Heparin and Low-Molecular-Weight Heparin

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This article about unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH) is part of the Seventh American College of Chest Physicians Conference on Antithrombotic and Thrombolytic Therapy: Evidence-Based Guidelines. UFH is a heterogeneous mixture of glycosaminoglycans that bind to antithrombin via a pentasaccharide, catalyzing the inactivation of thrombin and other clotting factors. UFH also binds endothelial cells, platelet factor 4, and platelets, leading to rather unpredictable pharmacokinetic and pharmacodynamic properties. Variability in activated partial thromboplastin time (aPTT) reagents necessitates site-specific validation of the aPTT therapeutic range in order to properly monitor UFH therapy. Lack of validation has been an oversight in many clinical trials comparing UFH to LMWH. In patients with apparent heparin resistance, anti-factor Xa monitoring may be superior to measurement of aPTT. LMWHs lack the nonspecific binding affinities of UFH, and, as a result, LMWH preparations have more predictable pharmacokinetic and pharmacodynamic properties. LMWHs have replaced UFH for most clinical indications for the following reasons: (1) these properties allow LMWHs to be administered subcutaneously, once daily without laboratory monitoring; and (2) the evidence from clinical trials that LMWH is as least as effective as and is safer than UFH. Several clinical issues regarding the use of LMWHs remain unanswered. These relate to the need for monitoring with an anti-factor Xa assay in patients with severe obesity or renal insufficiency. The therapeutic range for anti-factor Xa activity depends on the dosing interval. Anti-factor Xa monitoring is prudent when administering weight-based doses of LMWH to patients who weigh > 150 kg. It has been determined that UFH infusion is preferable to LMWH injection in patients with creatinine clearance of < 25 mL/min, until further data on therapeutic dosing of LMWHs in renal failure have been published. However, when administered in low doses prophylactically, LMWH is safe for therapy in patients with renal failure. Protamine may help to reverse bleeding related to LWMH, although anti-factor Xa activity is not fully normalized by protamine. The synthetic pentasaccharide fondaparinux is a promising new antithrombotic agent for the prevention and treatment of venous thromboembolism.

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Key words: heparin; low-molecular-weight heparin; pentasaccharide

Abbreviations: aPTT = activated partial thromboplastin time; AT = antithrombin; BMI = body mass index; CI = confidence interval; CrCl = creatinine clearance; DVT = deep-vein thrombosis; HC = heparin cofactor; HIT = heparin-induced thrombocytopenia; LMWH = low-molecular-weight heparin; MI = myocardial infarction; PF = platelet factor; RR = relative risk; rt-PA = recombinant tissue plasminogen activator; SC = subcutaneous; TBW = total body weight; UFH = unfractionated heparin

1.0 Heparin: Historical Perspective, Chemical Structure, and Mechanism of Action

Heparin, a heterogeneous mixture of branched glycosaminoglycans, was discovered to have antithrombotic properties by McLean almost 90 years ago.[1] Brinkhous and associates[2] then demonstrated that heparin is an indirect anticoagulant, requiring a plasma cofactor. This cofactor was subsequently named antithrombin (AT) III by Abildgaard in 1968[3] and now is referred to simply as AT. The main anticoagulant action of heparin is mediated by the heparin/AT interaction. The mechanism of this interaction was elucidated by Rosenberg and colleagues[4,5] and Lindahl et al[6] in the 1970s. Heparin binds to lysine sites on AT, producing a conformational change at the arginine reactive center, which converts AT from a slow, progressive thrombin inhibitor to a very rapid inhibitor. The arginine reactive center on the AT molecule binds covalently to the active center serine of thrombin and other coagulation enzymes, thereby irreversibly inhibiting their procoagulant activity.[4] Heparin then dissociates from the ternary complex and is reutilized[4] (Fig 1). Subsequently, it was discovered that heparin binds to AT through a unique glucosamine unit[4–7] that is contained within a pentasaccharide sequence.[8] The pentasaccharide has been synthesized and has been developed into a promising new anticoagulant.[9–13] The development of low-molecular-weight heparin (LMWH) in the 1980s introduced the concept that the ability of heparin molecules to inactivate thrombin and other activated coagulation factors are chain length-dependent, whereas the inactivation of factor Xa only requires the presence of the high-affinity pentasaccharide.

1.1 Heparin: structure and mechanism of action

Heparin is heterogeneous with respect to molecular size, anticoagulant activity, and pharmacokinetic properties (Table 1). Its molecular weight ranges from 3,000 to 30,000, with a mean molecular weight of 15,000 (approximately 45 monosaccharide chains) [Fig 2].[14–16] Only about one third of an administered dose of heparin binds to AT, and this fraction is responsible for most of its

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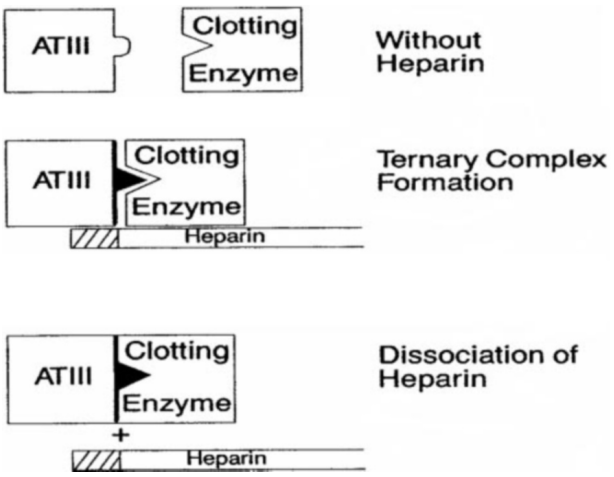

FIGURE 1. Inactivation of clotting enzymes by heparin. *Top*: ATIII is a slow inhibitor without heparin. *Middle*: heparin binds to ATIII through a high-affinity pentasaccharide and induces a conformational change in ATIII, thereby converting ATIII from a slow inhibitor to a very rapid inhibitor. *Bottom*: ATIII binds covalently to the clotting enzyme, and the heparin dissociates from the complex and can be reutilized.

anticoagulant effect.[17,18] The remaining two thirds of a dose has minimal anticoagulant activity at therapeutic concentrations, but at concentrations greater than usually obtained clinically both high-affinity and low-affinity heparin catalyze the AT effect of a second plasma protein, heparin cofactor (HC) II.[19] At even higher concentrations, low-affinity heparin impairs factor Xa generation through AT-independent and HCII-independent mechanisms[20] (Table 2).

