

A review of the two major regulatory pathways for non-proprietary low-molecular-weight heparins

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Summary

With the expiry or pending expiry of originator low-molecular-weight heparin (LMWH) patents, pharmaceutical companies have invested in developing non-proprietary versions of LMWHs. LMWHs are manufactured by depolymerising highly purified unfractionated heparin. In contrast to traditional synthetic drugs with well-defined chemical structures, LMWHs contain complex oligosaccharide mixtures and the different manufacturing processes for LMWHs add to the heterogeneity in their physicochemical properties such that the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) consider existing originator LMWHs to be distinct medicinal entities that are not clinically interchangeable. The FDA views LMWHs as drugs and has approved two non-proprietary (generic) LMWHs, using the Abbreviated New Drug Application pathway. In contrast, the World Health Organization and the EMA view LMWHs as biological medicines. Therefore, the EMA and also the Scientific and Standardization Subcommittee on

Anticoagulation of the International Society on Thrombosis and Haemostasis and the South Asian Society of Atherosclerosis and Thrombosis have all published specific guidelines for assessing non-proprietary (biosimilar) LMWHs. This manuscript reviews why there are two distinct pathways for approving non-proprietary LMWHs. Available literature on non-proprietary LMWHs approved in some jurisdictions is also reviewed in order to assess whether they satisfy the requirements for LMWHs in the three guidance documents. The review also highlights some of the significant difficulties the two pathways pose for manufacturers and an urgent need to develop a consensus governing the manufacture and regulation of non-proprietary LMWHs to make them more widely available.

Keywords

Low-molecular-weight heparins, biosimilars, biologics, patents, venous thromboembolism

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Introduction

Low-molecular-weight heparins (LMWHs) are antithrombotic drugs approved in various jurisdictions for clinical indications including venous thromboembolism (VTE) prophylaxis and treatment and acute coronary syndromes (ACS) (1–4). The original patents of some originator LMWHs have expired or are about to expire and, consequently, non-proprietary LMWHs are being developed. LMWHs are complex mixtures of highly sulfated oligosaccharides for which even minor alterations in the manufacturing process can cause significant physicochemical changes (5–11). Additionally, their pharmacokinetic parameters are not measured directly but are inferred from their measurable pharmacodynamic parameters. Further, the relationships between pharmacokinetic and pharmacodynamic parameters vary between LMWHs (12, 13) and may impact the development and assessment of non-proprietary LMWHs.

The US Food and Drug Administration (FDA) views heparin and LMWHs as drugs and thus classify a non-proprietary version

of an approved originator LMWH as a generic LMWH (14). In contrast, the European Medicines Agency (EMA) views heparin and LMWHs as biologics (14), and classify non-proprietary versions of originator LMWHs as biosimilar LMWHs. In order to address the perceived complexities surrounding their market approval, the EMA published specific guidelines for assessing biosimilar LMWHs in 2009 (15), as have the Scientific and Standardization Subcommittee on Anticoagulation of the International Society on Thrombosis and Haemostasis (ISTH) (16) and the South Asian Society of Atherosclerosis and Thrombosis (SASAT) (17). A comparable FDA guideline document for LMWHs is not yet available and instead the FDA has used an Abbreviated New Drug Application (ANDA) pathway for generic versions of chemically synthesised drugs to approve two generic LMWHs in 2010 and 2011 (18, 19). This manuscript reviews why there are two distinct pathways for regulating non-proprietary LMWHs and the potentially unnecessary hurdles faced by a manufacturer aiming to enter both the US and European Union (EU) markets. Additionally, available literature on non-proprietary LMWHs approved in several juris-

Thrombosis and Haemostasis 107.2/2012

ditions is reviewed in order to assess the extent to which they satisfy the requirements found in the three biosimilar LMWH guidelines.

LMWHs: A brief historical perspective

The antithrombotic activities of unfractionated heparin (UFH) were reported early in the 20th century (20, 21). In 1976, investigators at the National Institute for Biologic Standardization and Control (NIBSC) reported that gel filtration of UFH yielded a fraction with a lower average molecular weight of 9,000 Da (compared to 15,000 Da for UFH) and a lower anticoagulant potency than UFH (22). This fraction also catalysed antithrombin-mediated inactivation of both thrombin and factor Xa but had lower catalytic activities than UFH, i.e. it had a weaker ability than UFH, per mg, to prolong the activated partial thromboplastin time (APTT) and to catalyse the inactivation of factor Xa and thrombin by antithrombin (22). Further, when injected into healthy volunteers, the bioavailability of this LMWH fraction was at least three times greater than that of UFH. This LMWH fraction also had a significantly longer half-life than UFH, and the LMWH present in *ex vivo* plasmas catalysed antithrombin-mediated factor Xa inhibition (anti-factor Xa activity) longer than thrombin inhibition (antithrombin [factor IIa] activity) (22). These observations indicated that if LMWHs had antithrombotic activity, their longer half-life and greater bioavailability potentially made LMWHs more convenient for clinical use than UFH. Subsequent studies conducted in animals, healthy human volunteers and patients established the antithrombotic properties of LMWHs (22–28).

Following several phase 3 trials that demonstrated their superiority over placebo (many involving relatively small numbers of patients), some LMWHs have been approved in various jurisdictions for a range of cardiovascular indications, including thromboprophylaxis and treatment of VTE and ACS as listed in ► Table 1 (1–4, 25–50). There are now at least eight approved originator LMWHs with their own international non-proprietary names (INNs) (► Table 2) (12, 51–54), including enoxaparin (Lovenox), dalteparin (Fragmin), nadroparin (Fraxiparin), reviparin (Clivarin) and tinzaparin (Innohep). The original patents of several LMWHs have expired or are about to expire in some jurisdictions, prompting strong interest in the manufacture of non-proprietary versions of LMWHs. Classical generic drugs are non-proprietary synthetic drugs with identical chemical structures as the originator drugs. LMWHs are complex mixtures of highly sulfated oligosaccharides (55). As noted previously, the distinction between classification of non-proprietary LMWHs as generic or biosimilar versions arises from the FDA viewing them as drugs, whereas the World Health Organization (WHO) and EMA view them as biologics (14). To further cement this difference, the US Congressional legislation, passed in March 2010, that defines a “biologic” specifically excludes carbohydrate-derived medicines from the list of medicinal substances deemed to be biologic in nature in the USA (56). These two different viewpoints on the nature of

LMWHs lead to two distinct pathways for approving non-proprietary versions of LMWHs by the FDA and the EMA.

