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Heparin and low-molecular-weight heparin

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Summary

Heparin is one of the oldest biological medicines, and has an established place in the prevention and treatment of venous thrombosis. Low-molecular-weight heparins (LMWH) have been developed by several manufacturers and have advantages in terms of pharmacokinetics and convenience of administration. They have been shown to be at least as effective and safe as unfractionated heparin and have replaced the latter in many indications. In this article the chemistry, mechanisms of action, measurement of anticoagulant activities, and clinical status of heparin and LMWH are reviewed.

Keywords

Heparin, low-molecular-weight heparin

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Introduction

Heparin is one of the oldest biological drugs in the field of thrombosis and haemostasis. Following its discovery in 1916 and early clinical trials in the 1930s and 1940s, it became the mainstay of prevention and treatment of venous thromboembolism (VTE). In the 1980s the development of low-molecular-weight heparin (LMWH) extended and enhanced the usefulness of this class of drug, and for many indications LMWH has replaced its parent compound.

In this review the chemical characteristics, mechanism of action, measurement of anticoagulant activities, and clinical status of heparin and LMWH will be described.

Chemical characteristics of heparin products

Unfractionated heparin (UFH)

All heparin preparations are polydisperse linear polymers, so that their molecular weights (MWs) cannot be described by a single number. A convenient way to express the MW profile of a polymer such as a heparin sample is to take the number average MW M_n (defined below) and the weight average MW M_w. The ratio M_w/M_n (known as the polydispersity) expresses the spread of MWs in the sample. M_n for porcine mucosal UFH is about 12 to 16 kDa and M_w about 17 to 20 kDa, giving a polydispersity (M_w/M_n) of about 1.3–1.4 (1). Bovine lung heparin has a lower MW (M_n about 11 kDa; M_w about 15 kDa) (1), but is now hardly used. The polydisperse nature of heparin can be an important issue whenever a property of heparin depending on molar concentration is measured, including all measurements of binding and kinetic constants.

The number average MW, $\overline{M}_n$, is defined:

$$\overline{M}_n = \frac{\sum_i N_i M_i}{\sum_i N_i}$$

where N_i is the number of molecules at MW M_i.

The weight average MW, $\overline{M}_w$, is defined:

$$\overline{M}_w = \frac{\sum_i g_i M_i}{\sum_i g_i}$$

where g_i is the weight of the sample at MW M_i.

The physicochemical characteristics of UFH vary only a little between products. However, some relatively subtle changes, such as an increase in mean MWand specific activity, have been noted in some UFH products over the lifetime of the 4th International Standard heparin (1984–1998) (1). It may be the case that the introduction of LMWH influenced changes in the manufacture of UFH, which is used as a parent stock for partial depolymerisation to LMWH as well as a therapeutic agent in its own right.

Besides its polydispersity, UFH is heterogeneous in sequence, but consists predominantly of the trisulfated disaccharide repeating unit (Fig. 1).

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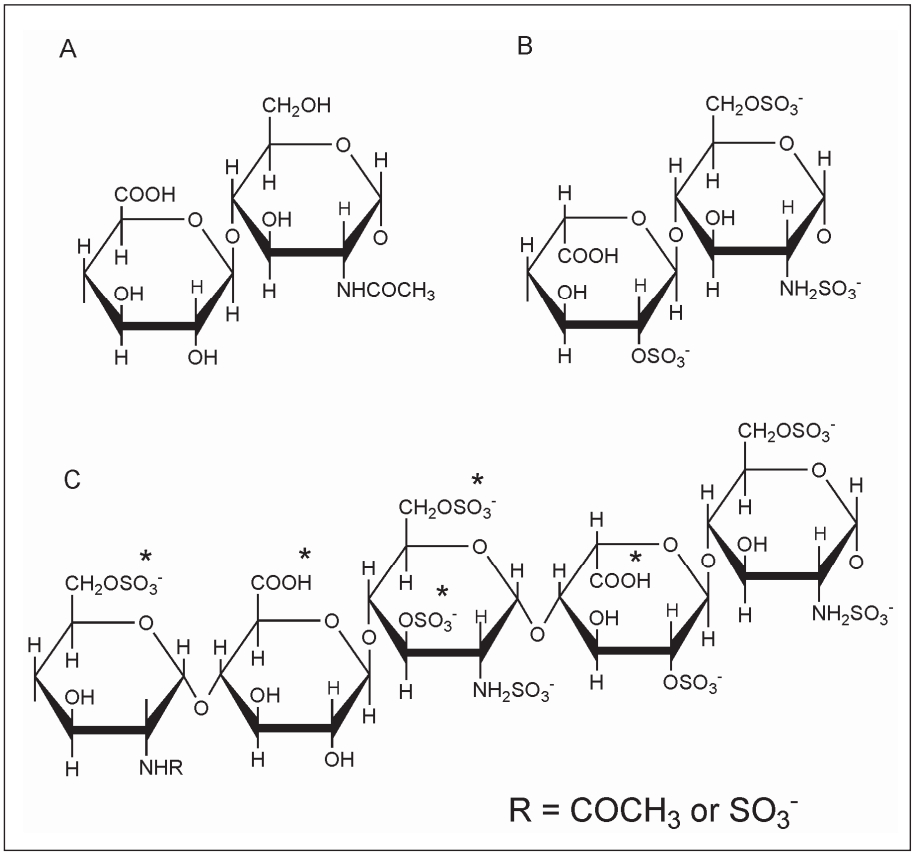

Figure 1: Structures of the repeating disaccharide motifs in heparin (and heparan sulphate), and the pentasaccharide with high affinity for antithrombin. A) The main repeating unit of heparan sulphate, [–4)-β-D-GlcA-(1→4)-α-D-GlcNAc-(1–]. This structure forms the precursor polysaccharide which is transformed by a series of enzymes into B) the main repeating unit of heparin [-4)-α-L-IdoA(2SO_3^-)-(1→4)-α-D-GlcNSO_3^-(6SO_3^-)-(1-]. The structural distinction between heparin and heparan sulphate is subtle; broadly, heparin contains 70% or more of structure B, heparan sulphate much less, with a higher proportion of the many intermediate structures arising from incomplete action of the post-polymerization enzymes. C) The pentasaccharide in heparin which is the minimal structure with high affinity for antithrombin: [-4)-α-D-GlcNAc(6SO_3^-)-(1→4)-α-D-GlcA-(1→4)-α-D-GlcNSO_3^-(3,6diSO_3^-)-(1→4)-α-L-IdoA(2SO_3^-)-(1→4)-α-D-GlcNSO_3^-(6SO_3^-)-(1-]. Substituents essential for this affinity are marked with an asterisk.

