College of American Pathologists Conference XXXI on Laboratory Monitoring of Anticoagulant Therapy

The Clinical Use and Laboratory Monitoring of Low-Molecular-Weight Heparin, Danaparoid, Hirudin and Related Compounds, and Argatroban

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● *Objective.*—To review the role of the laboratory in monitoring therapy with low-molecular-weight heparin, danaparoid, hirudin, and argatroban, as reflected in the medical literature and the consensus opinion of recognized experts in the field.

Data Sources.—Review of the medical literature and current clinical practice by a panel of 6 international experts in the field of anticoagulant therapy.

Data Extraction and Synthesis.—The experts made an extensive review of the published literature and prepared a draft manuscript, which included preliminary recommendations. The draft manuscript was circulated to participants in the College of American Pathologists Conference XXXI on Laboratory Monitoring of Anticoagulant Therapy prior to the conference. The manuscript and recommendations were then presented at the Conference for discussion. Recommendations were accepted if a consensus of the 26 experts attending the Conference was reached. The results of the discussion were used to revise the manuscript into its final form.

Conclusions.—This report reviews the mechanism of action and potential uses of these newer anticoagulant agents. General guidelines for monitoring these agents and 9 specific recommendations for laboratory monitoring of low-molecular-weight heparin and danaparoid are provided, along with citation of the appropriate supporting literature. Issues for which a consensus was not reached at the Conference are also discussed.

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This review describes the clinical use and laboratory monitoring of several anticoagulants, with special emphasis on low-molecular-weight (LMW) heparin. Consensus recommendations agreed upon at this conference regarding the use and monitoring of LMW heparin, danaparoid, hirudin and related compounds, and argatroban are also included. The information in this report is intended to improve the laboratory monitoring of patients treated with these anticoagulants (Tables 1 and 2).

LOW-MOLECULAR-WEIGHT HEPARIN

Pharmacology of LMW Heparins

Heparins are glycosaminoglycans consisting of chains of alternating residues of D-glucosamine and uronic acid.[1] Low-molecular-weight heparins are prepared from un-fractionated heparin by the chemical or enzymatic depolymerization of the parent molecules. The pharmacokinetics of LMW heparins differ from unfractionated heparin in a number of important aspects. When injected subcutaneously, the bioavailability of unfractionated heparin ranges from 10% to 90%, depending on the dose given. In contrast, the bioavailability of LMW heparin is greater than 90% and is independent of dose.[2] This difference is due to the propensity of unfractionated heparin to bind to plasma proteins, endothelial cells, platelets, and macrophages.[3,4] Low-molecular-weight heparins exhibit much less binding to plasma proteins[5] and do not accumulate in the liver or spleen,[6] which probably accounts for their longer plasma half-life (3–4 hours) as compared to un-fractionated heparin (30–150 minutes, depending on the dose).[7] Because of these pharmacokinetic properties, the dose-response curve of LMW heparins tends to be linear.[8] This implies that the anticoagulant effects of a given dose should be highly predictable, lessening the need for monitoring.

Heparins exert their anticoagulant activity predominantly by greatly increasing the activity of plasma antithrombin (formerly designated antithrombin III).[9] While unfractionated heparin is able to form a trimolecular complex with antithrombin and thrombin, and thereby exert a powerful inhibition of thrombin, only a portion of the chains of the LMW heparins are sufficiently long (>18 saccharide units) to form this complex.[10] In comparison,

Table 1. Levels of Evidence for Consensus Recommendations

Level 1	The recommendation is based on well-designed prospective studies, preferably more than 1.
Level 2	The recommendation is based on retrospective studies or multiple anecdotal studies that reach consensus.
Level 3	The recommendation is based on isolated anecdotal studies or the consensus of expert practitioners.

Table 2. Consensus Recommendations

1. Clinically stable patients receiving low-molecular-weight (LMW) heparin preoperatively or postoperatively for prophylaxis of venous thromboembolism do not require laboratory monitoring. (Level 1)
2. Uncomplicated patients being treated for venous thromboembolism by a weight-adjusted, fixed-dose regimen of LMW heparin do not require laboratory monitoring. (Level 1)
3. Laboratory monitoring using an anti–factor Xa assay may be of value in certain clinical settings (see text). (Level 3)
4. Pediatric patients receiving LMW heparin should be monitored. (Level 2)
5. When LMW heparin is monitored, the sample should be obtained 4 hours after subcutaneous injection. (Level 3)
6. The target concentration for the peak LMW heparin level in patients treated with twice daily dosing for venous thromboembolism should be 0.5–1.1 IU/mL when measured by an anti–factor Xa method. (Level 3)
7. The chromogenic anti–factor Xa method is recommended for monitoring LMW heparin. (Level 2)
8. A calibrated LMW heparin should be used to establish the standard curve for the assay to measure LMW heparin. Unfractionated heparin cannot be used to establish the standard curve for monitoring LMW heparin. (Level 2)
9. Danaparoid should be used to establish the standard curve for the assay to measure danaparoid with an anti–factor Xa method. (Level 2)

enhancement of antithrombin inhibition of factor Xa is not affected by the molecular weight of the heparin, as long as the specific pentasaccharide sequence that binds antithrombin is present on the heparin chains. Inhibition of factor Xa is therefore used to assess the anticoagulant activity of LMW heparins. However, it should be remembered that these drugs also have other antithrombotic actions that are not routinely measured, such as the in vivo release of tissue factor pathway inhibitor from endothelium.[11]

Clinical Situations for Which LMW Heparin Should be Considered

Low-molecular-weight heparins and heparinoids are currently licensed by the Food and Drug Administration (FDA) in the United States for the prevention of thrombosis in patients undergoing hip and knee replacement, and for patients undergoing abdominal and pelvic surgery. In Canada, LMW heparins have been approved for the treatment of deep vein thrombosis. For all these indications, LMW heparins have been shown to have equal, if not superior, efficacy to unfractionated heparin; LMW heparins also show no increase in bleeding complications.[12,13] A number of other indications for these agents are presently under study. These indications include the prevention of thromboembolism in patients with various types of trauma,[14] as well as hip fracture[15] and spinal cord injury.[16] In addition, recent trials have addressed the use of LMW heparin in unstable angina.[17,18] In one study with patients suffering from unstable coronary artery disease (the Essence Trial), enoxaparin was shown to be superior to unfractionated heparin.[18] A protocol for enoxaparin use in unstable angina has recently been approved by the FDA (Table 3). Low-molecular-weight heparins have also been investigated for the treatment of pulmonary embolism, outpatient treatment of deep vein thrombosis, and acute ischemic stroke.[19–23] Low-molecular-weight heparin has also been evaluated as an anticoagulant during pregnancy.[24] While most of the above-mentioned studies have been of short duration, some studies have found that longer term use of LMW heparin was efficacious and had a low frequency of bleeding. These investigations have evaluated LMW heparin for up to 1 month after joint replacement,[25] 3 months after deep vein thrombosis,[26] or for years in patients with cancer-associated thrombosis.[27]

The FDA-approved prophylactic doses administered by subcutaneous administration are as follows: 30 mg of enoxaparin twice a day for knee or hip replacement surgery, 40 mg of enoxaparin once a day for abdominal surgery, 2500 to 5000 IU of dalteparin once a day for abdominal surgery, and 50 U/kg of ardeparin twice a day for knee replacement surgery (Table 3). Full anticoagulant doses for treatment of venous thromboembolism are not yet approved by the FDA. However, clinical trials have commonly used subcutaneous administration of 1 mg/kg enoxaparin twice a day or dalteparin at a dose of 200 IU/kg administered once a day or 100 IU/kg twice a day. Protamine sulfate (1% solution) cannot reverse all of the anti-Xa activity, but it is recommended when emergent reversal of anticoagulation is required. The protamine dose is 1 mg per mg enoxaparin, 1 mg per 100 units ardeparin, or 1 mg per 100 units dalteparin by slow intravenous injection. If the activated partial thromboplastin time (aPTT) is prolonged 2 to 4 hours later, a second dose may be infused (0.5 mg per mg enoxaparin, 0.5 mg per 100 IU dalteparin, unspecified for ardeparin).