Non-proprietary drug development in the USA

The FDA has issued regulatory guidelines, essentially based on the Hatch-Waxman Act (The Drug Price Competition and Patent Term Restoration Act of 1984), that delineate the steps necessary for establishing the clinical equivalence of synthetic generic and related originator drugs (57). As recently summarised by Frank (58), the Hatch-Waxman Act creates an abbreviated approval process (an ANDA) for synthetic generic drugs. By demonstrating that a generic and its originator drug are chemically identical, the generic drug manufacturer can utilise the registration data provided by the originator drug manufacturer to seek marketing approval for the generic drug without the need for large-scale clinical trials (57, 58). The tremendous growth in the number of generic drug manufacturers since the passing of the Hatch-Waxman Act, the exponential growth in the use of generic drugs, and the growth in the investment for research and development by US pharmaceutical companies from \$26 billion in 2000 to about \$43 billion in 2006 (59) demonstrate that the goal of bringing about price competition in prescription medicines as envisaged by the Act has been accomplished. A recent study by the Blue Cross Blue Shield of Michigan provides data showing that the cost savings associated with a switch from branded (originator) to generic drugs vary from 23.7% (for the immunosuppressant Prograf versus its generic tacrolimus) to 98.9% (for valium versus its generic diazepam) (60).

The FDA used the ANDA pathway to approve two generic enoxaparins in 2010 and 2011 (18, 19). FDA approval of the first generic LMWH in 2010 required determining whether the complex chemical nature of a LMWH allowed the FDA to accept an ANDA for generic LMWHs. A second challenge was demonstrating the “sameness” of a generic and the originator LMWH, and evaluating the immunogenicity of a generic LMWH (18). Immunogenicity is an important consideration, as heparin-induced thrombocytopenia occurs in a minority of patients treated with LMWHs with potentially serious adverse clinical outcomes (60–63). Finally, the potential for contamination of the heparin source by the presence of oversulfated chondroitin sulfate had to be considered (18). If present in the UFH used to manufacture LMWHs, oversulfated chondroitin sulfate would survive unchanged in LMWHs produced by depolymerisation with nitrous acid (e.g. dalteparin), treatment with heparinase 1 (e.g. tinzaparin), or periodate (e.g. centaxarin). Thus, these three types of LMWH would be contaminated with intact oversulfated chondroitin sulfate. In contrast, any oversulfated chondroitin sulfate present in UFH depolymerised by β -elimination (e.g. enoxaparin) or hydrogen peroxide (e.g. parnaparin) would be partially and fully depolymerised, respectively (64). FDA scientists used five criteria to establish that the generic enoxaparin contained the *same* active ingredient as its originator. The active ingredient in the originator enoxaparin (Lovenox) has not yet been

Table 1: Recommended uses of LMWH from the ACCP and AHA/ACC guidelines (1–4, 29).

Patients and procedure	Patient risk status	Setting and duration	Recommendation
Thromboprophylaxis – surgical patients			
Major general or gynaecologic surgery for benign disease or cancer	Moderate risk to higher risk	Inpatient	LMWH (Grade 1A)
General surgery	High risk, e.g. has undergone major cancer surgery or previously had VTE	≤28 days	LMWH (Grade 2A)
Gynaecologic surgery			LMWH (Grade 2C)
Major vascular surgery, laparoscopic procedures or burn victims	Additional thromboembolic risk factors	Inpatient	LMWH (Grade 1C)
Arthroscopic knee surgery			LMWH (Grade 1B)
Major, open urologic surgery; bariatric surgery; major thoracic surgery; CABG			LMWH (Grade 1C)
HFS	No additional thromboembolic risk factors stated	Inpatient	LMWH (Grade 1B)
THR or TKR			LMWH (Grade 1A)
TKR or HFS		≥10 days	
THR		≥10 days, ≤35 days	
TKR or HFS			LMWH (Grade 1C)
Thromboprophylaxis – medical patients			
Acutely ill medical patients with CHF or SRD	Confined to bed plus additional thromboembolic risk factors (including previous VTE, sepsis, IBD or acute neurologic disease)	Inpatient	LMWH (Grade 1A)
VTE treatment			
Acute DVT	No additional thromboembolic risk factors stated	≥5 days and until the INR is >2.0 for 24 hours	LMWH SC OD or BID (Grade 1C)
		Outpatient (if possible), instead of IV UFH	Initial treatment with LMWH SC OD or BID (Grade 1C)
		Inpatient (if necessary), instead of IV UFH	Initial treatment with LMWH SC OD or BID (Grade 1A)
DVT	Cancer	Outpatient, 3 to 6 months	LMWH (Grade 1A)
		Outpatient, indefinitely or until the cancer is resolved	LMWH (Grade 1C)
PE	No additional thromboembolic risk factors stated	Inpatient	LMWH SC (Grade 1C)
Ancillary therapy in ACS			
UA/NSTEMI patients – invasive or conservative strategy	No additional thromboembolic risk factors stated	Inpatient	Enoxaparin* (Class 1, LOE A)
STEMI patients – fibrinolysis			Enoxaparin* (Class 1, LOE A)
STEMI patients – PCI			Enoxaparin* (Class 1, LOE B)