The anticoagulant activity of UFH *in vitro* is overwhelmingly dependent on the presence of a specific sequence with high affinity for the plasma serpin antithrombin (Fig. 1C). This sequence, characterised by its central, essential pentasaccharide motif containing the unusual 3,6 di-*O*-sulfated, 2-*N*-sulfated glucosamine residue, does not occur in every heparin molecule. In order to potentiate the inhibition of thrombin, a heparin molecule must contain the HA sequence and also have sufficient chain length to bind to both antithrombin and thrombin. The combination of HA sequence with this extra chain length has been termed the "C-region", the "C" standing for Choay (2).

Full linear sequences for heparin (or heparan sulfate) molecules are not available – scarcely two molecules are alike – but an overall picture of the proportions of different residue and sequence types may be determined by several methods: degradative, such as enzymic depolymerisation and oligosaccharide profiling (3), and non-destructive, such as ^{1}H or ^{13}C NMR (4). Either type of assay can give an estimate for HA sequence content, but not of C-region. It is also possible to estimate the HA sequence content of a heparin sample by titration of the native fluorescence of purified antithrombin, which is enhanced on binding to heparin (2, 5).

LMWH

LMWH products all have mean MWs less than half that of UFH; the defining characteristic that all have in common is that 60% or more by weight must be below 8,000 (6). However, as shown in Table 1, there is considerable diversity in the MW parameters of the various LMWH products.

As the minimum MW for anti-IIa activity is about 5,000 (see subsequent section), the anti-IIa specific activity of LMWH is drastically reduced. The ratio of anti-Xa to anti-IIa activity is greater than 1 and, like the MW distribution, is a defining characteristic of each separate LMWH product (see Table 1).

LMWH may in principle be prepared by fractionation of UFH or by its depolymerisation. None of the current LMWH products are prepared by fractionation, though early work on LMWH used fractions rather than depolymerised fragments (8). Several methods of partial depolymerisation are used (9), of which the most common is deaminative cleavage with nitrous acid or an organic nitrite. This method breaks the heparin chain at N-sulfated glucosamine residues, leaving a characteristic anhydromannose reducing-end residue which is usually reduced to anhydromannitol. Chemical or enzymatic beta-elimination is the second most common depolymerisation method; this leaves an unsaturated uronic acid residue at the non-reducing terminus. Oxidative depolymerisation is a third method in use; this does not leave a specific „signature" structure in the product. The chemical changes briefly outlined here are the most obvious effect of depolymerisation; there may be other, more subtle effects on the detailed sequence characteristics of LMWHs produced by different methods.

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Biochemistry and mechanism of action

The basic biochemistry of heparin's anticoagulant activity was unravelled during the 1970s. It was known that heparin required a plasma co-factor, and studies by the group of Rosenberg and others identified this as antithrombin III, now known simply as antithrombin; heparin was shown to markedly accelerate the inhibition of thrombin, and also factors (F) Xa, IXa, XIa and XIIa by antithrombin (10). Binding of heparin induces a conformational change in the antithrombin molecule which greatly facilitates the interactions with its serine protease targets. Less than half of heparin molecules bind with high affinity to antithrombin (11), and the structural basis for this binding, requiring a specific pentasaccharide, was described in the previous section. The molecular basis of heparin's anticoagulant activity was confirmed by chemical synthesis of the antithrombin-binding pentasaccharide, first described by Choay et al. (12).

Table 1: Molecular weight (MW) data and anticoagulant activities of currently available LMWH products. [1]Values measured at NIBSC (mean of 3 batches). [2]Characteristic value of *Mw* from monograph in European Pharmacopoeia. [3]Value measured at NIBSC (single batch [7]). [4]Value of M_w from manufacturer.

LMWH (INN)	Weight average molecular weight (M_w)	Ratio anti-Xa/anti-IIa activity[1]
Enoxaparin	4500[2]	3.9
Dalteparin	6000[2]	2.5
Tinzaparin	6500[2]	1.6
Parnaparin	5000[2]	2.3
Nadroparin	4300[2]	3.3
Certoparin	5400[3]	2.4
Bemiparin	3600[4]	9.7
Reviparin	4400[3]	4.2

MW dependence

Anti-Xa and anti-IIa activities

Early studies of the relationship between anticoagulant activity and MW revealed a puzzling anomaly, in that the activity in the traditional APTT assay decreased with decreasing MW, but when measured by the newer anti-Xa assay the activity was maintained or even increased in the lower MW fractions (13). Subsequent studies using purified antithrombin confirmed these trends, and it became clear that acceleration of inhibition of thrombin and FXa by heparin had different MW requirements (14, 15). This difference was emphasised in studies by Holmer (16) and in our laboratory (17, 18); high-affinity (for antithrombin) oligosaccharides with chain lengths mostly below 16 units, had very high anti-Xa activity, over 1,000 IU/mg, but their activity in thrombin inhibition and APTT assays was less than 10 IU/mg.

The minimum chain length required for potentiation of thrombin inhibition was defined more precisely by Lane et al., using a series of homologous oligosaccharides, and was shown to be 18 saccharides (19). The reason for this is that inhibition of thrombin takes place via a template mechanism, with both enzyme and inhibitor binding adjacently to the same heparin chain (20, 21). The implication of the minimum size requirement is that there must be at least 13 saccharides next to the pentasaccharide for thrombin binding. Since the distribution of the pentasaccharide is random, the lower MW fragments, e.g. 22 saccharides, will have a significant proportion of molecules with the pentasaccharide in the middle so that there is insufficient room for thrombin binding; this situation will pertain until the chain length reaches 32 saccharides, and accounts for the continual increase in thrombin inhibitory activity in the MW range 5,000 – 10,000.

