

The direct thrombin inhibitor hirudin

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Summary

This review discusses the pharmacology and clinical applications of hirudin, a bivalent direct thrombin inhibitor (DTI). Besides the current major indication for hirudin – anticoagulation of patients with heparin-induced thrombocytopenia (HIT) – the experience with hirudin in other indications, especially acute coronary syndromes, are briefly presented. Hirudins have been formally studied prior to their regulatory approval; however, impor-

tant information on their side effects and relevant preventative measures only became available later. Therefore, current recommendations and dosing schedules for hirudin differ considerably from the information given in the package inserts. Drawbacks of hirudin and important precautions for avoiding potential adverse effects are discussed in detail in the third part of this review.

Keywords

Thrombin, deep vein thrombosis, hirudin, HIT

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Hirudin

Hirudin extracted from leeches was the first anticoagulant used in humans in 1905. Today hirudins are produced as recombinant proteins based on the leech anticoagulant protein sequence. The recombinant hirudin (r-hirudin), lepirudin, was the first direct thrombin inhibitor (DTI) to be approved for clinical use, first by the European Medical Evaluation Agency (EMEA) in 1997, and then by the U.S. Food and Drug Administration (FDA) in 1998, for the treatment of heparin-induced thrombocytopenia (HIT) complicated by thrombosis. Subsequently, another recombinant hirudin, desirudin, was approved for thrombosis prophylaxis after major orthopedic surgery.

Pharmacology

Hirudin, the most potent natural thrombin inhibitor, is a 65-amino-acid polypeptide (molecular mass, ~7 kDa) produced by the parapharyngeal glands of the medicinal leech, *Hirudo medicinalis*. The molecule is stabilized by three disulfide bridges. Its three-dimensional structure (1) reveals three distinct regions: a central core (residues 3–30, 37–46, 56–57), a "finger" (residues 31–36), and a loop (residues 47–55).

Hirudins for therapeutic use are produced by recombinant biotechnology using yeast. Recombinant hirudins (r-hirudin) differ from natural hirudin by lacking the sulfate group at Tyr-63. Although this structural change results in a lower affinity of desulfato-hirudins to thrombin, r-hirudins nevertheless are highly specific inhibitors of thrombin, with an inhibition constant for thrombin in the picomolar range (2).

Effective inhibition of thrombin by antithrombin and heparin cofactor II require the catalytic actions of heparins. In contrast, hirudins effectively inhibit thrombin independently of any cofactor (3). They form noncovalent—but irreversible--1:1 complexes with thrombin. r-Hirudins bind to at least two sites on thrombin, and are thus classified as *bivalent* DTI's (Fig. 1). A further difference from heparin-antithrombin and heparin-heparin cofactor II is that r-hirudin inhibits both free and clot-bound thrombin (4, 5), as well as thrombin bound to fibrin split products (6). In contrast, heparin-antithrombin complexes are relatively poor at accessing and inactivating clot-bound thrombin. This could explain why hirudin is more effective than heparin in promoting dissolution of mural thrombi in experimental models (7). Another important difference to heparin is that hirudin shows virtually no interaction with plasma proteins (8), whereas heparin binding to plasma proteins is the major explanation for many limitations of heparin, including its

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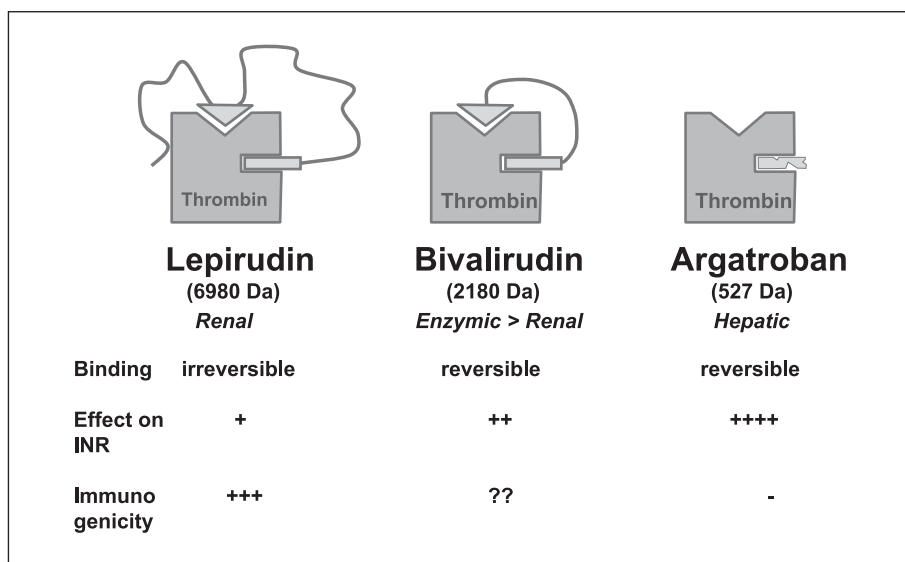