*Enoxaparin is the only LMWH indicated in ACS. ACCP, American College of Chest Physicians; ACC, American College of Cardiology; ACS, acute coronary syndromes; AHA, American Heart Association; BID, twice daily; CABG, coronary artery bypass graft; CHF, congestive heart failure; DVT, deep vein thrombosis; HFS, hip fracture surgery; IBD, inflammatory bowel disease; INR, international normalized ratio; IV, intravenous; LMWH, low-molecular-weight heparin; LOE, level of evidence; OD, once daily; PCI, percutaneous coronary intervention; PE, pulmonary embolism; SC, subcutaneous; SRD, severe respiratory disease; STEMI, ST-elevation myocardial infarction; THR, total hip replacement; TKR, total knee replacement; UA/NSTEMI, unstable angina/non-ST-elevation myocardial infarction; UFH, unfractionated heparin; VTE, venous thromboembolism.

defined. The criteria used by the FDA included demonstrating equivalence in 1: heparin source material and mode of depolymerisation; 2: physicochemical properties; 3: disaccharide building blocks, fragment mapping, and sequences of the oligosaccharide species; 4: biological and biochemical assays; 5: *in vivo* pharma-

dynamic profiles in healthy human volunteers (65). The last criterion is an important modification to the normal ANDA pathway as the FDA normally does not require any clinical data to approve non-proprietary versions of patented drugs. The FDA has concluded that the five criteria above are sufficient to ensure that the

two generic enoxaparins it has approved have the *same* active ingredient as originator enoxaparin, and therefore no additional clinical studies are necessary to demonstrate the equivalence of their clinical effectiveness and safety in all the clinical indications for which Lovenox has marketing approval in the USA (18, 19).

Physicochemical methods are available for identifying the animal sources and modes of depolymerising heparins into LMWHs, the percent composition of disaccharide building blocks, the sequences of oligosaccharide species in and fragment mapping of LMWHs, and impurities, if any, in LMWH preparations (9–11, 64, 66–70). The methods their manufacturers used to establish the chemical “sameness” of Lovenox and its two generic versions the FDA approved have not been disclosed.

Reasons for a biosimilar pathway for regulatory approval of non-proprietary LMWHs

As noted previously, the EMA asserts that LMWHs are biological medicines and that making identical copies of biological molecules is fraught with many difficulties and, thus, EMA considers copies of LMWHs as biosimilars. This position has led the EMA to require potentially extensive clinical trials to establish equivalency of clinical effectiveness and safety of biosimilar and originator LMWHs (15, 71).

Biologic medicines have active ingredients that are isolated from animal tissues, human plasmas or are made by recombinant DNA technology. Therapeutic proteins and other biologic medicines, such as LMWHs, may have one or more biologic activities in humans, and the relative contribution of each chemical moiety to the clinical effects and safety profile of each originator LMWH is unknown. Thus, without modifications, such as the requirements for comparative pharmacodynamic parameters and immunogenicity (65), the FDA's ANDA may not necessarily be an ideal route for establishing the safety and bioequivalence or otherwise of therapeutic agents as complex as LMWHs and recombinant therapeutic proteins (15, 57, 72–74). For example, it is well recognised that even minor changes in the formulations in which recombinant therapeutic proteins are dissolved can have severe clinical consequences (75, 76).

The omission of heparin and LMWHs from the definition of a “biologic” found in the 2010 US Congressional Legislation (56) clearly implies that the new legislated abbreviated pathway for approving follow-on biologics (i.e. biosimilars) in the USA cannot be applied to LMWHs. Guidelines for the authorisation of subsequent-entry biologics (i.e. biosimilars), also strictly applicable to the regulation of biologics with protein-based active substances, have been released by Health Canada (77). Additionally, the EMA has published specific guidelines detailing the necessary steps to be followed to characterise biosimilar proteins that are produced by recombinant DNA technology before they may receive regulatory approval (71). The WHO has also published guidelines aiming to provide globally acceptable principles for the evaluation and licensing of similar biotherapeutic products (SBPs) (78). The WHO guidelines also apply to well-established and well-characterised

biotherapeutic products, such as recombinant DNA-derived therapeutic proteins, and which, by the WHO definition, includes products defined as “biosimilar”, “follow-on” and “subsequent-entry”. The six key principles identified for licensing SBPs are 1: the requirement for stepwise comparability exercises, in particular demonstration of similar quality characteristics; 2: demonstrated similarity in quality, clinical and non-clinical parameters; 3: if there are any differences in these parameters, a product will not qualify to be licensed as an SBP; 4: likewise, a product will not be an SBP if comparability exercises are not conducted; 5: that SBPs are not generic medicines; and 6: that effective regulatory control is needed to manage any risks and benefits of SBPs (78). Several countries (including China, India, Brazil and Argentina) have also published guidelines or draft guidelines on production, preclinical and clinical evaluation of biosimilar biotechnological/biologic products (79). For the reasons cited above, these guidelines applicable to copies of recombinant therapeutic proteins are also not suitable for the approval of non-proprietary LMWHs. The complexities surrounding the marketing approval process of biosimilar LMWHs in regions where LMWHs are considered to be biologics and not drugs, have been addressed in the three guidelines for the manufacture and assessment of biosimilar LMWHs published by the EMA (15), ISTH (16) and SASAT (17).

An outline of the necessary steps for obtaining the regulatory approval of biosimilar LMWHs

The EMA, the Therapeutic Goods Administration (TGA) of Australia, ISTH and SASAT guidelines on biosimilar LMWHs (15–17, 80) state that a manufacturer can seek regulatory approval for a biosimilar LMWH by providing data that show the equivalence of the non-proprietary and originator LMWHs in a discrete set of tests. The TGA guidance document is identical to the EMA guidance document (80). The tests found in the three guidelines are in some ways analogous to the ANDA adapted in 2010 and 2011 by the FDA to approve two generic enoxaparins. In particular, the guidelines require the tests proposed to be appropriately designed, comparative in nature, and statistically powered to investigate equivalency between a proposed biosimilar and originator LMWH. Before comparing these three LMWH guidelines in detail below, it would be appropriate to briefly review the chemistry of UFH, manufacture of LMWHs from UFH, and the physicochemical characteristics of LMWHs. This knowledge is a useful prerequisite for appreciating the reasons for the several requirements found in these three guideline documents on biosimilar LMWHs.