Potentiation of FXa inhibition does not require a template mechanism, hence the very low MW fragments, down to the specific pentasaccharide, still have anti-Xa activity. However, it should be recognised that anti-Xa activity is usually measured *in vitro* in the absence of calcium ions, i.e. in citrated plasma or in the presence of purified antithrombin. When calcium ions are present, as shown by Ellis et al (22), there is a consistent increase in anti-Xa activity from 5–30 saccharides. Studies in our laboratory showed that calcium ions potentiated the anti-Xa activity of heparin, the degree of potentiation increasing with MW (23). Thus, whilst binding of FXa to heparin, unlike that of thrombin, is not an absolute requirement for activity, it appears that, in the presence of physiological calcium concentrations, such binding enhances the activity of the higher MW heparin chains. A recent study by Wagenvoord et al. (24) also found evidence for a MW dependency of the anti-Xa activity of heparin, even in the absence of calcium ions. A further complication of heparin's anti-Xa activity is that, *in vivo*, FXa usually occurs as part of the prothrombinase complex rather than in the free form. Several studies have found that, when in prothrombinase, inhibition of FXa by heparin increases with MW, but whatever the MW of the heparin a higher concentration is needed to inhibit FXa in prothrombinase than in free form (25–27).

Other enzymes

Holmer et al. (28) studied the influence of heparin MW on its ability to potentiate the inhibition of FIXa, FXIa, FXIIa and kallikrein by antithrombin. It was found that FIXa and FXIa behaved like thrombin, in requiring a template mechanism with enzyme and antithrombin bound to the same heparin chain, so that the low MW fragments had very little activity. However, FXIIa and kallikrein behaved like FXa, in that activity was retained down to low MW. However Colman et al. (29) found that the accelerating effect of heparin on inhibition of contact system enzymes was much less than that on thrombin or FXa, and it is doubtful whether the effect of LMWH on FXIIa and kallikrein inhibition makes much contribution to its overall anticoagulant activity.

Overall inhibition of thrombin generation

Biochemical studies with isolated enzymes are useful in defining detailed kinetic parameters such as rate constants, but cannot give information on the relative importance of inhibition of the various serine proteases to heparin's overall anticoagulant effect, which can be considered as its ability to delay and/or diminish

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the generation of thrombin. Three research groups have approached this problem using different methodology.

In the studies of Ofosu et al. (30–32), ELISA and gel techniques were used to measure activation of prothrombin and inhibition of the formed thrombin by heparins of various MW. It was found that UFH was very effective in delaying prothrombin activation via its inhibitory effect on thrombin feedback loops (thrombin activation of FVIII and FV). LMWH was much less effective at the same mass concentrations, and an octasaccharide fragment had hardly any activity in this system, despite its high anti-Xa activity. Thus it was postulated that inhibition of thrombin generation by heparin was mainly dependent on its ability to potentiate thrombin inhibition, and in particular the inhibition of the thrombin feedback loops.

These findings were supported by studies at the National Institute for Biological Standards & Control (NIBSC, Potters Bar, UK), in which thrombin generation was measured directly in plasma using a clotting method in the presence of procoagulant phospholipids after surface activation (33). It was found that LMWH was much less effective than UFH, and whereas oligosaccharides having only anti-Xa activity were largely ineffective, those having also thrombin inhibitory activity were much more active.

Subsequent studies with the synthetic pentasaccharide found that, although there was some inhibitory effect on thrombin generation, it required much higher concentrations than UFH, and maximum inhibition was 50% (34)

Hemker et al. introduced more sophisticated methods for measuring thrombin generation in plasma, using chromogenic, or more recently fluorogenic substrates (35–37). In these studies the effects of heparin and LMWH fragments on both prothrombin activation and thrombin decay could be measured simultaneously. It was shown that inhibition of prothrombinase, i.e. the FXa/FV/phospholipid complex, made little contribution to the overall effect of heparin. The major effects were due to inhibition of thrombin feedback loops (activation of FVIII and FV) and enhanced inhibition of FIXa; hence LMWH was less effective than UFH. The synthetic pentasaccharide could inhibit thrombin generation to a limited extent because in the absence of molecules which can potentiate thrombin inhibition its scavenging effect on free FXa, formed before and in equilibrium with prothrombinase, comes into play.

Studies in platelet-rich plasma showed that UFH was less effective in reducing thrombin generation than in platelet-poor plasma; this is because of neutralisation of heparin by platelet factor 4 (PF4). Since the ability of PF4 to neutralise heparin decreases with decreasing heparin MW, it might be expected that LMWH would have a proportionately greater effect, compared to UFH, in platelet-rich plasma – this was indeed shown to be the case (38).

In-vitro and in-vivo anticoagulant effects; relevance of anti-Xa assays

The studies outlined above, and particularly the elegant studies of the groups of Ofosu and Hemker, have emphasised that inhibition of thrombin generation in plasma by heparin is largely due to its ability to inhibit the thrombin feedback loops. The potentiation of inhibition of FXa alone, such as occurs with heparin oligosaccharides below 16 units, and with the synthetic pentasaccharide, whilst not completely ineffective, is a less efficient mechanism for inhibition of thrombin generation. The commercially available non-synthetic LMWH all contain a substantial proportion of molecules which are able to potentiate thrombin inhibition; it may be wondered therefore whether measurement of anti-Xa activity of such LMWH has any value at all – such a view has indeed been expressed (39).

Considering anticoagulant activities *in vitro*, it is clear from the studies mentioned earlier that measuring anti-Xa activity of LMWH in the absence of calcium overvalues their activities, as emphasised by Hemker (40). Furthermore, when FXa is bound in prothrombinase, the concentrations of LMWH required for its inhibition become even higher. Nonetheless, when appropriate concentrations are used, molecules up to 18 saccharides, which have no thrombin inhibitory activity, are able to inhibit thrombin generation, and all LMWH contain a substantial proportion of such molecules; in the case of some of the recent products, such as bemiparin, these are the predominant molecular species.