Figure 1: Schematic representation of the thrombin molecule and its inhibition by hirudin, bivalirudin, and argatroban. The active-site pocket catalyzes most of the functions of the thrombin molecule, whereas the fibrinogen-binding exosite mediates the binding of thrombin to fibrinogen. Hirudin and bivalirudin bind to the active-site pocket and the fibrinogen-binding exosite of thrombin, i.e. they are bivalent direct thrombin inhibitors. The arginine derivative argatroban binds competitively to only the active binding site pocket of thrombin. Hirudin and argatroban are univalent direct thrombin inhibitors. (Adapted from Hermann et al. 1997 [89]).

unpredictable dose-response relationship, the potential for heparin "resistance", and the induction of neoantigens after binding to PF4 (resulting in HIT). The activity of hirudin is standardized in thrombin inhibitory units (TIU), where 1 TIU is the amount of hirudin inhibiting 1 U of thrombin at 37°C.

Hirudins are administered parenterally, most often by intravenous (iv) injection. Hirudin distributes into the extracellular space. Only 20% of hirudin is found in the plasma, while the remaining 80% is in the extravascular compartment (8). The terminal plasma elimination half-life ($t_{1/2\beta}$) ranges from 0.8 to 1.7 hours (h) (mean, ~1.3 h, or 80 minutes [min]) following iv injection of bolus doses of 0.01–0.5 mg/kg and 1.1–2.0 h following continuous iv infusions over 6 h. Maximum activated partial thromboplastin time (aPTT) ratios occur about 10 min after iv bolus, 3–6 h following 6-h continuous iv infusion, and 2–3 h following subcutaneous (sc) administration (in patients with normal renal function). During iv infusion, therapeutic levels are usually reached within 30–60 min. Renal clearance (160–200 ml/min for an adult with normal body surface area of 1.73 m²) and degradation account for approximately 90% of the systemic clearance of lepirudin. The $t_{1/2}$ of r-hirudin lengthens with renal dysfunction (9–14); in nephrectomized patients, it approaches 5 days (15–18).

Bioavailability is almost 100% with sc administration. In healthy volunteers its concentration in the blood reaches 0.3–0.5 µg/ml after a subcutaneous (sc) dose of lepirudin of 0.5 mg/kg and approximately 0.7 µg/ml after a sc dose of 0.75 mg/kg. Thus, twice-daily sc injections provide effective anticoagulation (19–21). When administered sc, this drug is usually injected into an abdominal skin fold, reaching peak concentration after 2–3 h.

Monitoring

Numerous tests have been evaluated for monitoring anticoagulation by DTIs, ranging from the aPTT and the activated clotting time (ACT) to the newer ecarin clotting time (ECT), as well as enzyme-

immunoassay (EIA) techniques for directly measuring the hirudin concentration (22), and the ecarin chromogenic assay (ECA) which overcomes the problem of prothrombin dependency (23, 24). Like all clotting tests used for anticoagulant monitoring, they are at best a surrogate marker for the patient's haemostatic system.

The aPTT is a global coagulation assay and is the current method of choice for monitoring DTI therapy in most situations. However, for aPTT values above ~60–70 seconds (s) (depending on the reagent), the hirudin concentration-aPTT curve flattens, and even major increases in plasma levels cause only a minor change in the aPTT (Fig. 2). Because the sensitivities of different aPTT reagents vary (25), it is strongly recommended that each laboratory involved in monitoring of DTIs should generate its own standard dose-response curve for their aPTT reagent using "spiked" normal pooled plasma samples (e.g. with 0.25, 0.50, 0.75, 1.0, 1.25, 1.5, and 2.0 µg/ml lepirudin). This will define the expected range over which the aPTT reliably reflects changes in the DTI plasma concentration. At concentrations above this range, the ECT is more reliable for DTI monitoring. This is especially true for very high doses, such as those used during cardiopulmonary bypass (CPB).