Adverse Effects of Unfractionated and LMW Heparin

Heparins have 3 major adverse effects: bleeding, heparin-induced thrombocytopenia (HIT), and osteoporosis. As already mentioned, most of the clinical trials have reported similar or decreased bleeding with LMW heparin as compared to unfractionated heparin.[28,29] This was most dramatically shown in the treatment trial of Hull et al,[30] in which 11 of 219 patients treated with unfractionated heparin experienced major bleeding, as compared with only 1 of 213 receiving the LMW heparin preparation tinzaparin ($P = .006$). The low bleeding rate with tinzaparin may have been partly due to the fact that the drug was given only once daily. In subsequent trials using enoxaparin or dalteparin, twice daily injections were given. There are a number of theoretical reasons for less bleeding with LMW heparins. These include less inhibition of thrombin, less binding to platelets and endothelial cells, and no inhibition of von Willebrand factor.[31] Less binding to platelets and less release of platelet factor 4 by LMW heparins are considered to be the reasons for the lower incidence of HIT.[32,33] Although the incidence of HIT is lower with LMW heparin than with unfractionated heparin, it is advisable to follow the platelet count in patients receiving LMW heparin also. It is not currently known how often the platelet count should be determined in such patients. In patients who develop HIT from unfractionated

Table 3. Food and Drug Administration–Approved Indications, Dosages, and Laboratory Monitoring for Low-Molecular-Weight Heparins, Danaparoid, and Lepirudin

Drug Name	Indication	Dose	Laboratory Monitoring
Enoxaparin (Lovenox)	DVT prophylaxis for knee or hip replacement surgery	30 mg twice daily subcutaneously	Anti-Xa levels are not routinely necessary, particularly at prophylactic doses; anti-Xa levels may be used if significant renal impairment (see text for additional precautions)
	DVT prophylaxis for abdominal surgery	40 mg once daily subcutaneously	
	Unstable angina and non–Q-wave myocardial infarction	1 mg/kg twice daily subcutaneously, with asprin	Periodic monitoring of the complete blood count (including platelet count) and occult fecal blood is recommended
Dalteparin (Fragmin)	DVT prophylaxis for abdominal surgery	2500–5000 IU once daily subcutaneously	
Ardeparin (Normiflo)	DVT prophylaxis for knee replacement surgery	50 U/kg twice daily subcutaneously	
Danaparoid (Orgaran)	DVT prophylaxis for hip replacement surgery	750 U twice daily subcutaneously	
Lepirudin (Refludan) (a brand of hirudin)	Anticoagulation in patients with heparin-induced thrombocytopenia and associated thromboembolism	0.4 mg/kg (up to 110 kg) bolus, then 0.15 mg/kg (up to 110 kg) continuous intravenous infusion (lower dose for renal impairment; avoid if creatinine >6 mg/dL or hemodialysis)	Check aPTT 4 hours after start of infusion, then at least once daily. Target aPTT is 1.5–2.5 times mean of normal range; adjust dose if outside the target range

* DVT indicates deep venous thrombosis; aPTT, partial thromboplastin time.

heparin, the HIT antibody frequently cross-reacts with LMW heparin. Therefore, LMW heparin should not be used in patients with HIT. Less bone resorption by LMW heparins may account for the decreased frequency of osteoporosis relative to unfractionated heparin.[34–36] These properties would favor widespread use of LMW heparin. However, a dose of LMW heparin in the United States is significantly more expensive than a dose of unfractionated heparin. Despite the higher cost per dose, LMW heparin may actually be more cost-effective than unfractionated heparin.[37,38]

Laboratory Monitoring of LMW Heparin

Low-molecular-weight heparin has excellent bioavailability when administered subcutaneously, leading to a predictable response in most patients. Therefore, clinically stable, uncomplicated patients receiving low doses of LMW heparin preoperatively or postoperatively for prophylaxis of venous thromboembolism or higher doses for treatment of venous thromboembolism do not require laboratory monitoring. However, in some clinical settings, measurement of LMW heparin concentration in plasma using an anti-Xa activity assay may increase the safety or efficacy of the anticoagulant.[39]

Patients with renal insufficiency have delayed clearance of LMW heparin; consequently, they may benefit from monitoring.[39–41] In addition, patients receiving therapeutic levels (eg, 1 mg/kg enoxaparin q 12 hours or 200 U/kg dalteparin daily) of LMW heparin for prolonged periods of time may benefit from monitoring to prevent excessive or insufficient anticoagulation. Patients in this category would include long-term outpatients with malignancy (Trousseau's syndrome); patients with thrombosis refractory to warfarin, as found in myeloproliferative disorders or the antiphospholipid antibody syndrome; and patients who cannot take warfarin. Examples of the latter would

include pregnant patients and those with allergic reactions to coumarins. Treatment or prophylaxis of thrombosis in pregnancy may require intermittent monitoring because of changing requirements as the pregnancy proceeds, such as the need for an increased dose in the third trimester of pregnancy.[24] Patients who are treated for shorter periods but who have a high risk of bleeding, such as postoperative patients and those with a high likelihood of thrombotic recurrence, may also benefit from monitoring to avoid periods of over- or under-anticoagulation. The markedly obese patient and the patient with low body weight may also require intermittent monitoring because of possible differences in the pharmacokinetics of LMW heparin in such patients compared with patients closer to ideal body weight. Finally, as newborns may require a different dosage scheme than adults, monitoring may be necessary to assure adequate therapy. In a study with 25 patients, it was found that newborns required 1.6 mg/kg enoxaparin twice a day to bring them into the target range of 0.5 to 1.0 IU/mL, but that adult doses (1 mg/kg twice a day) sufficed for older children.[42]

Limited data are available on anti-Xa activity levels relative to time of injection of therapeutic doses of LMW heparin.[43,44] Maximal plasma concentrations typically occur 1 to 5 hours following administration of a dose, with the maximum peak varying slightly between LMW heparins. Anti-Xa levels at 4 hours after a dose (peak levels) have been measured in several studies, and the peak plasma concentrations varied rather widely. Plasma concentrations immediately prior to the administration of a daily LMW heparin dose averaged 0.1 IU/mL. In general, measurement of a heparin level near its peak (4 hours) seems to have a stronger correlation with safety and efficacy than trough levels obtained just prior to administration of a dose. Therefore, if LMW heparin therapy is monitored, the

sample should be drawn approximately 4 hours after subcutaneous administration.

The chromogenic anti-Xa method is the assay of choice for determining the plasma concentration of LMW heparin. Although it may be mildly prolonged during therapy, the aPTT is not helpful in monitoring LMW heparin. Anti-Xa clotting methods, such as *Heptest* (American Diagnostica, Greenwich, Conn), have been used, but they may give different results from the chromogenic method and they may be influenced by the anti-IIa activity of LMW heparins.[40,45]

Studies have shown that the LMW heparin preparation given to the patient is the best material for preparation of the standard curve.[46] There is no need to use the same lot that the patient is receiving; any lot supplied by the manufacturer can be used, provided that its anti-Xa activity in IU/mL has been calibrated directly or indirectly against the World Health Organization (WHO) standard for LMW heparin. Therefore, if different LMW heparins are used in the same hospital, the use of a single LMW heparin standard for each preparation is acceptable. It should be noted that danaparoid, which is not a LMW heparin, must have its own standard for use in the anti-Xa assay.