The true test of the relevance of anti-Xa activities can only be established from in-vivo studies in animals and man. Here a number of additional factors come into play:

- 1) After subcutaneous injection the higher MW fractions of a LMWH are less readily absorbed, so the MW distribution in the blood shifts towards the lower end. Hence the anti-Xa/anti-IIa ratio measured *ex vivo* is higher than that *in vitro*.
- 2) The MW dependence of binding to PF4 is very similar to that for thrombin inhibition. Therefore the molecules which are most effective in inhibiting thrombin are also most likely to be neutralised by PF4 in a situation where platelets are activated, whereas the molecules which have only anti-Xa activity are hardly affected by PF4.
- 3) Although FXa in prothrombinase is relatively protected from inhibition by antithrombin/heparin, the main protective agent is FVa, which is a transient species, being rapidly inactivated by activated protein C. Therefore free FXa will be liberated from the prothrombinase complex, and its inhibition by LMWH molecules with anti-Xa activity could be important for prevention of spread of a thrombus.
- 4) Both heparin and LMWH release tissue factor pathway inhibitor (TFPI) from the vessel wall into the blood after injection. As well as its ability to inhibit TF/FVIIa, TFPI also has direct anti-Xa activity and this contributes to the overall anti-Xa activity measured *ex vivo*. TFPI may also contribute to the overall anti-thrombotic action of heparin, since low-affinity heparin, which has the same ability to release TFPI as UFH, is not completely devoid of anti-thrombotic activity in animal studies (41).

The initial animal studies confirmed that LMWH fragments with only anti-Xa activity were relatively poor inhibitors of thrombosis compared to fragments with both anti-Xa and anti-IIa activity (42). However, when the dosage was adjusted in proportion to their ability to inhibit FXa in prothrombinase, effective anti-thrombotic activity was obtained. The most definitive evidence of the importance of anti-Xa activity comes from clinical studies of the synthetic pentasaccharide, which have shown it to be an effective antithrombotic drug (43, 44).

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Measurement of anticoagulant activities

The measurement of anticoagulant activities of heparin and LMWH is described in detail elsewhere (41), and will only be considered briefly here. The methods established over many years for UFH are all based on its ability to delay the clotting time of animal or human plasma, except for the US Pharmacopoiea (USP) method, in which the strength of the clot in sheep plasma is assessed. The European Pharmacopoeia (EP) method is based on measurement of the APTT in sheep plasma, and the method used at NIBSC is similar, but using human plasma instead of sheep. Other methods based on thrombin inhibition have been described, and in 1999, the World Health Organisation (WHO) initiated a harmonisation programme of measurement of anticoagulant activity of UFH and introduced a "global" method, which is based on a chromogenic assay measuring potentiation of inhibition of thrombin by purified antithrombin The USP is currently working on the replacement of the sheep plasma clot based method with this global method.

In all these methods the activity of samples of heparin is measured by comparison with a reference standard. The 1st International Standard for unfractionated heparin (UFH) was established by WHO in 1942, and this has been replaced at regular intervals – the current WHO Standard is the 5th (45). The EP and the USP both issue working standards; the EP Standard is calibrated in International Units against the current WHO Standard, but the USP unit, as defined by the USP Standard, differs from the International Unit by approximately 7% (46). It is hoped that the introduction of the global method and the new generation of UFH which has higher specific activity than the previous USP reference standard will minimise or abolish this difference.

Despite the considerable technical differences, when different methods have been compared in international collaborative studies of UFH, the potencies given by the various methods have agreed to within a few percent (45). This is a corroboration of one of the basic principles of biological assays, i.e. that when standard and test are similar in composition ("like vs. like"), the potencies are largely independent of the method used. However, when the first samples of LMWH were assayed against the UFH Standard this was clearly not the case – there was large variability between laboratories, even when ostensibly using the same method. For instance, the coefficient of variation (CV) among seven laboratories carrying out a chromogenic method on the same LMWH sample was 43% (47). There was also a tendency to non-parallelism between the log dose – response lines of the LMWH and UFH Standard, rendering many of the assays statistically invalid. In addition, as expected from the known properties of LMWH, there was a large difference in potency between methods based on inhibition of FXa, and those based in thrombin inhibition or delay of clotting times. The anti-Xa/anti-IIa ratio differed widely among the various LMWH products, and continues to do so; the ratio ranges from 1.6 to 9.7 (Table 1).

Because of all these problems it became clear that the UFH Standard was unsuitable for measurement of the anticoagulant activities of LMWHs. It was therefore decided to establish a separate standard for LMWH, on the basis that "like vs. like" would give better reproducibility. It was recognised that LMWHs as a group were not identical to each other, and so the appropriate material for a standard had to be carefully chosen to be "in the middle" of the group with regard to its MW and anticoagulant properties. Following a preliminary study, two of eight LMWHs were identified as giving the least inter-laboratory variability when used as a standard for assay of the other preparations, with CVs in the range of 4–14% (47). These two preparations were then subjected to a large international collaborative study, and one of the materials was established by WHO as the 1st International Standard for LMWH in 1986 (48). Although WHO Standards are traditionally assigned a single potency, this would have been inappropriate in the case of LMWH, because of the large difference between potencies by anti-Xa and anti-IIa assays (around 2.5 fold). Accordingly the LMWH Standard was assigned two values, one for anti-Xa assays and another for thrombin inhibition assays (including APTT and anti-IIa chromogenic methods).

The 1st International Standard, and the 2nd International Standard which has recently replaced it have been used by manufacturers of all LMWHs to calibrate their products, with the exception of the synthetic pentasaccharide, fondaparinux, which is measured and dosed in mg. It should be noted that the anti-Xa activity of fondaparinux can be validly estimated against the International Standard for Low Molecular Weight Heparin and it is found to be in the range of 800–900 IU/mg. In addition, the EP issues a working standard for LMWH, calibrated against the WHO Standard.

It should be emphasised that the aim of the LMWH standard is to allow reproducible and consistent measurements of in-vitro anticoagulant activities of the various products. The clinical dosage of the various products has to be established on an individual product basis and for this purpose the various products have rightly been treated as individual drugs by the regulatory authorities. The relationship between anticoagulant activity, dosage and clinical efficacy will be discussed in a later section.