Chemistry of UFH

UFH is a polysulfated mucopolysaccharide currently extracted primarily from porcine intestinal mucosa (6, 81, 82). Over the past

Table 2: Physicochemical heterogeneity between the LMWHs (12, 51–54).

LMWH	Depolymerisation process	Associated chemical change	Mean (Mw)*	Sulfate/carboxyl ratio
Enoxaparin	Benzylation, β -eliminative cleavage of benzyl ester by alkaline hydrolysis	2-O-sulfated uronic acid (unsaturated at the 4–5 position) at non-reducing ends	4,500	~2.0
Dalteparin	Deaminative cleavage with nitrous acid	2,5-anhydromannitol residue at reducing ends	6,000	2.0–2.5
Tinzaparin	β -eliminative cleavage by heparinase	Same as enoxaparin	6,500	1.8–2.5
Certoparin	Deaminative cleavage with isoamyl nitrite	Same as dalteparin	5,400	2.5
Parnaparin	Radical-catalysed depolymerisation	No systemic chemical changes to terminal residues	5,000	2.0–2.6
Nadroparin	Deaminative cleavage with nitrous acid	Same as dalteparin	4,300	~2.0
Reviparin	Deaminative cleavage with nitrous acid	Same as dalteparin	4,400	-
Bemiparin	Deaminative cleavage with nitrous acid	Same as dalteparin	3,600	Not reported
UFH	Not applicable	Not applicable	16,000	2.5

LMWH, low-molecular-weight heparin; UFH, unfractionated heparin. Only the first three LMWHs are approved in the USA. *Mw, weight-average molecular weights (Daltons). The value given for each LMWH is the characteristic value of molecular weight from the monograph in European Pharmacopoeia (54).

50 years, both the average molecular weights and the specific activities of clinical lots of UFH have increased significantly (82). Presently, the individual oligosaccharide chains in UFH range in size from 3,000 to 30,000 Da (mean molecular weight 15,000 Da) (82, 83). The nature, number and arrangement of the disaccharide units determine, in part, the structure of each individual oligosaccharide chain (6, 7, 9–11, 66, 67, 81, 82, 84, 85).

About a third of the molecules in UFH contain one or more of a pentasaccharide sequence that binds the plasma protein cofactor of heparin, antithrombin, with a high affinity (6, 7, 83, 86, 87). The high-affinity binding of UFH to antithrombin accounts for its ability to accelerate antithrombin-mediated inactivation of several activated clotting factors, particularly factor Xa and thrombin (6, 9, 11, 66, 69, 83, 86, 87). The highly acidic UFH binds to antithrombin with a higher affinity than it binds to thrombin (a basic protein), factor Xa and other activated vitamin K clotting factors and their zymogens (86–89). However, UFH chains that are more than nine disaccharides long and also contain the pentasaccharide with high affinity for antithrombin can transiently bind, by a bridging mechanism, both antithrombin and the highly basic enzyme thrombin. This is why UFH has a greater absolute catalytic effect on the inactivation of thrombin by antithrombin, compared to the rates of inhibition of factor Xa and other activated clotting factors (83, 86–90). By convention, the ratio for the catalytic effects of UFH on the inactivation of factor Xa and thrombin by antithrombin is 1.0 (i.e. the anti-factor Xa : antithrombin ratio) (12, 83).

Physicochemical heterogeneity among LMWHs

LMWHs are manufactured by chemically or enzymatically truncating highly purified UFH (5, 6, 91–94). The weight distribution

of the molecular entities in LMWH preparations vary from 2,000 to 9,000 (mean molecular weight 4,000 to 4,500 Da) (► Table 2). Further, the distribution of oligosaccharides differs significantly among LMWHs (9, 95). Only between 25% and 50% of the molecules in LMWH preparations able to bind antithrombin are long enough to simultaneously bind both antithrombin and thrombin. This is the reason why LMWHs catalyse antithrombin-mediated inactivation of factor Xa more effectively than that of thrombin (5, 83, 86, 88–90) and the anti-factor Xa to antithrombin ratios of LMWHs always exceed 1.0, varying between 1.6 and 9.7 (12, 83). Compared to UFH, LMWHs have greater bioavailability (~90%), slower clearance *in vivo* and have more predictable *ex vivo* anticoagulant effects. For these reasons, in contrast to UFH use, laboratory monitoring of the concentrations of LMWHs in patient plasmas is generally not required (83).

The techniques used to depolymerise highly purified UFH into LMWHs are summarised in ► Table 2 (5, 6, 91–94). The chemical and enzymatic processes for depolymerising heparin introduce significant structural changes in the LMWH products and also regulate the size of the polysaccharides found in LMWH preparations. As a result, LMWHs have diverse physicochemical characteristics (5, 6, 8–12, 51, 91, 94–96). For example, each technique used to depolymerise UFH produces specific end-groups (► Table 2) (6, 8, 91). Different end-groups apparently influence the pharmacodynamic and pharmacokinetic parameters (97). The size of the oligosaccharide fragments in LMWHs and their affinities for antithrombin (and hence their pharmacologic activities) are governed largely by the manufacturing process (9, 83, 98–100). Reversal of anticoagulation caused by the injection of a LMWH may be required under some clinical circumstances (83). LMWHs are variably neutralised by protamine sulfate depending on the sulfate content and molecular weights of the constituent fragments (8, 95, 101, 102) (► Table 2). Specifically, the maximum percentage of the larger fragments in LMWH preparations that also have anti-

Table 3: Pharmacodynamic heterogeneity between the LMWHs (12, 83, 107–109).