The heparin/AT complex inactivates thrombin factor IIa and factors Xa, IXa, XIa, and XIIa.[4] Thrombin and factor Xa are most sensitive to inhibition by heparin/AT, and thrombin is about 10-fold more sensitive to inhibition than factor Xa. Heparin inhibits thrombin by binding both to the coagulation enzyme (through a nonspecific charge effect) and to AT through the high-affinity pentasaccharide. In contrast, the inhibition of factor Xa requires that heparin bind only to the AT via the high-affinity pentasaccharide.[7] Molecules of heparin with < 18 saccharides lose their ability to bind simultaneously to thrombin and AT, and, therefore, are unable to catalyze thrombin inhibition.

Table 1—*Heterogenicity of Heparin*

Attribute	Characteristics
Molecular size	Mean molecular weight, 15,000 Range, 3,000–30,000
Anticoagulant activity	Only one third of heparin molecules contain the high-affinity pentasaccharide required for anticaogulant activity
Clearance	High-molecular-weight moieties are cleared more rapidly than lower molecular weight moieties

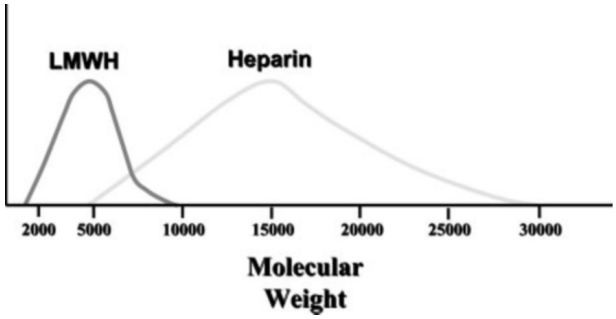

FIGURE 2. Molecular weight distributions of LMWHs and heparin.

In contrast, very small heparin fragments containing the high-affinity pentasaccharide sequence catalyze the inhibition of factor Xa by AT.[21–24] By inactivating thrombin, heparin not only prevents fibrin formation but also inhibits thrombin-induced activation of platelets and coagulation factors factor V and factor VIII.[25–27]

Heparin activates HCII and thereby inactivates thrombin through a second mechanism. This interaction is charge-dependent, but is pentasaccharide-independent and requires a higher concentration of heparin than that required for AT-mediated inactivation. HCII-mediated inactivation of thrombin is also molecular weight-dependent, requiring a minimum of 24 saccharide units. The HCII-mediated anticoagulant effect of heparin could operate in cases of severe AT deficiency.

The third anticoagulant effect of heparin results from an AT-independent and HCII-independent modulation of factor Xa generation. It is charge-dependent, is mediated by heparin binding to factor IXa, and requires very high doses of the sulfated polysaccharide to produce an anticoagulant effect.[20] Although not important clinically, this direct anticoagulant effect has complicated the development of non-anticoagulant heparin preparations for the prevention of restenosis after angioplasty.

The anticoagulant activity of heparin is heterogeneous for the following reasons: (1) only one third of heparin molecules contain the high-affinity pentasaccharide; and (2) the anticoagulant profile and clearance of heparin is influenced by the chain length of the molecules. Thus, the higher molecular weight species are cleared from the circulation more rapidly than the lower molecular weight species, resulting in the accumulation of the lower molecular weight species, which have a lower ratio of anti IIa activity to anti-factor Xa activity. *In vitro*, heparin binds to platelets and, depending on the experimental conditions, can either induce or inhibit platelet aggregation.[28,29] High-molecular-weight heparin fractions with low affinity for AT have a greater effect on platelet function than LMWH fractions with high AT affinity.[30] Heparin prolongs the bleeding time in humans[31] and enhances blood loss from the microvasculature in rabbits.[32–34] The interaction of heparin with platelets[32] and endothelial cells[33] may contribute to heparin-induced bleeding by a mechanism that is independent of its anticoagulant effect.[34]

In addition to anticoagulant effects, heparin increases

Table 2—*Antihemostatic Effects of Heparin*

Effect	Comment
Binds to ATIII and catalyzes inactivation of factors IIa, Xa, IXa, and XIIa	Major mechanism for anticoagulant effect, produced by only one third of heparin molecules (those containing the unique ATIII-binding pentasaccharide)
Binds to heparin cofactor II and catalyzes inactivation of factor IIa	Anticoagulant effect requires high concentrations of heparin and occurs to the same degree whether the heparin has high or low affinity for ATIII
Binds to factor IX and inhibits factor Xa activation	Requires very high concentration of heparin, and is AT- and HCII-independent

vessel wall permeability,[33] suppresses the proliferation of vascular smooth muscle cells,[35] suppresses osteoblast formation, and activates osteoclasts, with these last two effects promoting bone loss.[36,37] Of these three effects, only the osteopenic effect has been shown to be relevant clinically, and all three are independent of its anticoagulant activity.[38] Warkentin et al, in another article in this supplement, discuss heparin-induced thrombocytopenia (HIT) as another clinically important side effect.

1.2 Heparin: pharmacokinetics

The two preferred routes of administration of unfractionated heparin (UFH) are continuous IV infusion and subcutaneous (SC) injection. When the SC route is selected, the initial dose should be approximately 10% higher than the usual IV dose to overcome the reduced bioavailability associated with SC administration.[39] When heparin is given by SC injection in a dose of 35,000 U over 24 h in two divided doses,[40] the anticoagulant effect is delayed for approximately 1 h, and the peak plasma levels occur at approximately 3 h. If an immediate anticoagulant effect is required, the initial dose should be accompanied by an IV bolus injection.