Clinical status of heparin and LMWH

Indications and status of UFH

UFH is one of the oldest established antithrombotic drugs. The first clinical studies were carried out in Sweden in the 1930s and 1940s, and it soon became clear that heparin was highly effective in preventing post operative thrombosis (49, 50). Subsequently the indications were extended to treatment of established venous thrombosis, and in a landmark clinical trial in 1960, Barritt and Jordan showed that heparin was highly efficacious in treatment of pulmonary embolism (51). In the 1970s the concept of prophylaxis was refined by Kakkar (52), who introduced low-dose heparin, administered subcutaneously three times daily before and after surgery. In 1988 Collins et al. (53) reviewed the results of over 70 randomised trials, involving more than 16,000 patients, in general, orthopaedic and urological surgery. They concluded that the use of perioperative subcutaneous heparin could prevent about half of all pulmonary emboli and about two thirds of all deep vein thromboses (DVTs), and that twice daily injections were as effective as three times daily; however, there was a slightly increased risk of bleeding compared with the control groups receiving no heparin.

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The main clinical indications for UFH are in prevention and treatment of VTE, in certain types of coronary artery syndrome, particularly unstable angina, and in thrombotic stroke. It has also been used for a number of other minor indications, including haemodialysis. LMWHs has now been studied in all these indications. Given the undoubted efficacy of UFH, there are only three ways in which LMWHs might provide an improvement: greater efficacy (lower rate of thrombotic events); improved safety (less bleeding or other side effects); greater convenience (fewer injections).

Early clinical studies of LMWH for prophylaxis of DVT

General surgery

Following promising results in animal studies, the first clinical trials of LMWH for prophylaxis of DVT took place in the early 1980s. Kakkar et al. (54) were the first to show that a single daily dose of LMWH was able to prevent DVT in 97 of 100 patients, though there was no control group in this pioneering study. In a double-blind study versus UFH in 400 patients, the same group found increased efficacy for the LMWH, with a DVT rate of 2.5% compared to 7.5% for UFH (55). In the latter study there were no significant differences in bleeding rates between UFH and LMWH groups, but in two subsequent studies excessive bleeding was found in patients given LMWH (56, 57). These studies were carried out before the measurement of anti-Xa activity of LMWH had been standardised, and in retrospect it is clear that patients were given too high doses of LMWH. This was emphasised in a second study by Koller et al. (57), where the dosage was reduced to a third of that in the first study – the bleeding rates were then comparable between UFH and LMWH groups.

These studies emphasise the importance of the International Standard for LMWH in ensuring that the anti-Xa activity of different LMWHs when measured for dosage purposes has the same basis. However, it was already clear that the optimum dose of each LMWH had to be evaluated carefully for each preparation, and that LMWH was not a "magic" anticoagulant which could be given at any dose without risk of bleeding.

Two double-blind studies of surgical prophylaxis were carried out by Bergqvist et al. In the first study (58), 5,000 anti-Xa units once daily of LMWH had equivalent efficacy to UFH twice daily but the bleeding rate was significantly higher (11.6% vs. 4.6%). In the second study (59) the same doses were used but the interval between the first dose and surgery was prolonged; the bleeding rates with LMWH were lower (5.9%) but still higher than with UFH (3%).

The studies of Bergvist et al. used the same LMWH (Fragmin, dalteparin) as those of Koller, but at twice the dose (5,000 units vs. 2,500 units). A French multicentre study of Fragmin found 2,500 units to be as effective as UFH with no differences in bleeding rate (60). In another French study of a different LMWH (enoxaparin, Clexane/Lovenox), doses of 20, 40 and 60 mg (≈ 2,000, 4,000, 6,000 anti-Xa units) were compared against UFH. The 20 and 40 mg doses were both equally effective and safe as UFH, but the 60 mg dose was associated with significant decreases in haematocrit and haemoglobin, and the authors concluded that a once-daily dose of 20 mg enoxaparin was as safe and effective as UFH given three times daily (61).

These early studies in the 1980s showed that LMWH could be as effective as UFH in prophylaxis of DVT, but it was clear that the bleeding rate was not reduced, and could be higher if too high a dose was given. Thus the main improvement given by LMWH was the greater convenience of one injection a day instead of two or three. These conclusions were confirmed in a number of other studies in general surgery (for review see [62]).

Orthopaedic surgery

In the more difficult situation of orthopaedic surgery, where DVT rates are higher than general surgery, even with UFH prophylaxis, the early results were also quite promising, though there were some differences in outcome in the various trials. Turpie et al. (63) found a major reduction in DVT in patients undergoing hip surgery, compared to a placebo group (12% vs. 42%), when giving prophylaxis with enoxaparin 30 mg (≈ 3,000 anti-Xa units) twice a day, and Eriksson et al. (64) also found improved efficacy with a twice-daily dose of 2,500 anti-Xa units of Fragmin (DVT 20%), compared to dextran (45%). In contrast Mätzsch et al. (65), using a different LMWH (Logiparin) found no reduction in DVT rate (28%) compared to a dextran group (39%) – in this study the LMWH was given once daily at 35 anti-Xa units/kg (i.e. an average of around 2500 units). However Planes et al. (66) gave 40 mg enoxaparin (≈ 4,000 anti-Xa units) once daily in total hip replacement, and found a significantly reduced DVT rate (12.5%) compared to UFH three times daily (25%). Somewhat surprisingly, Levine et al. (67), using the same LMWH as Planes and a twice-daily dose of 30 mg, found no reduction in overall DVT compared to UFH. One reason for this may be the fact that Levine et al. started prophylaxis 12–24 hours after surgery, whereas Planes et al. gave the first dose 12 hours before surgery. A number of additional studies are reviewed elsewhere (62).

The different results in the various trials could be due to a number of factors, including the use of different LMWHs, different dosage and administration regimens, as well as the fact that most of the trials were fairly small. However, it seems clear from these early studies that LMWH is at least as effective in preventing DVT as UFH, and several studies found it to be more effective, particularly in preventing proximal DVT. Most of the studies found no difference in bleeding rates between LMWH and UFH groups, but those of Planes (66) and Levine (67) found a reduced haemorrhagic tendency in the LMWH groups.