Study	Pharmacodynamic variable (units)	Heparin-based anticoagulant						
		Enoxaparin	Dalteparin	Tinzaparin	Certoparin	Nadroparin	Reviparin	UFH
Gerotziapas 2005 (107)	Thrombin generation Tmax (anti-Xa IU/ml)*	0.58	0.65	0.28	-	0.75	-	0.10
	Lag time (anti-Xa IU/ml)**	0.62	0.65	0.35	-	0.80	-	0.05
Fareed et al 2008 (108)	anti-Xa : anti-IIa ratio	3.8	2.7	2.2	3.2	3.6	4.1	1.1***
Gray et al 2008 (12)	anti-Xa : anti-IIa ratio	3.9	2.5	1.6	2.4	3.3	4.2	1.0
Jeske et al 2008 (109)	anti-Xa (U/mg)	105	130	83	88	95	-	-
	anti-IIa (U/mg)	27	58	45	32	27	-	-
	anti-Xa : anti-IIa ratio	3.9	2.2	1.8	2.8	3.5	-	-

*The concentrations of the various agents to decrease thrombin generation by 50%; ** the concentrations of the various agents to double the lag time preceding thrombin generation; ***data from Hirsh et al 2008 (83). IU, international units; LMWH, low-molecular-weight heparin; Tmax, therapeutic maximum; U, units; UFH, unfractionated heparin.

thrombin activity and are readily neutralised by protamine sulfate (84–96%) exceeds that of the smaller fragments with only anti-factor Xa activity which varies from 37, 46, 51, 59, to 81% for reviparin, enoxaparin, nadroparin, dalteparin and tinzaparin, respectively (95).

The concentrations of LMWHs in patient plasmas cannot be determined by simple chemical means and, therefore, pharmacokinetic parameters can only be inferred from pharmacodynamic parameters such as anti-factor Xa and anti-thrombin activities *ex vivo*, and LMWH-induced tissue factor pathway inhibitor (TFPI) release (103, 104). However, these pharmacodynamic parameters neither predict efficacy nor safety of LMWHs (105, 106). The contents of the various measurable functional entities in LMWHs vary widely, such that, for equivalent anti-factor Xa activity levels, the anti-thrombin activities can vary significantly for different products (12, 13) (► Table 3) (12, 83, 107–109). The anti-factor Xa activity varies from 83 to 130 U/mg, while the anti-thrombin activity varies from 27 to 58 U/mg (► Table 3). While the anti-factor Xa and anti-thrombin activities are used as standard measures of anticoagulation (110, 111), LMWHs also affect other haemostatic proteins, for example, von Willebrand factor (VWF) and TFPI release (99, 104, 112). The basis for the reported anti-inflammatory, anti-neoplastic and anti-angiogenic activities of LMWHs has not been determined (113, 114).

For the above reasons, therefore, the “active ingredients” in the eight originator LMWHs may not be quite the same (9, 10, 12, 18). Whether these approved originator LMWHs have equivalent clinical efficacy and safety profiles and can therefore be used interchangeably, was first addressed in 1999 and much debate has followed primarily because of lack of data obtained from large prospective studies (45, 47, 108, 115–119). As each LMWH has specific structural features and consequently unique pharmacodynamic profiles (5, 9, 10, 12, 21) (► Table 3), the FDA, EMA, WHO, American College of Chest Physicians (ACCP), and the American Heart Association/American College of Cardiology (AHA/ACC) view each LMWH as a distinct medicinal entity, and require clinical validation for each specific indication. Thus, the general consensus

of the AHA/ACC, EMA, FDA, North American Thrombosis Forum (NATF), ISTH, SASAT and WHO is that LMWHs may not be used interchangeably (3, 18, 83, 120). Only one (non-randomised) study involving over 8,000 patients has reported that switching from nadroparin to enoxaparin within an institution’s formulary did not compromise patient safety after major orthopaedic surgery (121). In fact, the clinical trials that led to approval of the eight LMWHs in ► Table 2 compared the incidence of distal and proximal deep-vein thrombosis rates and the incidence of pulmonary embolism in surgical and medical patients randomised to receive a LMWH or placebo (25–28, 30, 33, 40–42, 44, 49, 50, 122). The key motivating factors for developing these eight originator LMWHs were the clinical efficacy and safety of each product, not bioequivalence with another LMWH. Relatively few of the studies were head-to-head comparisons of two LMWHs (45, 123–127). A brief review of the recommendations found in the three specific guidance documents on biosimilar LMWHs now follows.

Guidelines on physicochemical equivalency

The ISTH and SASAT guidelines recommend several experiments for demonstrating physicochemical equivalency of biosimilar and originator LMWHs (16, 17) (► Table 4). The 2009 EMA guidelines do not, however, provide explicit descriptions of the physicochemical equivalency points (15). The writers of the EMA guidance document may have had the expectation that manufacturers of non-proprietary versions of LMWHs in the EU will make products with similar physicochemical attributes as their originators. Both the ISTH and SASAT require that biosimilar LMWHs are produced using exactly the same methods as the originator LMWHs. Thus, it is expected that each biosimilar LMWH has the same attributes as found in the originator LMWH in the following areas: mean molecular weight and molecular weight distribution; proportion of molecules containing the antithrombin binding domain; carboxylate and sulfate group density, and end-group se-

Table 4: Summary of the SASAT, ISTH and EMA guidelines for establishing equivalency of a biosimilar and originator LMWH (15–17).