Unlike global coagulation tests, the ECT monitors prolongation of clotting time caused by thrombin inhibition alone (26–33). The ECT can be performed with plasma or with whole blood. Most concentrations are reported as whole blood concentrations. Therefore, for both methods the standard curve should be obtained with spiked whole blood. For the plasma method plasma should then be obtained by centrifugation. This allows to compare the concentrations between both methods. The ECT shows a linear correlation to plasma hirudin levels over a wide range. At present, this assay is recommended for monitoring anticoagulation when higher concentrations of DTIs are used. It is mandatory for monitoring of DTIs during CPB.

Recently, an automated assay, the ecarin chromogenic assays (ECA), has been introduced that provides a linear dose-response curve for all DTIs independently of the patient's prothrombin and fibrinogen levels (23, 24).

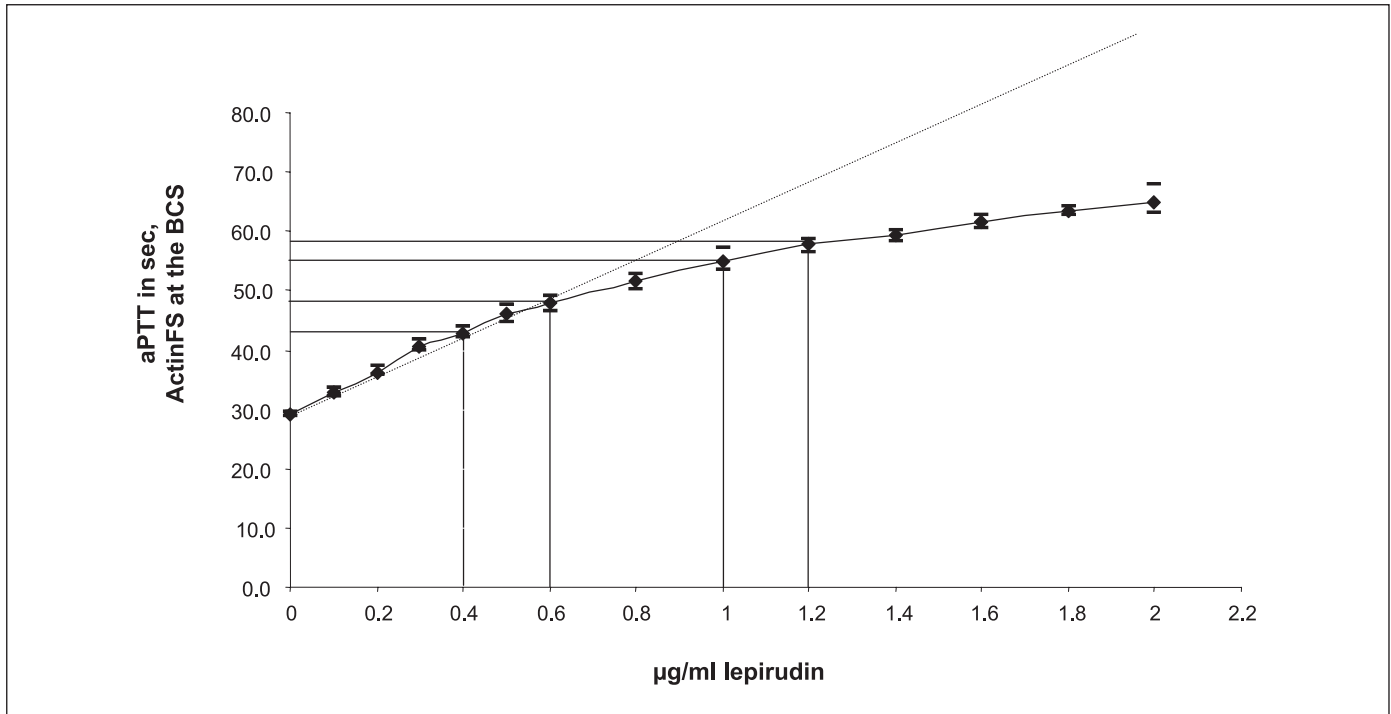


Figure 2: Lepirudin standard curve. This curve was generated using seven normal plasmas spiked with various concentrations of lepirudin ($\mu\text{g/ml}$) using reagent Actin FS and the BCS analyzer (Dade-Behring, Germany). Note that incremental changes in activated partial thromboplastin time (aPTT) become much smaller at greater plasma lepirudin concentrations, i.e. the dose-response curve flattens.