As the commercial availability of LMW heparin standards for controls and standard curve construction is limited, plasma samples supplemented with LMW heparin may be used for these purposes at the present time. Plasma samples supplemented with LMW heparin may give lower results in the anti-Xa assay than samples collected from patients given LMW heparin; however, the differences are not as great in the anti-Xa assay as they are for the aPTT. Commercial controls will probably become more available as more laboratories perform anti-Xa assays.

Manufacturers are expected to calibrate an in-house standard of their own material against the WHO standard, then use this in-house material to assign potency to individual lots of LMW heparin. It is recommended that manufacturers include information on LMW heparin units referenced to the WHO standard in the package insert. Some European manufacturers may use the European Pharmacopoeia standard, which has been calibrated against the WHO standard. The WHO standard cannot be supplied in adequate amounts for routine use as a standard in clinical laboratories, but it can be used as an occasional confirmation of calibrator or control samples. The WHO LMW heparin standard has been useful as a reference material for the current range of LMW heparin products. Its anti-Xa/anti-IIa ratio of 2.5 is in the middle of the range of 1.5 to 4.0 for the various products.[47] The need for additional standards will be considered as other LMW heparins with very different anti-Xa to anti-IIa ratios are introduced.

Since all manufacturers calibrate, directly or indirectly, against the WHO standard, there is no need for a conversion factor to other units. However, there may be some complications with enoxaparin, for which dosage recommendations are in mg, and with nadroparin, for which dosage recommendations have previously been expressed in Institut Choay Units (ICU), which differ approximately threefold from International Units of anti-Xa activity. However, provided that they have been calibrated for anti-Xa activity against the WHO standard for LMW heparin, these products can be used for constructing standard curves in anti-Xa assays.

The therapeutic range has not been rigorously defined for LMW heparin, as many of the studies performed to date have not reported anti-Xa levels. It is suggested that performance of anti-Xa levels be included in future clinical trials, even if the levels are not used for monitoring, to further define a therapeutic range to benefit future patients who may need monitoring (eg, renal failure, newborn, pediatric, and pregnant patients). For twice-a-day dosing in the treatment of venous thromboembolism, an acceptable target range for a sample collected 4 hours after subcutaneous injection (peak level) is 0.4 to 1.1 IU/mL; a more conservative range would be 0.6 to 1.0 IU/mL. In one study using twice-a-day administration, an increased risk of bleeding was suggested at anti-Xa activities above the 0.8 to 1.0 IU/mL range.[48] The therapeutic range is even less clear for once-a-day dosing, but the target range will likely be higher, possibly 1.0 to 2.0 IU/mL in samples collected 4 hours after subcutaneous injection (peak level).[49–54] At this point, there are insufficient data to determine if there should be individual target ranges for each individual LMW heparin. However, there does appear to be sufficient similarity between the commercially available LMW heparins to aim for one overall target range.

Specimens for LMW heparin assays should be collected according to the same protocol used for other coagulation assays.[55,56] In view of the recent recommendations by the National Committee for Clinical Laboratory Standards, specimens should be collected in 0.109 mol/L (3.2%) sodium citrate tubes with a whole blood-anticoagulant ratio of 9:1. The sample must be centrifuged to separate the cells from the plasma within 1 hour of sample collection. The assay should ideally be performed within 2 to 4 hours after collection. However, the citrated platelet-poor plasma may be frozen at $-20°C$ and assayed at a later time; the platelet-poor plasma should have a platelet count of less than $10 \times 10^9/L$.

DANAPAROID

Actions, Clinical Indications, and Monitoring Requirements

Danaparoid (also called Org 10172, Orgaran, Lomoparan) is a heparinoid composed of a mixture of LMW glycosaminoglycans.[57] The mixture contains 84% heparan sulfate, 12% dermatan sulfate, and 4% chondroitin sulfate, and exhibits predominantly anti–factor Xa activity. The anti-Xa to anti-IIa ratio is approximately 28:1 and results in an anti-IIa activity below the limit of detection in most assays.[58] Drug monitoring is achieved with anti-Xa assays even though the anti-Xa activity of danaparoid on a milligram basis is approximately 10-fold less than that of LMW heparin. The half-life of the anti-Xa activity is 19 to 24 hours; the half-life of the anti-IIa activity is 2.0 to 3.5 hours.[59,60] The half-life of total antithrombotic activity is between the half-lives of anti-Xa and anti-IIa activities, at approximately 8 hours. Twice daily dosing is required when using the subcutaneous route.[59,60] Bioavailability of danaparoid for subcutaneous dosing is essentially 100%. The kidney plays a major role in the elimination of danaparoid, and its half-life is prolonged in renal failure.[59,61]

The mechanism of danaparoid action is only partially understood. Four percent of the heparan sulfate contains the pentasaccharide sequence found in heparin that binds antithrombin and enhances its anti-factor Xa activity. The remainder of the heparan sulfate also appears to contribute to the anticoagulant effect in some way. Dermatan sul-

fate enhances the activity of heparin cofactor II, which inhibits factor IIa.[58]

Danaparoid has been used in patients with HIT.[57,62] In most cases of HIT, the causative antibodies do not cross-react with danaparoid; in these cases, danaparoid appears to be a safe anticoagulant. However, approximately 10% of patients demonstrate in vitro cross-reactivity, and these patients may be at risk for HIT-associated thrombosis if danaparoid is used.[63] Therefore, a negative in vitro platelet aggregation or serotonin release test result in response to danaparoid prior to using danaparoid in HIT patients is ideal. Danaparoid should be discontinued if the thrombocytopenia persists or worsens. There are a growing number of reports of successful danaparoid use in patients for whom in vitro cross-reactivity was discovered retrospectively, suggesting that in vitro cross-reactivity commonly lacks clinical significance. At the present time, the commercially available enzyme-linked immunosorbent assays for heparin-induced thrombocytopenia cannot be used to determine danaparoid cross-reactivity.

For rapid full anticoagulation, the American College of Chest Physicians consensus panel recommends an intravenous bolus of 2500 U (1250 U if <55 kg and 3750 U if >90 kg), followed by 400 U/hr for 4 hours, then 300 U/hr for 4 hours, then 150 to 200 U/hr to maintain anti-Xa levels at 0.5 to 0.8 U/mL.[64] Alternatively, the intravenous bolus can be followed by subcutaneous administration of 1250 U subcutaneously, 2 or 3 times a day. The anti-Xa level is determined 6 hours after subcutaneous injection in an anti-Xa assay in which danaparoid, and not LMW or unfractionated heparin, is used to construct the standard curve.[57,64] Although anti-Xa monitoring is advised, it has been suggested that the dosing listed can be used without monitoring if anti-Xa monitoring is not available and renal function is normal, in as much as bleeding complications are uncommon.[64] One study has shown promising results, compared with a heparin treatment arm, for the treatment of venous thromboembolism with an intravenous bolus of 1250 or 2000 U of danaparoid, followed by subcutaneous administration of 1250 or 2000 U twice a day, with no danaparoid monitoring.[58]

Danaparoid has also been used successfully for deep venous thrombosis prophylaxis, generally with subcutaneous administration of 750 U twice a day. For patients who weigh more than 90 kg, a dose of 1250 U twice a day or 750 U three times a day may be used. Although anti-Xa monitoring of prophylactic dosing is generally not required, prophylactic dosing yields an anti-Xa level of 0.15 to 0.35 U/mL.[64] Danaparoid has been used for hemodialysis,[57,65–68] acute ischemic stroke,[69–71] and disseminated intravascular coagulation.[72] Additionally, more than 50 patients have undergone cardiopulmonary bypass with danaparoid anticoagulation.[68,73–77] A dosing protocol for the use of danaparoid during cardiopulmonary bypass in patients with HIT has been proposed by Magnani et al.[76] The protocol is based on the results from 53 patients who have undergone cardiopulmonary bypass with danaparoid. The initial protocol had to be modified to decrease postoperative bleeding. The current protocol suggests a 125 U/kg intravenous bolus after thoracotomy, with 3 U/mL in the priming fluid. An intravenous infusion of 7 U/kg/hr is started at the time of bypass hook-up. The infusion should be stopped 45 to 60 minutes before the anticipated completion of bypass. At the present time, the protocol does not require anti-Xa monitoring. There is an increased risk of bleeding when using danaparoid for cardiopulmonary bypass, owing to the long half-life of danaparoid and the inability to reverse danaparoid with protamine.