The plasma recovery of heparin is reduced[41] when the drug is administered by SC injection in low doses (*eg*, 5,000 U every 12 h) or moderate doses of 12,500 U every 12 h[42] or 15,000 U every 12 h.[39] However, at high therapeutic doses (*ie*, $>$ 35,000 U over 24 h) plasma recovery is almost complete.[43] The difference between the bioavailability of heparin administered by SC or IV injection was demonstrated strikingly in a study of patients with venous thrombosis[39] who were randomized to receive either 15,000 U heparin every 12 h by SC injection or 30,000 U heparin by continuous IV infusion. Both regimens were preceded by an IV bolus dose of 5,000 U. Therapeutic heparin levels and activated partial thromboplastin time (aPTT) ratios were achieved at 24 h in only 37% of patients who were given SC injections of heparin, compared with 71% in those who were given the same total dose by continuous IV infusion.

After entering the bloodstream, heparin binds to a number of plasma proteins, which reduces its anticoagulant activity, thereby contributing to the variability of the anticoagulant response to heparin among patients with thromboembolic disorders[44] and to the laboratory phenomenon of heparin resistance.[45] Heparin also binds to endothelial cells[46] and macrophages, a property that further complicates its pharmacokinetics. The binding of heparin to von Willebrand factor also inhibits von Willebrand factor-dependent platelet function.[47]

Heparin is cleared through the combination of a rapid saturable mechanism and a much slower first-order mechanisms[48–50] (Fig 3). The saturable phase of heparin clearance is thought to be due to binding to endothelial cell receptors[51,52] and macrophages,[53] in which it is depolymerized[40,54] (Fig 4). The slower nonsaturable mechanism of clearance is largely renal. At therapeutic doses, a considerable proportion of heparin is cleared through the rapid, saturable, dose-dependent mechanism. These kinetics make the anticoagulant response to heparin nonlin-

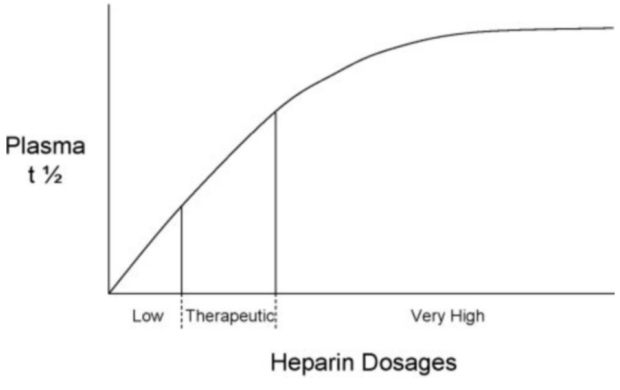

FIGURE 3. Low doses of heparin clear rapidly from plasma through a saturable (cellular) mechanism and the slower, nonsaturable, dose-independent mechanism of renal clearance. Very high doses of heparin are cleared predominantly through the slower nonsaturable mechanism of clearance. t½ = half-life.

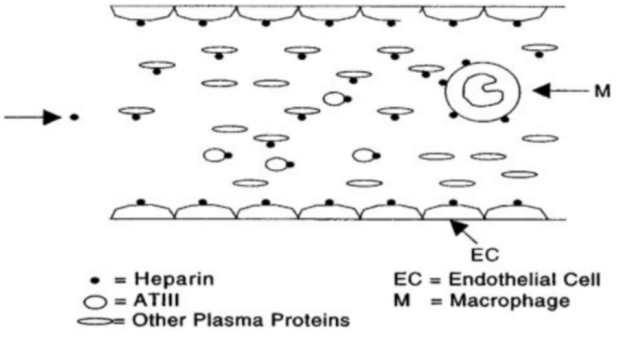

FIGURE 4. As heparin enters the circulation, it binds to heparin-binding proteins (ie, other plasma proteins), ECs, Ms, and ATIII. Only heparin with the high-affinity pentasaccharide binds to ATIII, but binding to other proteins and to cells is nonspecific and occurs independently of the ATIII binding site.

ear at therapeutic doses, with both the intensity and duration of effect rising disproportionately with increasing dose. Thus, the apparent biological half-life of heparin increases from approximately 30 min following an IV bolus of 25 U/kg, to 60 min with an IV bolus of 100 U/kg, to 150 min with a bolus of 400 U/kg.[48–50]

1.3 Heparin: initial dosing

The initial dosing of heparin for treatment of venous thromboembolism is weight-based (80 U/kg bolus and 18 U/kg/h infusion).[55] Doses of heparin that are administered to treat patients with coronary thrombosis syndromes are lower than those typically used to treat those with venous thromboembolism. The American College of Cardiology recommends a heparin bolus of 60 to 70 U/kg (maximum dose, 5,000 U) and the infusion of 12 to 15 U/kg/h (maximum dose, 1,000 U per hour) for unstable angina and non-ST-segment elevation myocardial infarction (MI),[56] and somewhat lower dosing (60 U/kg bolus [maximum dose, 4,000 U], and 12 U/kg infusion (maximum dose, 1,000 U per hour) in patients receiving recombinant tissue plasminogen activator (rt-PA) [alteplase] for acute ST-segment elevation MI.[57] In patients undergoing percutaneous coronary interventions, heparin is administered in conjunction with glycoprotein IIb/IIIa inhibitors as a bolus of 70 U/kg, with additional boluses administered to keep the activated clotting time (ACT) at > 200 s.[58]

The risk of heparin-associated bleeding increases with the dose,[59,60] and with thrombolytic therapy[61–64] or glycoprotein IIb/IIIa inhibitor therapy.[58,65] The risk of bleeding is also increased if the patient has recently undergone surgery, trauma, or invasive procedures, or has concomitant hemostatic defects.[66] A relationship has been reported between the dose of heparin administered and both its efficacy[39,55,67] and safety.[58,65] Therefore, the dose of heparin must be adjusted, usually by the measurement of the aPTT, or, when very high doses are given, by ACT. These tests are sensitive mainly to the AT effects of heparin.

1.4 Heparin monitoring

The anticoagulant effect of heparin is monitored by the aPTT when usual therapeutic doses are used and by the ACT when higher doses are used in association with percutaneous coronary interventions and cardiopulmonary bypass surgery. In the 1970s, an aPTT in the range of 1.5 to 2.5 times the control value was shown to be associated with a reduced risk for recurrent thromboembolism.[68] Thereafter, a therapeutic aPTT range of 1.5 to 2.5 times the control value gained wide clinical acceptance.

