHs such as enoxaparin can be derived from both bovine lung and intestinal mucosal sources and may exhibit bioequivalence. These studies also suggest that other commercially available LMWHs such as dalteparin and tinzaparin, which are produced by depolymerization of porcine heparin, can also be derived by using bovine heparin and may be biosimilar to their porcine counterpart. Additional in vivo validation of these observations is warranted for a stepwise development of depolymerized LMWHs obtained from nonporcine species.

Authors' Note

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Declaration of Conflicting Interests

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Supplemental Material

Supplementary material for this article is available online.

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