In 2005, a workshop compared several methods for monitoring DTIs: aPTT using local reagents and methods (Actin FS, Thrombolil I, Pathromtin SL, Synthasil aPTT, Automated aPTT, STA-PTT, STA – CK Prest 5); aPTT using a common reagent (C-aPTT; Actin FS; Aventis Pharma, Marburg, Germany); anti-IIa chromogenic assay with the S2238 chromogenic substrate from Instrumentation Laboratory/Haemochrom Diagnostica (Essen, Germany); ECT–wet chemistry (Wet ECT) reagents (University of Jena, Germany); ECT–dry chemistry reagent (Cardiovascular Diagnostics Inc., Raleigh, NC, USA) with two ecarin concentrations (low ecarin reagent card: dry ECT; higher concentration ecarin card: TIM); EIA kit (Immuno Bind, Hirudin Elisa kit, American Diagnostica Inc., Greenwich, CT, USA). The interlaboratory variations for measurement of lepirudin observed were (from lowest to highest): TIM < C-aPTT < Dry ECT < L-aPTT < wet ECT < anti-IIa < EIA (34).

Limitations of functional monitoring tests

Results obtained with the aPTT or ECT may be inaccurate in patients whose plasma has a reduced concentration of prothrombin (e.g. severe liver disease, disseminated intravascular coagulation [DIC], treatment with vitamin K antagonists [VKAs]) or in patients with fibrinogen depletion (e.g. post-thrombolysis, haemodilution during CPB) (32, 35). This is especially problematic during CPB. In the ECT, this can be overcome by addition of normal plasma 1:1 to the assay (30). EIAs which measure the plasma concentration of lepirudin independent of prothrombin concentration or the ECA can also be used to overcome this problem.

A systematic laboratory study on the effects of different DTIs on the International Normalised Ratio (INR) (36) revealed that

the differing effects of the DTIs on PT prolongation are primarily driven by their respective molar plasma concentrations required for clinical effect. DTIs with a relatively low affinity for thrombin (e.g. argatroban) require high plasma concentrations to double the aPTT, compared with those with a higher affinity for thrombin (e.g. lepirudin). These higher plasma concentrations of DTI, in turn, quench more of the thrombin generated in the PT, thereby prolonging the PT to a greater extent.

Clinical use of hirudins

Lepirudin has been investigated extensively in controlled clinical trials for acute coronary syndrome (ACS) ($n > 14,000$), including myocardial infarction (MI) (37, 38) and unstable angina pectoris (39, 40); and in pilot studies for prophylaxis and treatment of deep-vein thrombosis (DVT) (41, 42). Lepirudin has also been studied for anticoagulation in settings of artificial surfaces, such as haemodialysis and cardiac surgery utilizing the CPB.

Hirudins are approved for only two indications, HIT complicated by thrombosis (lepirudin), as well as for thrombosis prophylaxis after major orthopedic surgery, a clinical situation in which desirudin has been studied systematically (43, 44). However, despite this approval, the use of desirudin for post-surgery thromboprophylaxis is negligible (primarily due to high cost) and will not be discussed further. Current dosing recommendations for the different clinical applications are summarized in Table 1.

Table 1: Dosing schedules for lepirudin treatment of patients with heparin-induced thrombocytopenia (HIT). Reprinted with permission from Greinacher A. Lepirudin in heparin-induced thrombocytopenia. In: Heparin induced thrombocytopenia (4th ed), Informa Health Care, New York 2007.