However, the reagents and instruments used to determine the aPTT have changed in the past 25 years. Thus, there are now > 300 different laboratory methods in current use,[69] and as a result there is a wide variation in responsiveness to an anticoagulant among different laboratories. This variability is due to differences among thromboplastin reagents and coagulometer instruments.[70–78] The magnitude of this variability is highlighted by the observation that at plasma heparin concentrations of 0.3 IU/mL (by factor Xa inhibition) the mean aPTT results range from 48 to 108 s, depending on the laboratory method employed.[70,72] At therapeutic heparin levels (*ie*, 0.3 to 0.7 anti-factor Xa units), modern thromboplastin reagents produce aPTT ratios that range from 1.6 to 2.7 to 3.7 to 6.2 times the control value[70,72–74,78–84] (Table 3). It is clear, therefore, that the use of a standard aPTT therapeutic range of 1.5 to 2.5 for all reagents and methods of clot detection leads to the systematic administration of subtherapeutic doses of heparin.

Despite these shortcomings, the aPTT is the most common method used for monitoring its anticoagulant response. The aPTT should be measured approximately 6 h after the bolus dose of heparin, and the continuous IV dose should be adjusted according to the result. Various heparin dose-adjustment nomograms have been developed (Tables 4[85] and 5), but none are applicable to all aPTT reagents, and, for the reasons discussed above, the therapeutic range should be adapted to the responsiveness of the reagent used.

Such problems with standardizing APTT monitoring have been highlighted in a recent review[86] that examined the methodological quality of heparin administration in clinical trials comparing heparin and LMWH for the treatment of venous thrombosis. Of the 16 studies that met the inclusion criteria,[87–102] only 3 used a properly validated aPTT therapeutic range to make heparin dose adjustments. Eleven studies used aPTT ranges that included values of 1.5 times the control value, which is invariably associated with subtherapeutic heparin levels when modern thromboplastin reagents are used, and 7 studies reported that the steady-state dose of heparin was $< 30{,}000$ U per 24 h after adjustment based on aPTT measurement results. Thus, the true efficacy of heparin in clinical trials of venous thromboembolism has likely been underestimated because most of the studies used unvalidated aPTT therapeutic ranges and therefore suboptimal heparin dosing.

It is possible that similar problems with heparin monitoring could explain the results of studies[103] that have reported that the aPTT is not a good measure of heparin efficacy in patients with acute MI who were treated with thrombolytic therapy. The studies that provide the foundation for recommendations for UFH use in coronary thrombosis did not calibrate their therapeutic aPTT ranges by anti-factor Xa assays. Therefore, it is difficult to accurately reproduce the UFH dose adjustments used in other institutions. Simply generalizing aPTT therapeutic ranges would guarantee systematic errors in heparin administration in institutions with different thromboplastin reagents.

The College of American Pathologists[104] and others[69,71,73,75–77,79,80,82,105] have joined the American College of Chest Physicians consensus group in recommending against the generalized use of a fixed aPTT therapeutic range such as 1.5 to 2.5 times the control value. Instead, we recommend that the therapeutic aPTT range be calibrated specifically for each reagent lot/coagulometer by determining the aPTT values that correlate with therapeutic heparin levels (equivalent to 0.3 to 0.7 IU/mL by factor Xa inhibition for the treatment of venous throm-

Table 3—*Representative aPTT Therapeutic Ranges for Various Modern Thromboplastin Reagents, Determined by Recommended Methodology*

Reagent	Therapeutic aPTT	Therapeutic aPTT ratio	Reference
Actin*	59–84	2.3–3.2†	71
	49–92 to 49–109‡	1.9–3.7 to 2.1–4.6‡	69
Actin FS*	60–85	1.8–2.5	67
	66–109	2.2–3.6	70
	79–105	2.3–3.0	67
	64–112	2.2–3.9†	77
	55–78	1.9–2.7†	75
	81–185	2.6–6.0	79
	72–119 to 98–165‡	2.6–4.3 to 3.7–6.2‡	69
Actin FSL*	57–98 to 84–124‡	2.1–3.5 to 2.6–3.8‡	69
IL Test§	71–96	2.3–3.1	80
	49–109 to 63–101‡	1.7–3.8 to 1.9–3.3‡	69
Platelin L‖	75–105	2.8–3.9	78
	64–106	2.3–3.9	76
	55–97	2.1–3.7†	77
Syntasil¶	70–158	2.0–4.5	79
Thrombosil I¶	44–75 to 58–112‡	1.6–2.7 to 2.4–4.5‡	69

*Dade Diagnostics, Aguada, Puerto Rico.
†aPTT ratios were calculated by dividing the reported therapeutic aPTT range by the control value for the reagent reported in contemporaneous literature.
‡aPTT therapeutic ranges were obtained with the same reagent but with different coagulometers.
§Instrumentation Laboratories, Fisher Scientific, Unionville, ON, Canada.
‖Organon Teknika, Durham, NC.
¶Ortho Diagnostic Systems, Raritan, NJ.

bosis. The therapeutic range for coronary indications is unknown but is likely to have an upper limit of 0.6 IU/mL.

1.5 Heparin resistance

Heparin resistance is a term that is used to define patients who require unusually high doses of heparin in order to prolong their aPTT into a therapeutic range.[106–108]

Table 4—*Protocol for Heparin Dose Adjustment**

aPTT,† s	Repeat Bolus Dose, U	Stop Infusion, min	Change Rate (dose) of Infusion at 40/mL/h,‡ mL/h	Time of Next aPTT
< 50	5,000	0	+ 3 (+ 2,880)	6 h
50–59		0	+ 3 (+ 2,880)	6 h
60–85§		0	0 (0)	Next morning
86–95		0	− 2 (− 1,920)	Next morning
96–120		30	− 2 (− 1,920)	6 h
> 120		60	− 4 (− 3,840)	6 h

*Starting dose of 5,000 U as IV bolus followed by 32,000 U per 24 h as a continuous infusion (40 U/mL). First aPTT measurement was performed 6 h after the bolus injection, dosage adjustments were made according to the protocol, and aPTT measurement was repeated as indicated in the far right column. Table was adapted from Cruickshank et al.[85]
†Normal range for aPTT with Dade Actin FS reagent is 27 to 35 s.
‡Values in parentheses are U per 24 h.
§Therapeutic range of 60 to 85 s is equivalent to a heparin level of 0.2 to 0.4 U/mL by protamine titration or 0.35 to 0.7 U/mL as an anti-factor Xa heparin level. Therapeutic range will vary with responsiveness of the aPTT reagent to heparin.