Early studies of LMWH in treatment of VTE

For the first few years after the introduction of LMWH into clinical use most of the studies focused on its use on prophylaxis of DVT. It was initially thought that the relatively high anti-Xa activity of LMWH would lend itself best to prevention, whereas treatment of established thrombosis would require an agent with high anti-thrombin activity. It is now recognised that this is an over-simplification, and that LMWHs can inhibit thrombin generation by multiple pathways (see earlier section).

The first clinical trials of LMWH for treatment of DVT were rather small, and Levine and Hirsh (68) pooled and analysed the data from six such studies. In these studies 97 patients received LMWH, of which 61% had improvement in the venogram, 38% had no change, and 1% showed progression; this compared with

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57% improved, 36% unchanged, and 7% progression in another 97 patients receiving UFH. There were no differences in bleeding tendency between the two groups, and overall it was concluded that LMWH could be at least as effective and safe as UFH for treatment of DVT. Larger individual studies published by several groups (69–71) came to similar conclusions (for review see [72]). As was the case with the prophylaxis studies, there was no clear-cut evidence of a reduction in bleeding tendency with LMWH compared to UFH, but a major advantage was the ability to use twice-daily subcutaneous injections instead of continuous infusion, which is the usual regime with UFH, with the added advantage that no laboratory monitoring is necessary.

Current clinical status of LMWH

Since the early trials in the 1980s there has been a wealth of data published on the use of LMWH in both prophylaxis and treatment of thrombosis, and increasingly it has been replacing UFH in many of the latter's traditional indications. A detailed review is not possible in an article such as this, and attention is focused on the following issues.

- 1) Is LMWH better than UFH for prophylaxis and treatment of DVT?
- 2) What is the place of LMWH in treatment of arterial disease?
- 3) Does LMWH have any special role in cancer patients?
- 4) Is there a need for monitoring of LMWH?
- 5) Are all LMWH products the same in clinical terms?
- 6) Side effects of LMWH and UFH

Is LMWH better than UFH for prevention and treatment of DVT?

As previously noted, there are three ways in which LMWH could be superior to UFH: less thrombosis; less bleeding; more convenient. Although some authors consider that meta-analysis is inappropriate for LMWH because of potential or real differences between products (see subsequent section), it seems clear that LMWHs are a closely related family of drugs with a shared mechanism of action, and several meta-analyses have been carried out.

Leizorovicz et al. (73) analysed results from 52 randomised trials in which LMWH was compared with placebo, dextran or UFH for prophylaxis of DVT in general or orthopaedic surgery. LMWH was much more effective than placebo or dextran (odds ratio [OR] 0.31, 0.44, respectively) and was also slightly more effective than UFH (OR 0.85, p = 0.02). However, there was no significant difference in the incidence of major haemorrhage between LMWH and UFH. Nurmohamed et al. (74) using studies over the same time period, also found an overall benefit for LMWH (OR 0.74), though when only studies with strong methodology were considered the OR was reduced to a non-significant 0.91. They also found no difference in relative effectiveness between general or orthopaedic surgery, and no difference in major bleeding.

In a second meta-analysis of LMWH compared with UFH for treatment of DVT, Leizorovicz et al. (75) analysed 16 randomised trials with over 2,000 patients. There was a significant reduction of the incidence of thrombus extension (OR 0.51) in favour of LMWH. There were also trends in favour of LMWH for lower recurrence of thromboembolism, reduced incidence of major haemorrhage, and lower total mortality, but none of these was statistically significant.

Hirsh et al. (76) examined studies of LMWH versus UFH for treatment of DVT, and classified studies as level 1 or level 2 according to the degree of blinded assessment. In studies classified as level 1, the relative risk (RR) of recurrent VTE during the first 15 days and over the entire period of anticoagulant therapy was 0.24, (p = 0.02) and 0.39 (p = 0.006), respectively, in favour of LMWH treatment. The RR for major bleeding was 0.42 (p = 0.01), in favour of LMWH. In studies classified as level 2, no significant differences in the rates of recurrent VTE and major bleeding were observed. Pooling level 1 and level 2 studies, the RR for overall mortality and mortality in cancer patients was 0.51 (p = 0.01) and 0.33 (p = 0.01) respectively, in favour of LMWH.

Thus overall it is now recognised that LMWH is at least as effective as UFH in prophylaxis of DVT in both general and orthopaedic surgery; there are a number of individual trials which found a greater improvement in effectiveness in orthopaedic surgery, particularly in prevention of proximal thrombosis, but this trend was not significant in the meta-analyses. Although some individual studies of prophylaxis have found a reduced incidence of bleeding for LMWH (77, 78) the overall data do not indicate any improvement of safety with regard to haemorrhage. What is undoubtedly true, however, is that LMWH is more convenient, with one injection a day instead of two or three for UFH.

For treatment of established DVT, LMWH seems from the meta-analyses to offer improvement in all three aspects; greater efficacy, less haemorrhagic effects, and improved convenience. However, the reduced haemorrhagic incidence comes mainly from one large trial in which the dosage of UFH was unusually high (69). In another large individual trial with over 1,000 patients, there was no difference in incidence of bleeding in the LMWH and UFH groups (79). However, probably the greatest advantage of LMWH over UFH is the ability to use fixed dose (per kg body weight) subcutaneous injections, without monitoring, compared to the need for continuous infusion of UFH with frequent monitoring and adjustment of dosage. This has allowed large numbers of patients to be treated at home instead of in hospital, a major advantage both for the patients and in economic terms. As noted in a recent review by Hull, these advantages have led to LMWH also being the recommended treatment for pulmonary embolism, instead of UFH (80).

LMWH in arterial disease

A number of studies have shown that the use of UFH in the acute phase of treatment of unstable angina or non-Q wave myocardial infarction (NQMI) can considerably reduce the risk of recurrent ischaemic events (81, 82). However UFH has several drawbacks, including the need for frequent monitoring, neutralisation by activated platelets, and risk of haemorrhage. LMWH could be expected to have some advantage in these respects, and has been studied in a number of trials.