	Bolus	I.v. infusion^{a,b}	Target aPTT ratio^c
Dose recommended in all HIT patients without renal impairment	None^d	0.05–0.10 mg/kg BW/h^d	1.5–2.5 (0.6–1.0 mg/ml)
HIT with acute thrombosis (dose regimen B in HAT trials)	None ^e	0.10 mg/kg BW/h ^e	1.5–2.5 (0.6–1.0 mg/ml)
HIT with isolated thrombocytopenia (dose regimen A1 in HAT trials)	(0.40 mg/kg BW iv) ^e	0.15 mg/kg BW/h ^e	1.5–2.5 (0.6–1.0 mg/ml)
Thrombosis prophylaxis in patients with a history of HIT	15 mg sc twice daily ^f	-	-
HIT with thrombosis and concomitant thrombolysis (dose regimen A2 in HAT trials)	(0.20 mg/kg BW iv) ^e	0.10 mg/kg BW/h ^e	1.5–2.5 (0.6–1.0 mg/ml)
Renal dialysis every alternate day	0.10 mg/kg BW iv predialysis	-	2.0–2.5 (0.6–1.0 mg/ml)
CVVH (use bolus or i.v. infusion regimen)	intermittent i.v. boli 0.005–0.01 mg/kg	0.005 mg/kg BW/h (initial rate)	1.5–2.5 (0.6–1.0 mg/ml)
PCI (Metha et al. 2002); UA or acute MI without ST elevation (OASIS-2, 1999)	0.40 mg/kg BW iv	0.15 mg/kg BW/h	1.5–2.5 (0.6–1.0 mg/ml)
Vascular surgery (Hach-Wunderle, 2001)	0.40 mg/kg BW iv	0.10 mg/kg BW/h	1.5–2.5 (0.6–1.0 mg/ml)
Vascular surgery (intraoperative vessel flushes)	use up to 250 ml (0.1 mg/ml solution)	-	-
Postoperative anticoagulation	-	0.10 mg/kg BW/h	1.5–2.5 (0.6–1.0 mg/ml)
Cardiac surgery using CPB (dose regimen C in HAT trials)	0.25 mg/kg BW iv ^e 0.20 mg/kg in the priming fluid	0.50 mg/min ^{a, b, g}	Monitored by ECT: >2.5 mg/ml before start of CPB; 3.5–4.5 mg/ml during CPB ^h

Note: repeat aPTT determinations should be made 4–6 h after any dose adjustment. ^aA maximum body weight (BW) of 100 kg should be used for dose calculations. ^bAdjust for renal insufficiency. ^cThe ratio is based on comparison with the normal laboratory mean aPTT. If Actin FS or Neothrombin reagents are used, the aPTT target range is usually 1.5–3.0. ^dThis is the author's recommended starting dose in all HIT patients, unless life- or limb-threatening thrombosis is present. ^eUsed in the HAT-1, -2, and -3 trials. ^fTested in a prospective, randomized trial after orthopaedic surgery with desirudin (Eriksson et al., 1996, 1997). ^gStop 15 min before end of CPB; put 5 mg into CPB after disconnection to avoid clotting of pump. ^hThe target lepirudin level pre-CPB (>2.5 mg/ml) is lower than the level sought during CPB (3.4–4.5 mg/ml) because of the addition of lepirudin to the pump priming fluid (0.2 mg/kg BW). Abbreviations: aPTT, activated partial thromboplastin time; BW, body weight; CPB, cardiopulmonary bypass; CVVH, continuous venovenous hemofiltration; ECT, ecarin clotting time; iv, intravenous; MI, myocardial infarction; PCI, percutaneous coronary intervention; UA, unstable angina.

Lepirudin in HIT

In three prospective studies, named the Heparin-Associated Thrombocytopenia (HAT)-1, -2, and -3 trials, lepirudin was evaluated for anticoagulation in patients with HIT (45–48). The HAT studies included comparisons of clinical outcomes with a historical control group treated before lepirudin became available. Inclusion criteria were clinical suspicion of HIT and a positive functional test for HIT antibodies, the heparin-induced platelet activation (HIPA) test. This HIPA test is a "functional" (platelet activation) assay using washed platelets that has similar sensitivity and specificity characteristics as the "gold standard" platelet serotonin-release assay.

In all three studies, the risk for a new thrombosis was decreased dramatically (by 92.9%) once lepirudin was started – from 5.1% per patient day during the 1.3 day period between diagnosis of HIT and start of lepirudin treatment to 0.4% during active treatment. (29, 30, 49).

The composite endpoint occurred less often in the lepirudin-treated patients as compared to controls ($p = 0.04$), primarily due to a reduction in new thrombotic events ($p < 0.001$), while the risk for limb amputation ($p = 0.79$) and death ($p = 0.43$) did not

differ significantly. However, the risk for major bleeding was also increased in the lepirudin-treated patients ($p = 0.015$).