Several mechanisms for heparin resistance have been identified, including AT deficiency,[104] increased heparin clearance,[109] elevations in heparin-binding proteins,[110] and elevations in factor VIII levels[106,111] and fibrinogen levels.[111] Aprotinin and nitroglycerin have been reported as causes of drug-induced heparin resistance,[112,113] although the association with nitroglycerin is controversial.[114] Elevated factor VIII level is a common mechanism for apparent heparin resistance.[106] It causes a dissociation of the anticoagulant effect of heparin, as measured by the aPTT from heparin levels, as measured by anti-factor Xa activity.[104,108] The results of a randomized controlled trial[106] in patients with venous thromboembolism showed that patients with heparin resistance, which is indicated by a requirement for large doses of heparin, achieve equiva-

Table 5—*Weight-Based Nomogram**

aPTT	Dose
Initial dose	80 U/kg bolus, then 18 U/kg/h
< 35 s	80 U/kg bolus, then 4 U/kg/h
35–45 s	40 U/kg bolus, then 2 U/kg/h
46–70 s†	No change
71–90 s	Decrease infusion rate by 2 U/kg/h
> 90 s	Hold infusion 1 h, then decrease infusion rate by 3 U/kg/h

*Table was adapted from Raschke et al.[67]
†Therapeutic aPTT range of 46 to 70 s corresponded to anti-factor Xa activity of 0.3 to 0.7 U/mL at the time this study was performed. The therapeutic range at any institution should be established by correlation with anti-factor Xa levels in this range.

lent clinical outcomes with lower doses of heparin when heparin therapy is adjusted to achieve anti-factor Xa heparin concentrations of 0.35 to 0.7 IU/mL. The latter is a reasonable approach in patients with venous thromboembolism who require unusually high doses of heparin (*eg*, > 40,000 U per 24 h) to achieve a therapeutic aPTT.

1.6 Limitations of heparin

In addition to its well-known bleeding complications, heparin has limitations based on its pharmacokinetic and biophysical properties, its ability to induce immune-mediated platelet activation (leading to HIT), and its effect on bone metabolism (leading to heparin-induced osteoporosis). All of the nonhemorrhagic limitations are caused by the AT-independent, charge-dependent binding properties of heparin to proteins and surfaces. Pharmacokinetic limitations are caused by the AT-independent binding of heparin to plasma proteins,[115] to proteins released from platelets,[40] and possibly to endothelial cells, which result in the variable anticoagulant response to heparin and to the phenomenon of heparin resistance;[103] AT-independent binding to macrophages and endothelial cells also result in the dose-dependent mechanism of clearance.

The biophysical limitations occur because the heparin/AT complex is unable to inactivate factor Xa in the prothrombinase complex and thrombin bound to fibrin or to subendothelial surfaces. The biological limitations of heparin include osteopenia and HIT. Osteopenia is caused by the binding of heparin to osteoblasts,[36] which then release factors that activate osteoclasts, whereas HIT results from heparin binding to platelet factor (PF) 4, forming an epitope to which the HIT antibody binds.[116,117] The pharmacokinetic and non-anticoagulant biological limitations of heparin are less evident with LMWH,[118] while the limited affinity of the heparin/AT complex to fibrin-bound thrombin and factor Xa has been overcome by several new classes of AT-independent thrombin and factor Xa inhibitors.[119]

The anticoagulant effect of heparin is modified by platelets, fibrin, vascular surfaces, and plasma proteins. Platelets reduce the anticoagulant effect of heparin by protecting surface factor Xa from inhibition by heparin/AT complex[120,121] and by secreting PF4, a heparin-neutralizing protein.[122] Fibrin protects thrombin bound to its surface from inhibition by heparin/AT complex because heparin binds to fibrin, and bridges between fibrin and the heparin-binding site on thrombin.[123] As a result, heparin increases the affinity of thrombin for fibrin and, by occupying the heparin-binding site on thrombin, protects fibrin-bound thrombin from inactivation by the heparin/AT complex.[124,125] Thrombin also binds to subendothelial matrix proteins, where it is protected from inhibition by heparin.[126] These observations explain why heparin is less effective than the AT-independent thrombin and factor Xa inhibitors[123] for preventing thrombosis at sites of deep arterial injury in experimental animals,[127,128] and may explain why hirudin is more effective than heparin in patients with unstable angina and non-Q-wave MI.[129]

1.7 Reversing the anticoagulant effects of heparin

The treatment of clinically severe bleeding in the course of heparin therapy includes antiheparin therapy in addition to supportive care and transfusion therapy. The effects of UFH can be rapidly antagonized by an IV bolus of protamine. Protamine is a basic protein derived from fish sperm that binds to heparin to form a stable salt. One milligram of protamine will neutralize approximately 100 U UFH. Therefore, a patient who bleeds immediately following an IV bolus of 5,000 U UFH will require the administration of 50 mg protamine. When UFH is given as an IV infusion, only heparin given during the preceding several hours needs to be included in this dose calculation, since the half-life of IV UFH is short (approximately 60 min). Therefore, a patient receiving a continuous IV infusion of 1,250 U per hour will require approximately 30 mg protamine. The neutralization of an SC dose of UFH may require a prolonged infusion of protamine. The aPTT can be used to assess the effectiveness of antiheparin therapy.[130]

The risk of severe adverse reactions, such as hypotension and bradycardia, can be minimized by administering protamine slowly (*ie*, over > 1 to 3 min). Patients who have previously received protamine-containing insulin, have undergone a vasectomy, or have a known sensitivity to fish are at an increased risk to develop antiprotamine antibodies and to experience allergic reactions, including anaphylaxis.[131,132] Patients who are at risk for protamine allergy can be pretreated with corticosteroids and antihistamines.

A number of other methods have been used to neutralize the effects of UFH. These include hexadimethrine,[133,134] heparinase (neutralase),[135] PF4,[136,137] extracorporeal heparin-removal devices,[138,139] and synthetic protamine variants.[140] These therapies are not widely available.