There is no reversal agent for danaparoid. Protamine provides little if any clinical improvement in bleeding complications. There is one case report of successful emergent reversal of danaparoid after bypass using plasmapheresis.[78] Unlike unfractionated heparin, ''heparin-resistance'' is not a significant problem because danaparoid is not readily neutralized by plasma proteins.[76] Danaparoid produces small linear effects on the prothrombin time, aPTT, thrombin time, and activated clotting time.

Danaparoid is supplied by Organon Inc (West Orange, NJ) under the trade name Orgaran. It has recently been approved by the FDA for prophylactic use in patients undergoing elective hip replacement (Table 3). Therefore, it is readily available in the United States at the present time.

HIRUDIN

Actions, Clinical Indications, and Monitoring Requirements for Hirudin and Its Related Compounds

Hirudin and its related compounds are direct thrombin inhibitors. Hirudin (HBW 023, CGP 39393, Revasc, desirudin, Lepirudin) is found in the medicinal leech *Hirudo medicinalis.* Recombinant hirudin differs from natural hirudin by the lack of a sulfate group on tyrosine 63, and is therefore also called desulfatohirudin.[79] Hirudin binds tightly to thrombin and blocks both its active (catalytic) site and its substrate-binding site.

Hirugen is a peptide fragment of hirudin, containing amino acids 53–64 of hirudin.[31] It is a weak thrombin inhibitor, inasmuch as it binds to the substrate recognition site of thrombin but not the active catalytic site. It inhibits the binding of high-molecular-weight substrates, such as fibrinogen and platelets, to thrombin, but it does not reduce the binding of low-molecular-weight substrates to thrombin.

Hirulog (bivalirudin) is synthesized by combining DPhe-Pro-Arg-Pro-(Gly4) to the amino terminal region of hirugen. The tetrapeptide DPhe-Pro-Arg-Pro is specific for the inhibition of the catalytic site of thrombin. Consequently, Hirulog binds to both the active catalytic site and the substrate binding site of thrombin and is a potent thrombin inhibitor. Its effect is transient because thrombin slowly cleaves the Pro-Arg bond within the tetrapeptide component of Hirulog, transforming Hirulog into a hirugen-like compound.[80]

Hirudin and Hirulog offer several advantages over heparin. First, the mechanism of action is independent of plasma antithrombin. They are not affected by platelet factor 4, histidine-rich glycoprotein, or other substances that can cause heparin resistance. Consequently, they have a more predictable dose response than unfractionated heparin.[31] Additionally, in contrast to unfractionated heparin, hirudin and Hirulog are capable of efficiently inhibiting clot-bound thrombin. Lastly, they do not cause HIT nor do they cross-react with the antibodies that cause HIT. Therefore, they have been reported to be useful for patients with HIT who require continued anticoagulation. A disadvantage of hirudin and related compounds is that there is no reversal agent. However, owing to the short half-life of hirudin and Hirulog, a reversal agent may not be necessary. A concern over the possibility of the formation of anti-hirudin antibodies has been raised; although, by some reports, this

has not been demonstrated to be an issue thus far.[79,81] By other reports, the formation of antihirudin antibodies can be common and may increase the anticoagulant effect of hirudin.

The half-life of intravenously administered hirudin is nearly 50 minutes.[82] The half-life of subcutaneously administered hirudin is longer, approximately 2 to 3 hours, owing to slow absorption.[79,83] The bioavailability of subcutaneous hirudin is approximately 90%.[83] The kidney is required for excretion of hirudin.[79] A recent study found that hirudin was not effectively removed by hemodialysis, and the mean half-life of hirudin in patients with end-stage renal failure was 52 hours.[84]

One hirudin dosing regimen has recently been approved by the FDA (Table 3). The hirudin dosages used in recent studies, which showed equivalent or improved results when compared with heparin, include the following:

Deep Venous Thrombosis Prophylaxis.—(1) Ten to 20 mg administered subcutaneously twice a day; the aPTT 1.5 to 3 hours after hirudin injection was prolonged 1.3 to 1.4-fold.[85] (2) Fifteen milligrams hirudin twice a day was found to be superior to 40 mg of enoxaparin.[86]

Treatment of Venous Thromboembolism.—(1) Intravenous bolus of 0.07 mg/kg over 3 minutes followed by intravenous administration of 0.05 mg/kg/hr for 5 days with no monitoring adjustments and no comparison to heparin; the aPTT remained 1.2 to 2.8 times control.[87] (2) Subcutaneous administration of 0.75 to 2.00 mg/kg twice a day with no monitoring adjustments; the aPTT 3 hours after hirudin injection remained at 2.2 to 2.7-fold above baseline. Immediately prior to subsequent injections, the aPTT was 1.5 to 2.0-fold over baseline.[88]

Coronary Angioplasty.—Intravenous bolus of 40 mg followed by intravenous administration of 0.2 mg/kg/hr for 24 hours, then subcutaneous administration of 40 mg twice a day for 3 days without monitoring or dosage adjustments.[89]

Unstable Angina or Acute Myocardial Infarction.—Intravenous bolus of 0.1 mg/kg followed by intravenous administration of 0.1 mg/kg/hr for 3 to 5 days with adjustments to maintain the aPTT between 60 and 85 seconds. Virtually all patients received aspirin, and thrombolytic therapy was added at the discretion of the attending physician.[90]

Acute Myocardial Infarction.—Intravenous bolus of 0.1 mg/kg, not to exceed a total dose of 15 mg, followed by intravenous administration of 0.1 mg/kg/hr, not to exceed 15 mg/hr, with adjustments to maintain the aPTT between 55 and 85 seconds. Patients also received aspirin and thrombolytic therapy.[91]

Acute Myocardial Ischemia Without ST-Segment Elevation.—Intravenous bolus of 0.2 to 0.4 mg/kg, then intravenous administration of 0.10 to 0.15 mg/kg/hr for 72 hours with adjustments to maintain the aPTT between 60 and 100 seconds.[92]

Three earlier trials using higher doses of hirudin (a 0.4–0.6 mg/kg bolus followed by 0.15–0.2 mg/kg/hr) versus heparin for myocardial ischemia or infarction, in most cases in conjunction with thrombolytic therapy and aspirin, were stopped early owing to increased rates of bleeding in the hirudin groups.[93–95] These results indicate that hirudin may have a narrow window of safety, especially when used in conjunction with thrombolytic therapy and

aspirin for acute coronary syndromes. Hirudin has also been used for anticoagulation in patients with HIT, including HIT patients who have successfully undergone cardiopulmonary bypass using hirudin.[96–99]

It appears that target levels of anticoagulation are more predictably achieved with hirudin than with heparin.[91,99] Nevertheless, some studies have indicated that monitoring and appropriate dosage adjustments of hirudin may be indicated.[87,90] The test used most often for laboratory monitoring is the aPTT. However, an in vitro study found that the aPTT varies depending on the aPTT reagent.[100] Other tests that have been suggested for monitoring hirudin therapy include the thrombin time, ecarin clotting time, chromogenic anti-factor IIa assays, or immunoassays for hirudin.[101] The thrombin time is generally too sensitive and the prothrombin time, although it is prolonged with hirudin therapy, is generally too insensitive.[79] The method for monitoring hirudin based on the ecarin clotting time test has been gaining greater acceptance. This assay involves the use of a prothrombin activator from the snake venom from *Echis carinatus*.[98,102]

Suppliers of hirudin include Ciba-Geigy (Summit, NJ), Knoll Pharmaceuticals (Mt Olive, NJ), Transgene (Strasbourg, France), and Hoechst-Marion Roussel (Kansas City, Mo). The Knoll preparation of hirudin contains polyethylene glycol, which gives the product a longer half-life and thereby allows for less frequent dosing. One brand of hirudin has recently been approved for clinical use by the FDA, and it should become readily available in the United States in the near future (Table 3).