Patients with HIT and thrombosis

Altogether, 235 patients with acute HIT and thrombosis were enrolled. From start of lepirudin treatment, the combined end point for new thrombosis, limb amputation, and death was significantly lower in the lepirudin-treated patients ($n = 235$) than in the controls ($n = 75$) (19.1% vs. 40.0%; $p = 0.002$). This difference was primarily due to a reduction in the number of new thrombosis (6.8% vs. 25.3%; $p = 0.001$), while incidences of limb amputation (5.5% vs. 8.0%; $p = 0.44$) and death (11.9% vs. 12.0%; $p = 0.98$) did not differ between the lepirudin group and the historical controls. The cumulative incidence of major bleeding was higher in the lepirudin group than in the control group (14.9% vs. 6.7%; $p = 0.064$). The risk for major bleeding was 1.05% per patient day (mean treatment duration 14.2 days) (Table 2).

Patients with isolated HIT

Ninety-one patients with acute HIT but no thrombosis ("isolated HIT") were enrolled into the prospective trials. During treatment with lepirudin, four (4.4%) patients experienced new throm-

Table 2: Studies of lepirudin for treatment of heparin-induced thrombocytopenia (HIT): Clinical description and results. *Composite endpoint of death, limb amputation, and new thrombus.

Author /Year	Intervention/ dosing (mean DTI duration)	# Patients analyzed (%)	Duration of follow-up	New throm- bosis (%) RR (95%CI)	Limb ampu- tation (%) RR (95%CI)	Composite end point* (%) RR (95%CI)	Major bleeds (%) RR (95%CI)	% Major bleeds / tx day RR (95%CI)	Comment
Lepirudin for treatment of thrombosis complicating HIT									
Cohort studies with historic controls									
Greinacher 1999a, Greinacher 1999b, Lubenow 2005	Lep: Bolus 0.4mg/kg; 0.15 mg/kg/h (15.8 days) Con: Varied [danaparoid, n=24; phenprocoumon, n=21; other, n=30]	Lep: 214/214 Con: 75/75	35 days	Lep: 15/214 (7.0%) Con: 19/75 (25.3%) P<0.001 RR: 0.28 (0.15, 0.52)	Lep: 12/214 (5.6%) Con: 6/75 (8.0%) P=0.58 RR: 0.70 (0.27, 1.80)	Lep: 41/214 (19.2%) Con: 30/75 (40.0%) P<0.001 RR: 0.48 (0.32, 0.71)	Lep: 33/214 (15.4%) Con: 5/75 (6.7%) P=0.072 RR: 2.31 (0.94, 5.71)	Lep: 0.97%	Pooled analysis of HAT-1, -2, and -3 studies; all patients tested positive for HIT antibodies; analysis from start of treatment
Case series (post-marketing study)									
Lubenow 2002	Lep: Bolus 0.4mg/kg; 0.15 mg/kg/h (12.1 days); mean infusion rate = 0.12 mg/kg/hr (no bolus, n=141)	Lep: 496	To end of treatment (mean, 12.1 days]	Lep: 26/496 (5.2%)	Lep: 29/496 (5.8%)	NR	Lep: 27/496 (5.4%)	Lep: 0.45%	77% of patients tested positive for HIT antibodies; thrombotic death rate = 1.8%; post-marketing study (lepirudin dose reductions began to be applied)
Lepirudin for treatment of HIT with isolated thrombocytopenia									
Lubenow 2004	Lep: 0.10 mg/kg/h [no bolus] (13.9 days) Con: Varied [nil, n=31; phenprocoumon, n=11; ASA, n=5]	Lep: 91/91 Con: 47/47	Lep: 24 days (median) Con: 15 days (median)	Lep: 4/91 (4.4%) Con: 7/47 (14.9%) P=0.045 RR: 0.30 (0.09, 0.96)	Lep: 3/91 (3.3%) Con: 0/47 (0%) P=0.55 RR: 3.65 (0.19, 69.27)	Lep: 18/91 (19.8%) Con: 14/47 (29.8%) ^{††} P=0.22 RR: 0.66 (0.36, 1.21)	Lep: 13/91 (14.3%) Con: 4/47 (8.5%) P=0.42 RR: 1.68 (0.58, 4.86)	Lep: 1.03%	Pooled data from subgroup of patients with isolated HIT from the three HAT studies; all patients tested positive for HIT antibodies

bores, three (3.3%) underwent limb amputation, and 13 (14.3%) died (Table 2). Most of the deaths were related to underlying disease, not to HIT or treatment with lepirudin. Since patients were counted only once if multiple events occurred, the incidence of the combined endpoint was 18/91 (19.8%). The median platelet count increased to $150 \times 10^9/l$ within four days of beginning lepirudin treatment. Episodes of major bleeding occurred in 13/91 (14.3%) of patients (risk for major bleeding per patient day, 1.3%). aPTT ratios above 2.5 and aPTTs > 60s were associated with an increased risk of bleeding ($p < 0.001$) (50). Nearly all patients with bleeding complications had impaired renal function.