An early single blind trial of nadroparin (Fraxiparine) found significant benefit compared to UFH (83), but a larger double blind trial of the same LMWH found no significant difference in

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efficacy between the two groups (84). Three studies of dalteparin (Fragmin) found no overall benefit over UFH, in terms of long-term outcome, in either short-term or long-term treatment (for review see [85]). In contrast, a large study of enoxaparin, in over 3,000 patients, found a reduced incidence of the composite end-point (death, MI, or recurrent angina), compared to UFH, both agents being used in conjunction with aspirin (86). This difference was apparent at 14 and 30 days, and was still evident at the one-year follow-up. In a second study of enoxaparin similar results were found, but there was no additional advantage of prolonging the administration of LMWH by outpatient treatment beyond the initial 2–3 days in hospital (87). It is not clear whether the different outcome in these two studies compared with the nadroparin and dalteparin studies are due to the different LMWHs used, or, more likely, to differences in the patient groups (there were more high-risk patients with NQMI and more prior aspirin users in the enoxaparin trials). However, in a recent retrospective analysis of over 6,000 patients treated with enoxaparin for acute ST-elevation myocardial infarction (STEMI), there was a significant reduction in rates of hospital death and re-infarction (88), compared to a similar group of patients receiving UFH.

LMWHs have also been used as adjunctive therapy with thrombolytics in acute MI, and with anti-platelet agents in high risk acute cormoary syndromes, in both cases with promising results (for review see [85]). However, studies of LMWH in thrombotic stroke have so far proved disappointing (89).

LMWH in cancer

Hypercoagualability is a common complication of malignancy, and UFH has been used extensively for prevention and treatment of thrombosis in cancer patients. Heparin is known to have a number of non-anticoagulant activities, including inhibition of tumour growth (90), and these may contribute to its overall therapeutic effect in cancer. Many of the trials of LMWH for treatment of thrombosis have included cancer patients, and in the study of Hull et al. (69), in which LMWH was associated with lower total mortality than UFH (risk reduction 51%), the effect was even more marked in cancer patients. Similar findings were noted in two meta-analyses of LMW versus UFH for treatment of DVT (91, 92), and this has led to the design of a number of clinical trials of LMWH specifically in cancer patients in recent years.

In a study of 136 cancer patients, Meyer et al. (93) found that LMWH (enoxaparin 1.5 mg/kg body weight once daily, ≈150 anti-Xa units/kg) was as effective as warfarin in prevention of recurrent thromboembolism, and there was a non-significant trend towards a reduction in haemorrhagic events.

In one of the largest of such studies, CLOT (94), patients with cancer who had acute, symptomatic proximal deep-vein thrombosis, pulmonary embolism, or both were randomly assigned to receive LMWH (dalteparin) at a dose of 200 IU/kg body weight subcutaneously once daily for 5–7 days and a coumarin derivative for six months (target international normalized ratio [INR], 2.5), or dalteparin alone for six months (200 IU/kg once daily for one month, followed by a daily dose of approximately 150 IU /kg for five months. Of 336 patients in the dalteparin group 27 had recurrent VTE, as compared with 53 of 336 patients in the oral-anticoagulant group (hazard ratio, 0.48; p = 0.002). There were no differences in the incidence of major bleeding but at the end of the trial there was no reduction of overall mortality.

As noted by Monreal et al. (95), many patients are excluded from randomised clinical trials because of complications such as chemotherapy. They carried out a prospective cohort study of 203 patients with disseminated metastatic cancer, of which 157 received chemotherapy. Following an initial dose adjusted for body weight during the first seven days, they gave a fixed daily dose of 10,000 anti-Xa units of dalteparin for three months. There was a reduction in dose to 5,000 Units/day in patients undergoing surgery, and those developing thrombocytopenia. Eleven patients (5.4%) developed major bleeding complications and 18 (8.9%) had recurrent venous thromboembolism. The authors conclude that LMWH is a safe and effective alternative to oral anticoagulants in general cancer patients.

Several recent meta-analysis have also indicated that both UFH and LMWH contribute to improvement of overall survival in cancer patients with and without venous thrombosis (96–101). In addition, there are indications that LMWHs improve response and survival in cancer patients on chemotherapy (102, 103).

Is there a need for monitoring of LMWH?

This question was reviewed by Boneu in 1994 (104), and the conclusion was reached that in the great majority of patients receiving LMWH for prophylaxis or treatment of DVT monitoring is not necessary. In the prophylaxis situation neither UFH nor LMWH have ever been routinely monitored, and in the treatment of DVT many clinical trials have shown LMWH to be effective and safe without the need for monitoring. However, in 1998 the College of American Pathologists (CAP) recommended monitoring in some groups of "non-standard" patients, i.e. those who are grossly under- or over-weight, children, pregnant patients, and those with renal insufficiency (105).

The debate was re-opened recently (106–109). Harenberg (106), whilst conceding that routine monitoring for treatment of DVT had not been shown to be of value in clinical trials, considered that monitoring would be desirable in the groups of patients identified by the CAP, and also in additional groups of patients such as those on long-term therapy for malignancy or other conditions. Harenberg points out that major haemorrhage tends to occur more frequently in patients who are older, lighter, or who have reduced creatinine clearance. Bonameaux and Moerloose (107), emphasise the technical limitations of monitoring, and point out that, in clinical trials where LMWH has been monitored, there was no relationship between the occurrence of major bleeding and high anti-Xa levels in individual patients. However, these authors concede that monitoring may occasionally be helpful in selected groups, i.e. pregnant women, small children, patients with extreme body weight, and those with renal insufficiency, and this conclusion was endorsed also by other authors (108, 109).

Are all LMWH products the same in clinical terms?

The debate about the similarities and differences of the various LMWH products has rumbled on since the early clinical studies, and resurfaces from time to time as more clinical evidence is accumulated.

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The two most important properties of any LMWH are its MW distribution and anticoagulant activities. In these respects it is clear that there are considerable differences among the various products, as can be seen from Table 1. Each manufacturer has developed their own manufacturing process, and for regulatory purposes each product has been treated as a separate drug, requiring its own toxicology, pharmacology, and clinical studies. However, in the opinion of the authors, the differences between products have sometimes been exaggerated, perhaps for commercial reasons, and their similarities ignored.