Hirulog has a half-life of approximately 36 minutes.[103] In a recent study, the mean half-life in 8 patients with severe renal failure was 3.5 hours between dialysis procedures and 1.3 hours during dialysis (B. Adelman, MD, written communication, October 1997). The optimal dose and laboratory monitoring protocols for Hirulog are not yet established. Dosages used in recent studies, which showed equivalent or improved results when compared with heparin, include the following:

Deep Venous Thrombosis Prophylaxis.—Subcutaneous administration of 1 mg/kg three times a day with no comparison to heparin; the aPTT 2 hours after injection was approximately 1.5 to 1.7 times control.[104]

Coronary Angioplasty.—Intravenous bolus of 1 mg/kg followed by intravenous administration of 2.5 mg/kg/hr for 4 hours, then 0.2 mg/kg/hr for 14 to 20 hours without monitoring adjustments, given with aspirin.[105]

Unstable Angina.—Intravenous administration of up to 1.0 mg/kg/hr for 72 hours with no comparison to heparin, given with aspirin; the aPTT remained up to 3.0 times the baseline value.[106]

Myocardial Infarction.—Intravenous bolus of 0.125 or 0.25 mg/kg, then intravenous administration of 0.25 or 0.5 mg/kg/hr for 12 hours, then 0.125 or 0.25 mg/kg/hr, given with streptokinase and aspirin.[107]

Thrombosis From HIT.—There are case reports of the successful use of Hirulog in such patients.[108]

Biogen (Cambridge, Mass) recently sold all rights of Hirulog to Medicines Co (Cambridge, Mass). Hirulog is not yet approved for use by the FDA.

ARGATROBAN

Actions, Clinical Indications, and Monitoring Requirements

Argatroban (also called MD-805, argipidine, MCI-9038, and Novastan) is a reversible synthetic direct thrombin

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inhibitor derived from arginine. Argatroban is competitive inhibitor of thrombin that binds to the active site of thrombin. It offers the same advantages over heparin as described for hirudin.[31,109] As with hirudin and Hirulog, there is no reversal agent for argatroban, but a reversal agent may not be necessary since argatroban has a short half-life of approximately 24 minutes.[110] Argatroban is cleared by hepatic metabolism and renal excretion.[109] Owing to its small size, it is not very immunogenic and is therefore unlikely to induce antibody formation.[111]

Optimal dosage and laboratory monitoring protocols for argatroban have not yet been fully established. Argatroban has been studied in small but promising trials for the following conditions:

Heparin-Induced Thrombocytopenia.—Intravenous administration of 2 to 10 μg/kg/min to maintain the aPTT at 1.5 to 3.0 times control; the aPTT is monitored daily as well as 2 hours after the start of the infusion and 2 hours after each dosage adjustment.[109]

Unstable Angina.—Intravenous administration of 0.5 to 5 μg/kg/min for 4 hours was effective, although angina returned in 21% of patients after discontinuation of argatroban.[112]

Percutaneous Transluminal Coronary Angioplasty.—During angioplasty, intravenous bolus of 0.35 mg/kg, then intravenous administration of 15 to 40 μg/kg/min to keep the activated clotting time between 300 and 450 seconds.[109,113] After angioplasty, intravenous administration at a rate of 0.6 to 1.0 mg/kg/hr for 4 days to maintain the aPTT at 1.5 to 2.0 times baseline.[114]

Hemodialysis.—Intravenous bolus of 10 mg plus 20 mg/hr continuous infusion was shown to elevate the aPTT 2.4-fold and yield a plasma argatroban level of 2.2 μg/mL.[115]

Acute Myocardial Infarction.—Intravenous bolus of 100 μg/kg, then intravenous administration at a rate of 3 μg/kg/min, given with alteplase.[116]

The results of 2 larger promising trials, the ARG911 trial in HIT patients and the Myocardial Infarction Novastan Trial (MINT) in myocardial infarction patients, will be published soon. Argatroban has been reported to be useful in a small number of patients with acute ischemic stroke[117] and disseminated intravascular coagulation.[115] The prothrombin time, aPTT, and activated clotting time are prolonged by argatroban. The aPTT is commonly used for therapeutic monitoring of argatroban, but the activated clotting time has been used in some studies. The prothrombin time cannot be used to monitor warfarin while patients on argatroban are being converted to oral anticoagulant therapy. It has been recommended that chromogenic factor X assays be used to monitor warfarin in these situations until the argatroban has been discontinued.[109] In the United States, argatroban is supplied by Texas Biotechnology (Houston, Tex) under the trade name Novastan. The product has not yet been approved for use by the FDA and is consequently not readily available in the United States at the present time.