SASAT guideline (17)	ISTH guideline (16)	EMA guideline (15)
Physicochemistry		
Produced exactly as in monograph	Produced exactly as in monograph	The EMA guideline does not explicitly list requirements for physicochemical tests, but must assume that all LMWH products comply with the physicochemical specifications in the appropriate European Pharmacopoeia monograph
Origin material specified	Origin material specified	
NMR and/or HPLC	NMR	
Lot-to-lot variation	Lot-to-lot variation	
Heparin unit composition	Analysis of internal and terminal sequences	
Sulfate to carboxyl group density ratio	Sulfate to carboxyl group density ratio	
% of total chains that contain antithrombin binding domain	% of total chains that contain antithrombin binding domain	
Heparan sulfate, glycosaminoglycans and other impurities	Dermatan sulfate, non-heparin glycosaminoglycans and other impurities	
Heparin cofactor II activity	-	
-	Mean MW and MW distribution	
In vitro and animal pharmacology		
Anti-factor Xa and antithrombin activities	Anti-factor Xa and antithrombin activities	Anti-factor Xa and antithrombin activities
Effects on the APTT	Effects on the APTT	-
Protamine neutralisation profiles	Protamine neutralisation profiles	-
Acute and chronic toxicology tests using different dosages in ≥ 2 animal species	Acute and chronic toxicology tests using different dosages in ≥ 2 animal species	A dose toxicity study conducted over the intended duration of clinical use (≥ 4 weeks)
Animal models of arterial thrombosis and VTE	Animal models of arterial thrombosis and VTE	-
Studies in healthy volunteers and in special populations		
Phase I single-dose, two-way crossover randomised controlled trials in healthy volunteers	Phase I single-dose, two-way crossover randomised controlled trials in healthy volunteers	Phase I single-dose, two-way crossover randomised controlled trials in healthy volunteers
1 study each at VTE prophylactic and treatment dosages for 5–7 days	1 study each at VTE prophylactic and treatment dosages for 5–7 days	Dosages appropriate for the intended clinical indication
Phase I study in patients with renal dysfunction	Phase I study in patients with renal dysfunction	-
Comparative clinical studies		
1 clinical trial to demonstrate equivalency for each intended clinical indication	1 clinical trial each for arterial thrombosis and VTE	1 clinical trial in the most sensitive, highest-risk population
APTT, activated partial thromboplastin time; EMA, European Medicines Agency; HPLC, high-performance liquid chromatography; ISTH, International Society on Thrombosis and Haemostasis; LMWH, low-molecular-weight heparin; MW, molecular weight; NMR, nuclear magnetic resonance; SASAT, South Asian Society of Atherosclerosis and Thrombosis; VTE, venous thromboembolism.		

quences. Lot-to-lot variation, and impurity levels in heparan sulfate, dermatan sulfate and other glycosaminoglycans, in the biosimilar and originator LMWH should also be comparable.

three guidelines has specific directions on how to determine whether biosimilar and originator LMWHs have equivalent toxicological, pharmacokinetic and pharmacodynamic profiles (15–17).

Guidelines on pharmacokinetic, pharmacodynamic and toxicological characteristics

Determining equivalency in the pharmacodynamic and hence pharmacokinetic characteristics of LMWHs is complex, which is why all three guidelines require comparative tests *in vitro*, in animals, and some testing in human volunteers and patients, in order for manufacturers of biosimilar LMWHs to establish comparability with originator LMWHs in as many areas as possible. Each of the

In vitro and animal pharmacology of LMWHs

For *in vitro* studies, the ISTH and SASAT guidelines require equivalent anti-factor Xa and anti-thrombin activities, and equivalent effects of LMWHs on the APTT of pooled human plasmas for biosimilar and originator LMWHs. The EMA guidance document requires equivalency of anti-factor Xa and anti-thrombin activities but not comparable effects on the APTT. Both the ISTH and SASAT guidelines require comparable protamine sulfate neutrali-

sation profiles for biosimilar and originator LMWHs, although the EMA guidelines do not. The ISTH and SASAT guidelines recommend use of platelet-factor 4-binding assays to compare originator and biosimilar LMWHs (16, 17).

All three guidelines require pharmacological testing in at least one relevant animal species. The EMA guidelines require a one-dose toxicity study conducted over the intended duration of clinical use of a biosimilar LMWH (at least 4 weeks) (15). In contrast, the ISTH and SASAT guidelines require both acute and chronic toxicology tests using different dosages in two or more animal species. Further, both the ISTH and SASAT guidelines recommend that comparability assessment of the biosimilar and originator LMWH be conducted in animal models of both arterial and venous thrombosis (16, 17).

Studies in healthy volunteers and in special populations

All three guidelines require phase 1 single-dose, two-way crossover randomised controlled trials in healthy human volunteers comparing several pharmacodynamic and pharmacokinetic parameters, such as *in vivo* recovery of anti-factor Xa and anti-thrombin activities, associated TFPI release and response-time curves for both originator and biosimilar LMWHs. While the EMA guidelines require a comparison of biosimilar and originator LMWHs given at dosages appropriate for the clinical indication for which manufacturers seek market authorisation (15), the SASAT and ISTH guidelines (16, 17) specify that one study comparing VTE prophylactic dosages and one study comparing VTE treatment dosages (twice daily) are each performed for 5–7 days. Because LMWHs are principally cleared by the kidneys and can accumulate in patients with renal impairment (83), the ISTH and SASAT guidelines also require that a similar phase 1 study be performed in patients with renal dysfunction (16, 17). The FDA only required comparative studies on the pharmacodynamic parameters and immunogenicity in healthy volunteers to approve the two generic enoxaparins (18).

Comparative clinical studies of originator and biosimilar LMWHs

All three guidelines specify comparative randomised clinical trials of a biosimilar and originator LMWH (15–17). The basis for this requirement is that the relationship between surrogate pharmacodynamic parameters of any LMWH and its clinical efficacy and safety profile is poorly understood (105, 106) and further non-proprietary versions and originator LMWHs have similar, and not identical, contents of the “active ingredient”. The guidelines require the clinical trials to be appropriately powered to detect non-inferiority or therapeutic equivalence. The ISTH guidelines require a minimum of two clinical trials; one for VTE and one for arterial

thromboembolism (16). The SASAT guidelines require one clinical trial to demonstrate equivalency for each indication for which manufacturers of biosimilar LMWHs seek regulatory approval (17). The EMA guidelines recommend that the comparative clinical trial is performed in the most sensitive, highest-risk population, such as in patients undergoing elective major orthopedic surgery (15). The suggested efficacy endpoints in all three guidelines are incidence of deep vein thrombosis and pulmonary embolism, and VTE-related death. The approaches for establishing the comparative safety profiles of biosimilar LMWHs recommended in all three guidelines are major and minor bleeding, effects on platelet counts, the incidence of heparin-induced thrombocytopenia and their effects on liver enzymes (15–17).