2.0 LMWHs: Historical Perspective and Overview

LMWHs are derived from UFH by chemical or enzymatic depolymerization. The development of LMWHs for clinical use was stimulated by the following three main observations: (1) LMWHs have reduced anti-factor IIa activity relative to anti-factor Xa activity[15,141]; (2) LMWHs have a more favorable benefit/risk ratio[142,143] in animal studies; and (3) LMWHs have superior pharmacokinetic properties.[144–149] Of these potential advantages, only the superior pharmacokinetic properties are of clear clinical importance.[118,150]

LMWH fractions prepared from standard commercial-grade heparin have been shown to have a progressively lower effect on aPTT as they are reduced in molecular size, while still inhibiting activated factor X (*ie*, factor Xa).[15,145] The aPTT activity of heparin reflects mainly its anti-factor IIa activity. The disassociation of anti-factor Xa activity from its effect on thrombin (IIa) activity (expressed as an aPTT measurement), which was described in 1976,[16] challenged the prevailing biophysical model for the anticoagulant effect of heparin, which predicted that any heparin molecule, irrespective of chain length, would catalyze the inactivation of serine protease coagulation

Table 6—*Methods of Preparation for LMWHs and a Heparinoid*

Agent	Method of Preparation
Nadroparin calcium (fraxiparin)	Nitrous acid depolymerization
Enoxaparin sodium (lovenox/clexane)	Benzylation followed by alkaline depolymerization
Dalteparin (fragmin)	Nitrous acid depolymerization
Tinzaparin (innohep)	Enzymatic depolymerization with heparinase
Danaparoid sodium (organ)	Prepared from animal gut mucosa; contains heparin sulfate (84%), dermatan sulfate (12%), and chondroitin sulfate (4%)

enzymes equally, provided that it contained the high-affinity binding site for AT. The explanation for the difference in anticoagulant profile between LMWHs and heparin was elucidated in subsequent studies.[151–155]

2.1 LMWH: structure and mechanism of action

LMWHs are polysulfated glycosaminoglycans that are about one third the molecular weight of UFH. LMWHs have a mean molecular weight of 4,000 to 5,000 d (about 15 monosaccharide units per molecule), with a range of 2,000 to 9,000 d. The various LMWHs approved for use in Europe, Canada, and the United States are shown in Table 6. Because LMWHs are prepared by different methods of depolymerization, they differ to some extent in pharmacokinetic properties and anticoagulant profiles, and are not clinically interchangeable.

The depolymerization of heparin yields low-molecular-weight fragments with reduced binding to proteins or cells (Table 7). Indeed, all of the anticoagulant, pharmacokinetic, and other biological differences between heparin and LMWH can be explained by the relatively lower binding properties of LMWH. Thus, compared to heparin, LMWHs have a reduced ability to inactivate thrombin because the smaller fragments cannot bind simultaneously to AT and thrombin. On the other hand, since bridging between AT and factor Xa is less critical for anti-factor Xa activity, the smaller fragments inactivate factor Xa almost as well as larger molecules.[156–158] Reduced binding to plasma proteins is responsible for the more predictable dose-response relationship of LMWHs.[118] A lower incidence of binding to macrophages and endothelial cells increases the plasma half-life[150] of LMWH compared to UFH, whereas reducing binding to platelets and PF4 may explain the lower incidence of HIT.[159] Finally, the reduced binding of LMWH to osteoblasts results in a lower incidence of activation of osteoclasts and lower levels of bone loss.[36,37]

Like heparin, LMWHs produce their major anticoagulant effect by activating AT. The interaction with AT is mediated by a unique pentasaccharide sequence,[7,160] which is found on fewer than one third of LMWH molecules. Because a minimum chain length of 18 saccharides (which includes the pentasaccharide sequence) is required for the formation of ternary complexes among heparin chains, AT, and thrombin, only the 25 to 50% of LMWH species that are above this critical chain length are able to inactivate thrombin. In contrast, all LMWH chains containing the high-affinity pentasaccharide catalyze the inactivation of factor Xa. Because virtually all heparin molecules contain at least 18 saccharide units, heparin has an anti-factor Xa/anti-factor IIa ratio of 1:1. In contrast, commercial LMWHs have anti-factor Xa/anti-IIa ratios between 2:1 and 4:1, depending on their molecular size distribution.

LMWHs have been evaluated in a large number of randomized clinical trials and have been shown to be safe and effective for the prevention and treatment of venous thrombosis. More recently, LMWH preparations also have been evaluated in patients with acute pulmonary embolism and in those with unstable angina. The pharmacokinetic differences between UFH and LMWH can be explained largely by the decreased propensity for LMWH to bind proteins, endothelial cells, and macrophages, as discussed above.

2.2 LMWH: pharmacokinetics

In the 1980s, a number of investigators[148–153] reported that LMWH preparations had superior pharmacokinetic properties than UFH preparations. LMWHs demonstrated SC bioavailability approaching 100% at low doses. Peak anti-factor Xa activity occurred 3 to 5 h after SC injection, with a more predictable dose response.[161] Also, the elimination half-life of LMWHs was longer (3 to 6 h after SC injection) and was not dose-dependent, as was the elimination half-life of UFH. These findings provided the rationale for comparing unmonitored weight-adjusted LMWH with aPTT-monitored heparin in patients with established deep-vein thrombosis (DVT) and in patients with unstable angina. One pharmacokinetic limitation is

Table 7—*Biological Consequences of Reduced Binding to Proteins and Cells of LMWH Compared to UFH*

Binding Target	Biologic Effects	Clinical Consequences
Thrombin	Reduced anti-IIa to anti-factor Xa ratio	Unknown
Proteins	More predictable anticoagulant response	Monitoring of anticoagulant effect unnecessary
Macrophages	Cleared through renal mechanism	Longer plasma half-life. Once-daily SC treatment effective
Platelets	Reduced incidence of heparin-dependent antibody	Reduced incidence of heparin-induced thrombocytopenia
Osteoblasts	Reduced activation of osteoclasts	Lower incidence of osteopenia

that LMWHs are cleared principally by the renal route, and their biological half-life is prolonged in patients with renal failure.[162,163]