References

1. Barrowcliffe TW, Johnson EA, Thomas DP. *Low Molecular Weight Heparin.* New York, NY: John Wiley & Sons; 1992:209.
2. Bara L, Bilaud E, Gramond G, Kher A, Samama M. Comparative pharmacokinetics of low molecular weight heparin (PK 10169) and unfractionated heparin after intravenous and subcutaneous administration. *Thromb Res.* 1985;39:631–636.
3. Lane DA. Heparin binding and neutralizing proteins. In: *Heparin: Chemical and Biological Properties: Clinical Applications.* London: E. Arnold; 1989;17:363–391.
4. Barzu T, Molho P, Tobelem G, et al. Binding and endocytosis of heparin by human endothelial cells in culture. *Biochim Biophys Acta.* 1985;845:196–203.
5. Young E, Wells P, Holloway S, Weitz J, Hirsh J. Ex-vivo and in vitro evidence that low molecular weight heparins exhibit less binding to plasma proteins than unfractionated heparin. *Thromb Haemost.* 1994;71:300–304.
6. Mason L, Rischke J, Young E, Hirsh J. Administration of no affinity heparin in vivo alters the tissue localization and metabolism of unfractionated heparin. *Blood.* 1995;86:881. Abstract.
7. Hirsh J, Levine MN. Low molecular weight heparin. *Blood.* 1992;79:1–17.
8. Frydman A, Bara I, Leroux Y, et al. The antithrombotic activity and pharmacokinetics of enoxaparin, a low molecular weight heparin in humans given single subcutaneous doses of 20 to 80 mg. *J Clin Pharmacol.* 1988;28:609–618.
9. Rosenberg RD, Bauer KA. The heparin-antithrombin system: a natural anticoagulant mechanism. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. *Hemostasis and Thrombosis: Basic Principles and Clinical Practice.* 3rd ed. Philadelphia, Pa: Lippincott; 1994:837–860.
10. Danielsson A, Raub E, Lindahl U, Bjork I. Role of ternary complexes in which heparin binds both antithrombin and proteinase, in the acceleration of the reactions between antithrombin and thrombin or factor Xa. *J Biol Chem.* 1986;261:15 467–15 473.
11. Norrheim L, Abildgaard U, Larsen ML, Lindahl AK. Involvement of the extrinsic pathway in the activities of low molecular weight heparins. *Thromb Res.* 1991;14(suppl):19–27.
12. Green D, Hirsh J, Heit J, Prins M, Davison B, Lensing AWA. Low molecular weight heparin: a critical analysis of clinical trials. *Pharmacol Rev.* 1994;46:89–109.
13. Lensing AWA, Prins MH, Davidson BL, Hirsh J. Treatment of deep venous thrombosis with low molecular weight heparins: a meta analysis. *Arch Intern Med.* 1995;155:601–607.
14. Geerts WH, Jay RM, Code KI, et al. A comparison of low-dose heparin with low-molecular-weight heparin as prophylaxis against venous thromboembolism after major trauma. *N Engl J Med.* 1996;335:701–707.
15. Clagett GP, Anderson FA, Heit J, Levine MN, Wheeler HB. Prevention of venous thromboembolism. *Chest.* 1995;108(suppl):312S–334S.
16. Green D, Lee MY, Lim AC, et al. Prevention of thromboembolism after spinal cord injury using low-molecular-weight heparin. *Ann Intern Med.* 1990;113:571–574.
17. Firsc Investigators. Low-molecular-weight heparin during instability in coronary artery disease. *Lancet.* 1996;347:561–568.
18. Cohen M, Demers C, Gurfinkel EP, et al. A comparison of low-molecular-weight heparin with unfractionated heparin for unstable coronary artery disease. *N Engl J Med.* 1997;337:447–452.
19. The Columbus Investigators. Low-molecular-weight heparin in the treatment of patients with venous thromboembolism. *N Engl J Med.* 1997;337:657–662.
20. Simonneau G, Sors H, Charbonnier B, et al. A comparison of low-molecular-weight heparin with unfractionated heparin for acute pulmonary embolism. *N Engl J Med.* 1997;337:663–669.
21. Levine M, Gent M, Hirsh J, et al. A comparison of low-molecular-weight heparin administered primarily at home with unfractionated heparin administered in the hospital for proximal deep-vein thrombosis. *N Engl J Med.* 1996;334:677–681.
22. Koopman MMW, Prandoni P, Piovella F, et al. Treatment of venous thrombosis with intravenous unfractionated heparin administered in the hospital as compared with subcutaneous low-molecular-weight heparin administered at home. *N Engl J Med.* 1996;334:682–687.
23. Kay R, Wong KS, Yu YL, et al. Low-molecular-weight heparin for the treatment of acute ischemic stroke. *N Engl J Med.* 1995;333:1588–1593.
24. Hunt BJ, Doughty HA, Majumdar G, et al. Thromboprophylaxis with low molecular weight heparin (Fragmin) and high risk pregnancies. *Thromb Haemost.* 1997;77:39–43.
25. Bergquist D, Benoni G, Bjorgell O, et al. Low-molecular-weight heparin (enoxaparin) as prophylaxis against venous thromboembolism after total hip replacement. *N Engl J Med.* 1996;335:696–700.
26. Pini M, Aiello S, Manotti C, et al. Low molecular weight heparin versus warfarin in the prevention of recurrences after deep vein thrombosis. *Thromb Haemost.* 1994;72:191–197.
27. Walsh-McMonagle D, Green D. Low-molecular-weight heparin in the management of Trousseau's Syndrome. *Cancer.* 1997;80:649–655.
28. Leizorovicz A, Simonneau G, Decousus H, Boissel JP. Comparison of efficacy and safety of low molecular weight heparins and unfractionated heparin in initial treatment of deep venous thrombosis: a meta-analysis. *BMJ.* 1994;309:299–304.
29. Hirsh J, Siragusa S, Cosmi B, Ginsberg JS. Low molecular weight heparins (LMWH) in the treatment of patients with acute venous thromboembolism. *Thromb Haemost.* 1995;74:360–363.
30. Hull RD, Raskob GE, Pineo GF, et al. Subcutaneous low-molecular-weight heparin compared with continuous intravenous heparin in the treatment of proximal-vein thrombosis. *N Engl J Med.* 1992;326:975–982.
31. Weitz JI, Hirsh J. New anticoagulant strategies. *J Lab Clin Med.* 1993;122:364–373.
32. Greinacher A, Potzach B, Amiral J, et al. Heparin-associated thrombocy-

topenia: isolation of the antibody and characterization of a multimolecular PF4-heparin complex as the major antigen. *Thromb Haemost.* 1994;71:247–251.

33. Warkentin TE, Levine MN, Hirsh J, et al. Heparin-induced thrombocytopenia in patients treated with low-molecular weight heparin or unfractionated heparin. *N Engl J Med.* 1995;332:1330–1335.

34. Shaughnessy SG, Young E, Deschamps P, Hirsh J. The effects of low molecular weight and standard heparin on calcium loss from fetal rat calvaria. *Blood.* 1995;86:1368–1373.

35. Monreal M, Lafoz E, Olive A, del Rio L, Vedia C. Comparison of subcutaneous unfractionated heparin with a low molecular weight heparin (Fragmin) in patients with venous thromboembolism and contraindications to coumarin. *Thromb Haemost.* 1994;72:7–11.

36. Melissari E, Parker CJ, Wilson NV, et al. Use of low molecular weight heparin in pregnancy. *Thromb Haemost.* 1992;68:652–656.

37. Anderson DR, O'Brien BJ, Levine MN, et al. Efficacy and cost of low-molecular-weight heparin compared with standard heparin for the prevention of deep vein thrombosis after total hip arthroplasty. *Ann Intern Med.* 1993;119:1105–1112.

38. Hull RD, Raskob GE, Rosenbloom D, et al. Treatment of proximal vein thrombosis with subcutaneous low-molecular intravenous heparin: an economic perspective. *Arch Intern Med.* 1997;157:289–294.

39. Cadroy Y, Pourrat J, Baladre MF, et al. Delayed elimination of enoxaparin in patients with chronic renal insufficiency. *Thromb Haemost.* 1991;63:385–390.

40. Boneu B. Low molecular weight heparin: is monitoring needed? *Thromb Haemost.* 1994;72:330–334.

41. Goudable C, Saivin S, Houin G, et al. Pharmakokinetics of a low molecular weight heparin (Fraxiparine®) in various stages of chronic renal failure. *Nephron.* 1991;59:543–545.

42. Massicotte P, Adams M, Marzinotto V, Brooker LA, Andrew M. Low-molecular-weight heparin in pediatric patients with thrombotic disease: a dose finding study . *J Pediatr.* 1996;128:313–318.

43. Harenberg J, Giese Ch, Dempfle CE, Stehle G, Heene DL. Biological activity and safety of the subcutaneous administration of high doses of low molecular weight heparin for 8 days in human volunteers. *Thromb Haemost.* 1989;61:357–362.

44. Collignon F, Frydman A, Caplain H, et al. Comparison of the pharmacokinetic profiles of three low molecular mass heparins—daltepaarin, enoxaparin and nadroparin—administered subcutaneously in healthy volunteers (doses for prevention of thromboembolism). *Thromb Haemost.* 1995;73:630–640.

45. Samama MM. Contemporary laboratory monitoring of low molecular weight heparins. *Clin Lab Med.* 1995;15:119–123.

46. Sie P, Aillaud MF, de Prost D, et al. Measurement of low molecular weight heparin ex vivo activities in clinical laboratories using various anti Xa assays: interlaboratory variability and requirement of an agreed low molecular weight heparin standard. *Thromb Haemost.* 1987;58:879–883.

47. Barrowcliffe TW. Low molecular weight heparins. *Br J Haematol.* 1995;90:1–7.

48. Nieuwenhuis HK, Albada J, Banga JD, Sixma JJ. Identification of risk factors for bleeding during treatment of acute venous thromboembolism with heparin or low molecular weight heparin. *Blood.* 1991;78:2337–2343.

49. Simonneau G, Charbonnier B, Decousus H, et al. Subcutaneous low-molecular-weight heparin compared with continuous intravenous unfractionated heparin in the treatment of proximal deep vein thrombosis. *Arch Intern Med.* 1993;153:1541–1546.