LMWHs, as a group, share the same essential mechanism of action (binding to antithrombin) and show the following differences from unfractionated heparin:

- higher anti-Xa than anti-IIa activity;
- bioavailability approaching 100%, leading to administration once or twice daily;
- lesser interaction with heparin-binding proteins (PF4, protamine, lipase, histidine-rich glycoprotein etc).

The differences among products in these respects are much less than the differences of the group from UFH. Furthermore, the in-vitro differences in MW range among products will be reduced after subcutaneous injection, as the higher MW fractions of the various products will be absorbed less, so that the molecular size of the circulating components from the various products will be more similar than their in-vitro profiles. The various methods of manufacture do give rise to some chemical differences, but there is no evidence that these have any consequences for the overall biological activities of the products. Ostergaard found no differences in the antithrombotic activities of LMWH prepared by three different methods in an animal model (110).

The crucial question is whether the recognisable differences among products have any clinical relevance. The most appropriate way to answer this question would be by direct comparison of two or more LMWHs in the same clinical trial. However, for a variety of reasons, commercial, economic and ethical, there have been very few such studies, and almost all studies of LMWHs have compared their clinical effects with UFH or placebo. Trying to compare the different products indirectly from the results of these trials is fraught with difficulties. It is inevitable that there will be differences of design, methodology of assessment and patient groups among the various trials, and the number of trials with each LMWH is quite small.

Despite these difficulties, van der Heijden et al. (111) analysed the results of 16 trials of LMWHs for treatment of DVT using a method called meta-regression. The conclusion was that one of the products (dalteparin) showed differences in efficacy and safety, but there were no differences among the others. However, this conclusion has not been generally endorsed; in a more recent review, Prandoni (112) considers that LMWHs are largely interchangeable in clinical terms when used at the recommended doses. This view is contradicted by Nenci (113), but his view of the inequivalence of LMWHs is based largely on results of two trials of enoxaparin in arterial disease, in which it was found to be more effective than UFH; other LMWHs have only been found equivalent to UFH in the same indication. However, as already pointed out, and conceded by Nenci and other authors (114), differences in characteristics of the patients are as equally plausible an explanation for the results of these trials as differences among products.

In one of the few direct comparisons of two products, Planes compared tinzaparin and enoxaparin for prophylaxis of DVT in 499 patients undergoing elective hip surgery (115). Despite significant differences in anti-Xa levels, the two drugs were equivalent in terms of efficacy and safety. The same conclusion was reached in several other trials comparing clivarin, reviparin, bemiparin and nadroparin versus enoxaparin for prophylaxis in orthopaedic surgery (116–119).

The only product which might be expected to show some differences from the others is the synthetic pentasaccharide, fondaparinux. Unlike the other products, which are all prepared by depolymerisation of UFH and contain a mixture of fragments with different MW, the pentasaccharide is a homogeneous product, does not have any anti-IIa activity, does not contain any low-affinity (to antithrombin) material, and has minimal binding to heparin-binding proteins. In a meta-analysis of four clinical trials in which fondaparinux was compared to enoxaparin for prophylaxis of DVT in orthopaedic surgery, Turpie et al. found that fondaparinux was more effective (odds reduction 55%), and although major bleeding occurred more frequently in the fondaparinux group, the incidence of clinically relevant bleeding did not differ between groups (120). In trials of fondaparinux for treatment of DVT, it has been found at least as effective and safe as enoxaparin (121).

Side effects of heparin and LMWH

Over many years of clinical use, heparin has been a remarkably safe drug, especially considering its biological origin and its heterogeneity. The main concern, as with all anticoagulants, is excessive bleeding, and the issue of whether LMWH is associated with less bleeding than UFH has been dealt with in earlier sections. The methods of manufacture of LMWHs should not give additional safety concerns, and any possible differences in side effects between LMWH and UFH are likely to rest on MW differences, with LMWH having lesser interaction with heparin-binding proteins and cells.

Apart from bleeding, the main side effect of heparin is heparin-induced thrombocytopenia (HIT). The incidence is difficult to estimate and depends on the type of clinical indication and the duration of therapy, but is generally thought to be around 1–3%. The sharp drop in platelet count is caused by antibodies binding to a complex of PF4 and heparin. Since LMWHs generally display less interaction with PF4 it could be that they may give rise to a lower incidence of HIT; this was indeed found to be the case in a prospective study of UFH versus enoxaparin (122). However, it is clear that HIT can still occur during LMWH treatment, and since such patients still require anticoagulant therapy, the general recommendation is to switch to alternative forms of anticoagulation, such as thrombin inhibitors. The synthetic pentasaccharide fondaparinux has no reactivity with PF4, and thus may be associated with a much reduced incidence: it has also been suggested for therapy in patients with established HIT (123, 124). However, Warkentin found that HIT could also occur, albeit rarely, in patients undergoing treatment with fondaparinux (125), and therefore thrombin inhibitors such as hirudin or ar-

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gatroban are still the recommended anticoagulants for patients with established HIT (126).

A further side-effect which may be sometimes related to HIT is heparin-induced skin necrosis. In an extensive review of the literature, Handschin et al. (127) found that this was a rare complication of LMWH treatment, with only 21 cases reported. Skin necrosis occurred distant from the injection site, and heparin-induced antibodies occurred in a majority of patients, although severe thrombocytopenia was only found in four cases; other pathogenic mechanisms as well as that causing HIT may be involved. Milder skin reactions classified as delayed hypersensitivity are relatively common with subcutaneous administration of UFH, but do not usually occur when UFH is given intravenously (128). Patients with skin lesions induced by UFH or LMWH are usually switched to alternative anticoagulants such as thrombin inhibitors, although fondaparinux has recently been suggested as an alternative therapy for such patients (129).

The other main side effect of heparin is osteoporosis, which can occur, albeit rarely, during long-term therapy. Animal studies have shown that both heparin and LMWH decrease bone formation, but heparin also increases bone resorption, whereas LMWH does not (130). Studies in rats showed a lower incidence of osteoporosis with LMWH compared to UFH (131, 132). In a double-blind study of treatment of DVT, the incidence of spinal fracture was lower with LMWH (dalteparin) than UFH (133), but further studies are needed to substantiate this difference.

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