2.3 LMWH: monitoring the antithrombotic effect

LMWHs are typically administered in fixed doses, for thromboprophylaxis, or in total body weight (TBW)-adjusted doses, for therapeutic effect. Laboratory monitoring is not generally necessary. However, dose-finding trials have not been carried out in special populations, such as patients with renal failure or severe obesity. It has been suggested that monitoring should be considered in such patients.[164–167]

Several laboratory assays have been proposed for this purpose, including the anti-factor Xa assay, and more globally responsive tests, such as the Heptest (Kappes; Augsburg, Germany).[167,168] Anti-factor Xa activity monitoring by a chromogenic assay is the most widely available and is the test currently recommended by the College of American Pathologists.[169]

The relationship between anti-factor Xa levels and clinical outcomes is not clear-cut. Anti-factor Xa levels have been shown to be inversely related to thrombus propagation and the development of thrombosis,[170,171] but the minimal effective level remains uncertain. One study,[175] which utilized continuous IV infusion of dalteparin, showed that an increased risk for bleeding was associated with steady-state anti-factor Xa levels of > 0.8 u/mL. However, several other studies[176–178] in which the LMWH was given at currently accepted doses by SC injection failed to show a relationship between anti-factor Xa level and bleeding. A randomized controlled trial comparing monitored and unmonitored therapy of venous thromboembolism with dalteparin showed no benefit of monitoring.[179] Therefore, the routine measurement of anti-factor Xa levels is not indicated. Rather, it should be limited to particular patient groups (such as in obesity or renal failure) because they are potentially more prone to overdosing when weight-adjusted regimens are used.

After a therapeutic weight-adjusted dose of LMWH is administered by SC injection, the anti-factor Xa activity peaks at approximately 4 h, and this is the recommended time to perform monitoring assays.[167,169,180,181] It should be noted that the measured peak anti-factor Xa activity varies among individual LMWH preparations given in the same anti-factor Xa dose, due to variations in pharmacokinetics.[182] A conservative therapeutic range for peak effect with twice-daily administration of enoxaparin or nadroparin is 0.6 to 1.0 IU/mL[167,169,180,182] for patients being treated for venous thromboembolism. In order to avoid an increased risk of bleeding, levels of > 1.0 IU/mL should be avoided if the appropriateness of the dose is in question in patients with renal impairment or severe obesity. The target range for peak anti-factor Xa effect is less clear in patients treated with once-daily LMWH, but is likely to be > 1.0 IU/mL for enoxaparin.[169] In patients being treated for venous thromboembolism, the target mean anti-factor Xa level, measured approximately 4 h after administration, for a once-daily tinzaparin dose is 0.85 IU/mL, for nadroparin it is 1.3 IU/mL, and for dalteparin it is 1.05 IU/mL.[182]

2.4 Dosing and monitoring in special situations

2.4.1 Obesity

Large contemporary randomized controlled trials of LMWH have generally used weight-adjusted doses without any ceiling for patients with obesity. Since intravascular volume does not have a linear relationship with TBW, it is possible that the use of TBW-based doses in obese patients could lead to overdosing. Conversely, the use of fixed doses for thromboprophylaxis in obese patients could result in underdosing.

Despite these theoretical considerations, anti-factor Xa activity is not significantly increased when LMWH is administered to obese patients in doses based on TBW. This observation has been made for the following drugs: (1) enoxaparin in patients with TBW up to 144 kg (body mass index [BMI], 48 kg/m^2)[183]; (2) dalteparin in patients with TBW up to 190 kg (BMI, 58 kg/m^2)[184,185]; and tinzaparin in patients with TBW up to 165 kg (BMI, 61 kg/m^2).[186]

Furthermore, an increased number of bleeding complications have not been observed when LMWH is administered in doses based on TBW to obese patients. Thus, in a meta-analysis that included data on 921 patients with a BMI of > 30, there was no increase in major bleeding in obese patients who received LMWH doses adjusted by TBW.[187]

Since these studies included few patients with a TBW of > 150 kg and a BMI of > 50 kg/m^2, it would be reasonable to consider anti-factor Xa testing in such patients. Dose reduction should be considered if the anti-factor Xa activity measured 4 h after SC administration is excessive. (See section 2.4 for a discussion of appropriate peak anti-factor Xa levels for various LMWH preparations.) Since the potential source of the dosing error is based on an uncertain volume of distribution in obese patients, repeated testing is not necessary.

Data that address the issue of thromboprophylaxis with fixed-dose LMWHs in obese patients are now available. There is a strong negative correlation between TBW and anti-factor Xa activity ($R^2 = 0.63$) in obese patients receiving fixed-dose thromboprophylaxis therapy with enoxaparin.[188] This relationship has also been observed in obese patients who are critically ill ($r = -0.41$; $p < 0.03$).[189] The correlation between TBW and the anti-factor Xa activity of prophylactic doses of nadroparin is likewise negative, but is not linear, since weight-adjusted LMWH produced more than a proportional increase in its anti-factor Xa activity.[190] BMI also was demonstrated to show a positive correlation with the risk of postoperative venous thromboembolism in patients receiving fixed-dose enoxaparin for thromboprophylaxis after total knee or hip replacement.[191] These data suggest that weight-based prophylactic dosing might be preferable to fixed dosing in obese patients.

Two small prospective trials have examined this issue. A nonrandomized prospective study[192] of enoxaparin, 30 mg

every 12 h, vs enoxaparin, 40 mg every 12 h, in 481 patients undergoing bariatric surgery showed a reduction in the incidence of postoperative DVT (5.4% vs 0.6%, respectively; $p = 0.01$) in the group receiving the 40-mg dose, with no increase in bleeding complications. However, a smaller randomized controlled trial[193] in 60 patients who had undergone bariatric surgery showed no difference in the rate of postoperative DVT between patients assigned to receive either 5,700 or 9,500 IU SC nadroparin. Because of its small size, this study did not exclude clinically important differences in DVT between the two dosage groups.[193] In the absence of clear data, it seems prudent to consider a 25% increase in the thromboprophylactic dose of LMWH in very obese patients (*eg*, enoxaparin, 40 mg bid).

2.4.2 Renal failure

The safety of administering standard doses of LMWH to patients with severe renal insufficiency has not been clearly established. Large contemporary randomized controlled trials of LMWH have generally excluded patients with severe renal insufficiency or have failed to specify whether patients with renal insufficiency were recruited. However, pharmacokinetic and clinical data have become available that allow reasonable conclusions to be made regarding the use of LMWH in these patients.