50. Agnelli G, Iorio A, Renga C, et al. Prolonged antithrombin activity of low-molecular-weight heparins. *Circulation.* 1995;92:2819–2824.

51. Lindmarker P, Holstrom M, Granquist S, Johnsson H, Lockner D. Comparison of once-daily subcutaneous Fragmin with continuous intravenous unfractionated heparin in the treatment of deep vein thrombosis. *Thromb Haemost.* 1994;72:186–190.

52. Alhenc-Gelas M, Guernic JL, Vitoux JF, et al. Adjusted versus fixed doses of the low-molecular-weight heparin Fragmin in the treatment of deep vein thrombosis. *Thromb Haemost.* 1994;71:698–702.

53. Holstrom MC, Berglund S, Granquist G, Bratt G, Tornebohm E, Lockner D. Fragmin once or twice daily subcutaneously in the treatment of deep venous thrombosis of the leg. *Thromb Res.* 1992;67:49–55.

54. Handeland GF, Abildgaard U, Holm HA, Arnesen KE. Dose adjusted heparin treatment of deep venous thrombosis: a comparison of unfractionated and low molecular weight heparin. *Eur J Pharmacol.* 1990;39:107–112.

55. National Committee for Clinical Laboratory Standards. *Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays.* 2nd ed. Approved guideline. Villanova, Pa: NCCLS; 1991. NCCLS document H21-A2.

56. Ozawa T, Domagalski J, Mammen E. Determination of low molecular weight heparin by *Heptest* on the automated coagulation system. *Am J Clin Pathol.* 1993;99:157–162.

57. Ortel TL, Gockerman JP, Califf RM, et al. Parenteral anticoagulation with the heparinoid Lomoparan (Org 10172) in patients with heparin induced thrombocytopenia and thrombosis. *Thromb Haemost.* 1992;67:292–296.

58. de Valk HW, Banga JD, Wester JWJ, et al. Comparing subcutaneous danaparoid with intravenous unfractionated heparin for the treatment of venous thromboembolism. *Ann Intern Med.* 1995;123:1–9.

59. Danhof M, de Boer A, Magnani HN, Stiekema JC. Pharmacokinetic considerations on Orgaran (Org 10172) therapy. *Haemostasis.* 1992;22:73–84.

60. Stiekema JC, Wijnand HP, Van Dinther TG, et al. Safety and pharmacoki-

netics of the low molecular weight heparinoid Org 10172 administered to healthy elderly volunteers. *Br J Clin Pharmacol.* 1989;27:39–48.

61. ten Cate H, Henny CP, ten Cate JW, et al. Anticoagulant effects of a low molecular weight heparinoid (Org 10172) in human volunteers and haemodialysis patients. *Thromb Res.* 1985;39:211–222.

62. Magnani HN. Heparin-induced thrombocytopenia (HIT): an overview of 230 patients treated with Orgaran (Org 10172). *Thromb Haemost.* 1993;70:554–561.

63. Greinacher A, Amiral J, Dummel V, et al. Laboratory diagnosis of heparin-associated thrombocytopenia and comparison of platelet aggregation test, heparin-induced platelet activation test, and platelet factor 4/heparin enzyme-linked immunosorbent assay. *Transfusion.* 1994;34:381–385.

64. Hirsh J, Raschke R, Warkentin TE, Dalen JE, Deykin D, Poller L. Heparin: mechanism of action, pharmacokinetics, dosing considerations, monitoring, efficacy and safety. *Chest.* 1995;108:258–275S.

65. Greinacher A, Philippen KH, Kemkes-Matthes B, et al. Heparin-associated thrombocytopenia type II in a patient with end-stage renal disease: successful anticoagulation with the low-molecular-weight heparinoid Org 10172 haemodialysis. *Nephrol Dial Transplant.* 1993;8:1176–1177.

66. Henny CP, ten Cate H, ten Cate JW, et al. Use of a new heparinoid as anticoagulant during acute haemodialysis of patients with bleeding complications. *Lancet.* 1983;i:891–893.

67. Greinacher A, Drost W, Michels I, et al. Heparin-associated thrombocytopenia: successful therapy with the heparinoid Org 10172 in a patient showing cross-reaction to LMW heparins. *Ann Hematol.* 1992;64:40–42.

68. Rowlings PA, Mansberg R, Rozenberg MC, Evans S, Murray B. The use of a low molecular weight heparinoid (Org 10172) for extracorporeal procedures in patients with heparin dependent thrombocytopenia and thrombosis. *Aust N Z J Med.* 1991;21:52–54.

69. Massey EW, Biller J, Davis JN, et al. Large dose infusions of heparinoid Org 10172 in ischemic stroke. *Stroke.* 1990;21:1289–1292.

70. TOAST Investigators. Low molecular weight heparinoid, Org 10172 (danaparoid), and outcome after acute ischemic stroke: a randomized controlled trial. *JAMA.* 1998;279:1265–1272.

71. Turpie AGG, Gent M, Cote R, et al. A low molecular weight heparinoid compared with unfractionated heparin in the prevention of deep vein thrombosis in patients with acute ischemic stroke. *Ann Int Med.* 1992;117:353–357.

72. Nieuwenhuis HK, Sixma JJ. Treatment of disseminated intravascular coagulation in acute promyelocytic leukemia with low molecular weight heparinoid Org 10172. *Cancer.* 1986;58:761–764.

73. Wilhelm MJ, Schmid C, Kececioglu D, et al. Cardiopulmonary bypass in patients with heparin-induced thrombocytopenia using Org 10172. *Ann Thorac Surg.* 1996;61:920–924.

74. Marshall LR, Cannell PK, Herrmann RP. Successful use of the aPTT in monitoring of the anti-factor Xa activity of the heparinoid Organon 10172 in a case of HITS requiring open heart surgery. *Thromb Haemost.* 1992;67:587. Letter.

75. Deryck YLJM, Schepp RM, van Krugten RJ, Mochtar B. Heparinoid Org 10172 anticoagulation for cardiopulmonary bypass: a case report. *J Cardiothorac Vasc Anesth.* 1990;4(suppl 3):40.

76. Magnani HN, Beijering RJR, ten Cate JW, Chong BH. Orgaran anticoagulation for cardiopulmonary bypass in patients with heparin-induced thrombocytopenia. In: Pifarre R, ed. *New Anticoagulants for the Cardiovascular Patient.* Philadelphia, Pa: Hanley & Belfus, Inc; 1997:487–500.

77. Doherty DC, Ortel TC, de Bruijn N, Greenberg CS, van Trigt P III. ''Heparin-free'' bypass: first reported use of heparinoid (Org 10172) to provide anticoagulation for cardiopulmonary bypass. *Anesthesiology.* 1990;734:562–565.

78. Schmahl KS, Ganjoo AK, Karloff MG. Orgaran (Org 10172) for cardiopulmonary bypass in heparin-induced thrombocytopenia: role of adjunctive plasmapheresis. *J Cardiothorac Vasc Anesth.* 1997;11:262–264.

79. Wallis RB. Hirudins: from leeches to man. *Semin Thromb Hemost.* 1996;22:185–196.

80. Turpie AGG, Weitz JI, Hirsh J. Advances in antithrombotic therapy: novel agents. *Thromb Haemost.* 1995;74:565–571.

81. Eicher P, Olbrich K, Potzsch B, Greinacher A. Anti-hirudin antibodies in patients treated with recombinant hirudin for more than five days, a prospective study. *Thromb Haemost.* 1997;77(suppl 1):493. Abstract.

82. Meyer BH, Luus HG, Muller FO, Badenhorst PN, Roethig H. The pharmacology of recombinant hirudin, a new anticoagulant. *S Afr J Med.* 1990;78:268–270.