Are non-proprietary LMWHs equivalent to their originator comparators?

Non-proprietary LMWHs have also been approved in Argentina, Brazil, China and India, where, with the exception of Brazil, no specific regulatory guidelines or any detailed information for determining the bioequivalence of non-proprietary and originator LMWHs have been published. The guidance document for Brazil was published in 2010, a few years after biosimilar LMWHs had become available in Brazil (128). This Brazilian document provides information on the requirements on the raw materials, structure and purity, preclinical studies, phase 1 studies in healthy volunteers, and at least one double-blind randomised phase 3 study which aims to prevent arterial or venous thrombosis in line with the EMA recommendations on clinical trials with biosimilar LMWHs (128). It appears that the regulations governing regulatory approval of synthetic generic drugs were applied to license generic LMWHs in Argentina. Several products have already been withdrawn after some batches apparently failed to comply with specifications (17). Melo et al. (129) reported that UFH made by some Brazilian manufacturers after Roche discontinued its sale of UFH in Brazil coincided with higher rates of reoperation after cardiopulmonary bypass surgery due to bleeding and post-operative blood dyscrasia. All four heparins made by the unnamed Brazilian manufacturers had significantly lower specific activity (< 200 IU/mg) than the Roche product (254 ± 18 IU/mg) or the current International Standard for UFH (245 ± 18 IU/mg). The Brazilian UFHs in question also had lower mean molecular weight than the Roche UFH, had approximately 20% chemically degraded heparins (as determined from their NMR spectra), and were incompletely neutralised by protamine sulfate (129). The authors asserted there was a “lack of specific regulations for the analysis of preparations of heparin using modern appropriate methods” and that “suppliers from the domestic market also have little interest in controlling the quality of non-fractionated heparin” (129). Source materials derived from porcine and bovine intestinal mucosa used to manufacture UFH in Brazil (130) may have been contributing factors for the differences reported by Melo et al. above (129). Manufacturers and regulators of non-proprietary LMWHs in Argentina, China

Table 5: Pharmacodynamic differences between generic and originator enoxaparin in animal models (138).

Variable (unit)	Originator enoxaparin	Biosimilar enoxaparin			
		Clenox	Cutenox	Dripanina	Dilutol
RSTM ED50 ($\mu\text{g}/\text{kg}$)	72 ± 6	87 ± 8	91 ± 6	62 ± 6	-
REBM haemorrhagic effect (RBCs $\times 10^9/\text{L}$)	4.1 ± 0.6	5.6 ± 1.1	5.2 ± 0.9	3.0 ± 0.5	2.9 ± 0.8
Laser model of efficacy rank order	2	1	3	4	4
Clamping model of efficacy rank order	2	5	4	1	3
NO levels*	Increase of 56%	Range – Increase of 11–53%			
TFPI levels*	Increase of 143%	Range – Increase of 95–153%			

*Primate model. Data not reported separately for biosimilar versions of enoxaparin. NO, nitric oxide; RBCs, red blood cells; REBM, rabbit ear bleeding model; RSTM, rabbit stasis thrombosis model; TFPI, tissue factor pathway inhibitor.

and India have not provided access to the data used for establishing bioequivalence of the non-proprietary and originator LMWHs. The majority of published data about these products report results of physicochemical, *in vitro* and animal experiments (109, 131–133). For example, the oligosaccharides generated after enzymatic degradation (109, 131) and the affinities of the originator LMWH enoxaparin and its non-proprietary version for anti-thrombin and heparin cofactor II differed significantly (131). Jeske et al. investigated pharmacodynamic differences between non-proprietary and originator enoxaparin in human plasma (132). At prophylactic doses, there were no differences in anticoagulant or anti-protease activities, although significant differences ($p < 0.05$) in anti-factor Xa and antithrombin activities and the APTT became apparent when (the higher) treatment doses of non-proprietary and originator enoxaparin were compared (132, 133). Adiguzel et al. (134) compared inhibition of thrombin generation *in vitro* by originator enoxaparin and several non-proprietary versions and found that each LMWH had a distinct inhibitory profile and, more importantly, the inhibitory effects of the non-proprietary LMWHs were variable. Differential patterns of inhibition of fibrin clot formation have also been demonstrated between originator and non-proprietary enoxaparin (135). The extent to which enoxaparin and a non-proprietary counterpart could be neutralised by protamine sulfate also differed significantly (132, 133). Lot-to-lot variation was evident only for a non-proprietary version of originator enoxaparin (132). Another study demonstrated differences in salt content, oligosaccharide composition, antithrombin and heparin cofactor II binding affinities, as well as neutralisation by PF4 of non-proprietary and originator enoxaparin or dalteparin (133). In addition, the abilities of anti-heparin/PF4 antibodies to aggregate platelets in platelet-rich plasmas differed significantly between originator enoxaparin and two non-proprietary LMWHs, although there was no difference between originator dalteparin and its non-proprietary version (136). Finally, LMWHs can cause the release of chemical mediators such as cytokines and chemokines by white blood cells (113, 114), and the extent to which originator enoxaparin and two non-proprietary counterparts induced the release of these chemical mediators varied significantly (137). Notably, when evaluated in animal models, the originator enoxaparin and its counterparts had distinct pharmacological properties (► Table 5), such as the inhibition of tissue

factor-induced P-selectin expression on platelets and their *ex vivo* anti-factor Xa activity and efficacy profiles (138).

How these physicochemical differences influence the recovery and survival of non-proprietary versions of originator enoxaparin or dalteparin *in vivo* have not been reported. One study has compared the recovery of originator enoxaparin and a non-proprietary version in 20 healthy volunteers (139). A single dose of either drug (40 mg) was administered subcutaneously in a crossover design with a six-day washout period in between the injections of the two drugs. Pharmacodynamic parameters of the two drugs measured in the *ex vivo* plasma samples were equivalent (139). However, the authors debated whether these data were sufficient to demonstrate similar efficacy and reiterated the need for head-to-head comparative clinical studies (139). A similar crossover design was used in a second study, which found comparable pharmacodynamic parameters of a non-proprietary LMWH and enoxaparin in 22 healthy young male volunteers (140).