With few exceptions,[194,195] pharmacokinetic studies have demonstrated that the clearance of the anti-factor Xa effect of LMWH is strongly correlated with creatinine clearance (CrCl). This relationship has been shown in single-dose studies of nadroparin at a CrCl rate of < 50 mL/min[196] and of enoxaparin at a CrCl rate of < 20 mL/min.[197] The accumulation of anti-factor Xa activity after multiple doses is of particular concern, and several multidose pharmacokinetic studies have now been published. A strong linear relationship has been demonstrated between CrCl rate and enoxaparin clearance ($r = 0.85$; $p = 0.001$) in a large study[198] of patients receiving therapeutic doses of enoxaparin for coronary indications. A linear correlation was confirmed between CrCl and anti-factor Xa levels ($p < 0.0005$) after multiple therapeutic doses of enoxaparin, with significantly increased anti-factor Xa levels in patients with a CrCl rate of < 30 mL/min.[199] In patients who received multiple prophylactic doses of enoxaparin, it was shown that the anti-factor Xa clearance was reduced by 39%, and the exposure (*ie*, the area under the curve of anti-factor Xa activity over time) was increased by 35% in those with a CrCl rate of < 30 mL/min relative to those with a CrCl of ≥ 31 mL/min.[200] In full therapeutic doses, nadroparin clearance, but not tinzaparin clearance, is correlated with CrCl rate ($r = 0.49$; $p < 0.002$)[201] down to a CrCl rate as low as 20 mL/min.[194] This suggests that there are differences among LMWH preparations in regard to their dependence on renal clearance. A review[202] of the influence of renal function on anti-factor Xa activity of LMWH came to the following conclusions: (1) most well-designed studies demonstrate increased anti-factor Xa activity in patients with diminished renal function; (2) the pharmacokinetic effect of impaired renal function may differ among LMWHs; and (3) there is not a single CrCl cutoff value that correlates with an increased risk of bleeding for all LMWH preparations.

Renal insufficiency has been reported to increase the risk of bleeding complications for therapeutic doses of LMWHs. In a *post hoc* analysis of data from the Efficacy and Safety of Subcutaneous Enoxaparin in non-Q-wave Coronary Events and the Thrombolysis and Thrombin Inhibition in Myocardial Infarction IIB studies, a CrCl rate of ≤ 30mL/min was associated with an increased risk for major hemorrhage in patients receiving enoxaparin (relative risk [RR], 6.1; 95% confidence interval [CI], 2.47 to 14.88; $p = 0.0019$ [calculated from data provided]).[187] In another study[203] of patients with venous thromboembolism or acute coronary ischemia, therapeutic doses of enoxaparin or tinzaparin yielded a CrCl rate of < 20 mL/min, which was associated with an RR of 2.8 (95% CI, 1.0 to 7.8) for bleeding complications. Finally, in a retrospective study[204] of patients receiving multiple doses of enoxaparin, patients with renal insufficiency had an RR for bleeding complications of 2.3 ($p < 0.01$) and a RR for major hemorrhage of 15.0 ($p < 0.001$).

We recommend using UFH to provide full therapeutic anticoagulation therapy in patients with severe renal insufficiency. If LMWH is chosen, monitoring should be performed with therapeutic anti-factor Xa activity, as outlined in section 2.4. The exact cutoff value in terms of CrCl for these recommendations probably varies for different LMWHs, but a safe threshold is likely to be 30 mL/min.

Thromboprophylactic LMWH in patients with renal insufficiency requires separate consideration. Although increased anti-factor Xa activity was observed in patients with renal failure who received multiple thromboprophylactic doses of enoxaparin (*ie*, 40 mg daily), the mean peak anti-factor Xa level was only 0.6 IU/mL and the trough was < 0.2 IU/mL.[200] These levels have not been clearly associated with an increased risk of bleeding. An increased risk of bleeding complications has not been reported in patients receiving thromboprophylactic doses of LMWHs. If enoxaparin is chosen for thromboprophylaxis in a patient with renal failure, the dose of 40 mg daily seems preferable to the 30 mg bid dose.

2.5 Reversing the antithrombotic effects of LMWH

There is no proven method for neutralizing LMWH. Studies in animals and *in vitro* studies have demonstrated that protamine neutralizes the antithrombin activity of LMWH, normalizing the aPTT and thrombin time. However, protamine appears to only neutralize approximately 60% of the anti-factor Xa activity of LMWH.[205–208] The interaction of protamine and heparin is influenced by the molecular weight of heparin,[209] and it is likely that a lack of complete neutralization of anti-factor Xa occurs because of reduced protamine binding to the lower molecular weight heparin moieties in the preparation.[118]

The clinical significance of the incomplete anti-factor Xa neutralization of LMWH by protamine is unclear. In a small case series, protamine failed to correct clinical

bleeding associated with LMWH in two thirds of patients,[207] but there are no human studies that have convincingly demonstrated or refuted a beneficial effect of protamine on bleeding related to the use of LMWH. One animal study[210] reported a reduction in bleeding with protamine in a microvascular bleeding model, despite persistent anti-factor Xa activity. Another study[211] demonstrated incomplete attenuation of bleeding.

Recently, a case report[212] has been published in which activated factor VII therapy appeared to be effective in a patient with postoperative bleeding. In animal studies, synthetic protamine variants have been shown to be highly effective in neutralizing the anticoagulant effects of LMWH (including anti-factor Xa activity), and they appear to be less toxic than protamine.[213–216] Adenosine triphosphate completely reversed clinical bleeding related to LMWH in a rat model.[217] These agents are not yet available for clinical use.

The following approach is recommended in clinical situations in which the antithrombotic effect of LMWH needs to be neutralized. If LMWH was administered within 8 h, protamine may be given in a dose of 1 mg per 100 anti-factor Xa units LMWH (1 mg enoxaparin equals approximately 100 anti-factor Xa units). A second dose of 0.5 mg protamine per 100 anti-factor Xa units may be administered if the bleeding continues. Smaller doses are needed if the LMWH was injected > 8 h before the event requiring neutralization.

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