83. Agnelli G, Sonaglia F. Recombinant hirudin in the prevention of venous thromboembolism in patients undergoing elective hip surgery. *Semin Thromb Hemost.* 1997;23:143–148.

84. Vanholder R, Camez A, Veys N, Van Loo A, Dhondt AM, Ringoir S. Pharmacokinetics of recombinant hirudin in hemodialyzed end-stage renal failure patients. *Thromb Haemost.* 1997;77:650–655.

85. Eriksson BI, Ekman S, Kalebo P, et al. Prevention of deep-vein thrombosis after total hip replacement: direct thrombin inhibition with recombinant hirudin, CGP 39393. *Lancet.* 1996;347:635–639.

86. Eriksson BI, Wille-Jorgensen P, Kalebo P, et al. A comparison of recombinant hirudin with a low molecular weight heparin to prevent thromboembolic complications after total hip replacement. *N Engl J Med.* 1997;337:1329–1335.

87. Parent F, Bridey F, Dreyfus M, et al. Treatment of severe venous thromboembolism with intravenous hirudin (HBW 023): an open pilot study. *Thromb Haemost.* 1993;70:386–388.

88. Schiele F, Lindgaerd F, Eriksson H, et al. Subcutaneous recombinant hiru-

din (HBW 023) versus intravenous sodium heparin in treatment of established acute deep venous thrombosis of legs; a multicentre prospective dose-ranging randomized trial. *Thromb Haemost.* 1997;77:834–838.

89. Serruys PW, Herrman JP, Simon R, et al. A comparison of hirudin with heparin in the prevention of restenosis after coronary angioplasty. *N Engl J Med.* 1995;333:757–763.

90. The Global Use of Strategies of Open Occluded Coronary Arteries (GUS-TO IIb) Investigators. A comparison of recombinant hirudin with heparin for the treatment of acute coronary syndromes. *N Engl J Med.* 1996;335:775–782.

91. Antman EM, for the TIMI 9B Investigators. Hirudin in acute myocardial infraction: Thrombolysis and Thrombin Inhibition in Myocardial Infarction (TIMI) 9B trial. *Circulation.* 1996;94:911–921.

92. Organization to Assess Strategies for Ischemic Syndromes (OASIS) Investigators. Comparison of the effects of two doses of recombinant hirudin compared with heparin in patients with acute myocardial ischemia without ST elevation. *Circulation.* 1997;96:769–777.

93. The Global Use of Strategies to Open Occluded Coronary Arteries (GUS-TO IIa) Investigators. Randomized trial of intravenous heparin versus recombinant hirudin for acute coronary syndromes. *Circulation.* 1994;90:1631–1637.

94. Antman EM. Hirudin in acute myocardial infarction: safety report from the Thrombolysis and Thrombin Inhibition in Myocardial Infarction (TIMI) 9A trial. *Circulation.* 1994;90:1624–1630.

95. Neuhaus KL, von Essen R, Tebbe U, et al. Safety observations from the pilot phase of the randomized r-Hirudin for Improvement of Thrombolysis (HIT-III) study. *Circulation.* 1994;90:1638–1642.

96. Schiele F, Vuillemenot A, Kramarz P, et al. Use of recombinant hirudin as antithrombic treatment in patients with heparin-induced thrombocytopenia. *Am J Hematol.* 1995;50:20–25.

97. Riess F, Lower C, Seelig C, et al. Recombinant hirudin as a new anticoagulant during cardiac operations instead of heparin: successful for aortic valve replacement in man. *J Thorac Cardiovasc Surg.* 1995;110:265–267.

98. Potzch B, Madlener K, Seelig C, et al. Monitoring of r-hirudin anticoagulation during cardiopulmonary bypass—assessment of the whole blood ecarin clotting time. *Thromb Haemost.* 1997;77:902–925.

99. Greinacher A, Volpel H, Potzsch B. Recombinant hirudin in the treatment of patients with heparin-induced thrombocytopenia (HIT). *Blood* 1996;88(suppl 1B):281A.

100. Tripodi A, Chantarangkul V, Arbini AA, Moia M, Mannucci PM. Effects of hirudin on activated partial thromboplastin time determined with ten different reagents. *Thromb Haemost.* 1993;70:286–288.

101. Breddin HK, Radziwon P, Boczkowska-Radziwon B. Laboratory monitoring of new antithrombotic drugs. *Clin Lab Med.* 1994;14:825–846.

102. Nowak G, Bucha E. Quantitative determination of hirudin in blood and body fluids. *Semin Thromb Hemost.* 1996;22:197–202.

103. Fox I, Dawson A, Loynds P, et al. Anticoagulant activity of hirulog, a direct thrombin inhibitor, in humans. *Thromb Haemost.* 1993;69:157–163.

104. Ginsberg JS, Nurmohamed MT, Gent M, et al. Use of hirulog in the prevention of venous thrombosis after major hip or knee surgery. *Circulation.* 1994; 90:2385–2389.

105. Bittle JA, Strony J, Brinker JA, et al. Treatment with bivalirudin (hirulog) as compared with heparin during coronary angioplasty for unstable or post-infarction angina. *N Engl J Med.* 1995;333:764–769.

106. Fuchs J, Cannon CP. Hirulog in the treatment of unstable angina: results of the Thrombin Inhibition in Myocardial Ischemia (TIMI) 7 trial. *Circulation.* 1995;92:727–733.

107. White HD. Clinical trials of direct thrombin inhibitors in acute ischaemic syndromes. *Thromb Haemost.* 1997;78:364–366.

108. Chamberlin JR, Lewis B, Leya F, et al. Successful treatment of heparin associated thrombocytopenia and thrombosis using Hirulog. *Can J Cardiol.* 1995; 11:511–514.

109. Lewis BE, Walenga JM, Wallis DE. Anticoagulation with Novastan (argatroban) in patients with heparin-induced thrombocytopenia and heparin-induced thrombocytopenia and thrombosis syndrome. *Semin Thromb Haemost.* 1997;23:197–202.

110. Clarke RJ, Mayo G, Fitzgerald GA, Fitzgerald DJ. Combined administration of aspirin and a specific thrombin inhibitor in man. *Circulation.* 1991;83: 1510–1518.

111. Verstraete M. Direct thrombin inhibitors: appraisal of the antithrombotic/hemorrhagic balance. *Thromb Haemost.* 1997;78:357–363.

112. Gold HK, Torres FW, Garabedian HD, et al. Evidence for a rebound coagulation phenomenon after cessation of a 4-hour infusion of a specific thrombin inhibitor in patients with unstable angina pectoris. *J Am Coll Cardiol.* 1993;21: 1039–1047.

113. Lewis BE, Ferguson JJ, Grassman ED, et al. Successful coronary interventions performed with argatroban anticoagulation in patients with heparin-induced thrombocytopenia and thrombosis syndrome. *J Invas Cardiol.* 1996;8:410–417.

114. Sakamoto S, Hirase T, Suzuki S, et al. Inhibitory effect of argatroban on thrombin-antithrombin III complex after percutaneous transluminal coronary angioplasty. *Thromb Haemost.* 1995;74:801–802. Letter.

115. Matsuo T, Kario K, Kodama K, Okamoto S. Clinical application of the synthetic thrombin inhibitor, argatroban (MD-805). *Semin Thromb Hemost.* 1992; 18:155–160.

116. Vermeer F, Vahanian A, Fels PW, Besse D, Radzik D, Simoons ML. Intravenous argatroban versus heparin as co-medication to alteplase in the treatment of acute myocardial infarction: preliminary results of the ARGAMI pilot study. *J Am Coll Cardiol.* 1997;29:185–186. Abstract.

117. Kario K, Kodama K, Koide M, Matsuo T. Thrombin inhibition in the acute phase of ischaemic stroke using argatroban. *Blood Coagul Fibrinolysis.* 1995;6: 423–427.