Additional considerations surrounding the market for non-proprietary LMWHs

From the viewpoint of patients treated with Lovenox who pay for this drug themselves and that of other payers, a clear positive outcome from the approval of a generic enoxaparin in the USA is the approximately 30% reduction in the price of Lovenox in the Chicago area in 2010 (Fareed J, personal communication). Approval of the second generic enoxaparin may lead to a further price reduction of Lovenox and possibly even that of the first generic LMWH in the USA. Note, however, that additional considerations arising from recent developments in antithrombotic drugs may also have contributed to the Lovenox price reduction in the Chicago area. Furthermore, these new synthetic antithrombotic drugs may significantly reduce the proportion of patients currently treated with LMWHs in the future. Fondaparinux (a synthetic pentasaccharide with high affinity for antithrombin that catalyses factor Xa inhibition by antithrombin) has been approved for the prophylaxis (in surgical patients) and treatment of VTE (141, 142). Several direct thrombin inhibitors and direct factor Xa inhibitors have more recently been approved (or are undergoing late phase 2 or phase 3

clinical trials) for VTE prophylaxis after joint replacement surgery in Canada and the EU and in patients with ACS or atrial fibrillation (143–149). The FDA approved dabigatran for stroke prevention in patients with non-valvular atrial fibrillation in 2010 (150). Should these synthetic antithrombotic drugs under development also prove to have similar efficacy and safety profiles as the current standard of care, their large-scale approvals and subsequent clinical use may decrease the currently anticipated market size for LMWHs. The additional requirements for phase 1 and some phase 3 clinical trials prior to seeking marketing approval of biosimilar LMWHs in the EU and Australia impose significant additional financial burdens on their manufacturers. For these reasons, seeking regulatory approval for biosimilar LMWHs may well have already become financially less attractive.

Conclusions

Implementation of appropriate guidance documents by national/regional regulatory authorities and adherence to these guidelines by manufacturers seeking regulatory approvals preceded the successful launch of biosimilar versions of therapeutic proteins and hormones and their increasing use in Europe, Australia and the USA. Additionally, for both efficacy and safety considerations, appropriate comparative clinical trials were also required prior to approval. Three biosimilar epoetin alfas, two epoetin zetas, two somatropins, and seven filgrastims had been approved in the EU as of August 2011 (151). Thus, despite their high cost, the required clinical trials were conducted by their manufacturers, perhaps due to the significantly higher cost of therapeutic proteins compared to LMWHs. Since LMWHs, like therapeutic proteins, are complex molecules, widespread approval of biosimilar LMWHs currently has requirements in many jurisdictions that are in many ways comparable to protein-based biosimilars in Europe and Australia. Based on the requirements found in the EMA guidance document for LMWHs and the results of published studies, the non-proprietary LMWHs approved for use in Argentina, China and India are unlikely to receive marketing approval in the EU or Australia without, at a minimum, detailed information on the structure and chemical composition of the LMWHs and results of clinical trials in both volunteers and patients. Importantly, the EMA guidelines clearly acknowledge the distinction between LMWHs and the more easily characterised protein-based products. Significantly, not a single non-proprietary LMWH has been approved in the EU, perhaps because the EMA guidelines for LMWHs have only been available since 2009. However, the EMA requirement for phase 3 clinical trials (and their high cost) may also have impeded the development of non-proprietary LMWHs for use in the EU. Furthermore, given the detailed nature of the information the FDA required prior to approving two generic enoxaparins, FDA approval of other non-proprietary LMWHs will likely be slow. At least one other generic enoxaparin has been awaiting FDA approval for several years. How the approval of two non-proprietary enoxaparins by the FDA will ultimately affect the biosimilar pathway for

approving LMWHs as envisaged by EMA is unknown. However, harmonisation of the current EMA and FDA regulatory approaches can only be beneficial for manufacturers and consumers alike as harmonisation could speed up the pace of regulatory approvals of LMWHs in the EU.

Given the inherent difficulties manufacturers currently face with two distinct pathways for evaluating non-proprietary LMWHs for registration in the EU and Australia on the one hand and the USA on the other, one can only hope that serious efforts in consensus building, perhaps sponsored by the WHO, will be undertaken to reconcile the two significantly different regulatory approaches advocated by EMA and FDA. Based on precedents, a guidance document developed under WHO auspices would be welcomed by regulatory authorities in many developing countries as the basis for market approval of non-proprietary LMWHs. A first step towards this consensus development could be an agreement on the criteria to be used to establish comparability of originator and proposed non-proprietary versions of LMWHs using established modern physicochemical methods able to identify all the constituent entities and their percent composition. A clear benefit from this step would be an ability to identify potentially unsafe contaminants in LMWH preparations (152), and therefore to identify products that are suitable for performing *in vitro* comparability and subsequent studies. Given that no serious adverse events associated with the use of the first generic enoxaparin have thus far been reported, a year since its approval by the FDA in 2010, the criteria the FDA has used to approve two generic enoxaparins (18, 65) may provide building blocks that are important for the proposed consensus-building process. A second point for which consensus is necessary, is a definition of the minimum acceptable pre-clinical and clinical studies required for marketing approval. A third important issue that consensus building will have to resolve is the clinical indications for which non-proprietary LMWHs may be used. Data from extended use of the two generic enoxaparins in the USA should help resolve the second and third issues. The same safety and efficacy data on the use of the two generic enoxaparins in the USA will also probably influence the revisions to the EMA guidelines now in progress (EMA/CHMP/BMWP/572297/210). Reconciling the two regulatory approaches should ultimately increase the availability of non-proprietary LMWHs with good efficacy and safety profiles to benefit patients requiring anticoagulation with LMWHs whether they live in the developed or developing world. The limited availability of non-proprietary LMWHs may persist until the two distinct regulatory pathways are reconciled.

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Conflicts of interest

None declared.

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