ACS and percutaneous coronary intervention (PCI)

The ability of DTIs to inhibit clot-bound thrombin provides a strong rationale for their use as anticoagulants for ACS and PCI. Lepirudin was examined in large numbers of patients with unstable angina or suspected acute MI without ST-segment elevation in the OASIS-1 (51) ($n = 909$) and OASIS-2 ($n = 10,141$) trials (40). These trials concluded that lepirudin is superior to heparin in preventing ischemic outcomes. However, the OASIS-2 trial failed to reach a significant difference in the favor of lepirudin in the primary endpoint of cardiovascular death or new MI at day 7 (relative risk 0.84 [95% CI 0.69–1.02]; $p=0.077$), while the

combined events of cardiovascular death, new MI, or refractory angina at seven days were lower with hirudin compared with heparin 5.6% versus 6.7% (relative risk 0.82 [0.70–0.96]; $p=0.0125$). This benefit of efficacy was balanced by an excess of major bleeding requiring transfusion with hirudin compared with heparin (1.2% vs 0.7%; $p=0.01$). A meta-analysis of 11 ACS trials involving over 35,000 patients revealed a 15% reduction in death or MI when bivalent DTIs (lepirudin or bivalirudin) were used to treat ACS patients, compared with heparin (52). A retrospective subset analysis of the OASIS 2 trial examined the benefit of lepirudin in 117 ACS patients undergoing PCI within the first 72 h (53). Lepirudin was superior to heparin in reducing the risk of death or MI at 96 h ($p = 0.036$) and 35 days ($p = 0.02$). These studies underscore the concept that DTIs, with their ability to inhibit clot-bound thrombin, have greater therapeutic efficacy compared with heparin in the setting of thrombotic arterial occlusion. Based on these data, lepirudin would be an appropriate treatment option in an ACS patient with acute or previous HIT.

CPB and vascular surgery

Lepirudin was initially used to manage CPB surgery in patients with HIT (49, 54, 55) and has been investigated in a randomized pilot trial in 20 non-HIT patients undergoing CPB surgery in which lepirudin provided effective CPB anticoagulation, but was associated with higher postoperative blood loss compared with heparin (1.226 ± 316 ml vs. 869 ± 189 ml; $p = 0.007$) (56).

Lepirudin is an alternative for anticoagulation during CPB in patients with acute HIT, provided that ECT monitoring is performed (30, 57–62). A commercial ECT is not available, however. Neither the activated clotting time (ACT) nor the aPTT are appropriate for monitoring r-hirudin plasma levels in such high-dose situations.

Lepirudin levels should be maintained in the range of 3–4 $\mu\text{g/ml}$ (concentrations are given for whole blood) during surgery, which can be reached with 0.016–0.035 mg/kg/min (1.0–2.1 mg/kg/h). Elimination of the drug at the conclusion of CPB can be augmented through modified zero-balanced ultrafiltration and forced diuresis. However, patients with impaired renal function show prolonged elimination and are at increased bleeding risk.

For patients undergoing vascular surgery, the dosage of lepirudin should be adjusted depending on the perceived risk of reocclusion (63). In patients judged to be at low risk of reocclusion (e.g. surgery in the aortic, iliac, and carotid arteries), a bolus of 0.4 mg/kg (reduced in case of renal insufficiency) is given just before the vessel is clamped and is followed postoperatively by either an aPTT-adjusted infusion starting at 0.1 mg/kg/h or 15 mg injected sc twice daily (assuming normal renal function). In patients with an increased risk for reocclusion (e.g. undergoing calf-vessel reconstruction or bypass), a preoperative bolus of lepirudin (0.4 mg/kg [less in case of renal impairment]) should be administered, followed by a postoperative infusion of 0.1 mg/kg/h (aPTT-adjusted) for at least 3–4 days. For intraoperative flushing of the vessel during vascular surgery, up to 250 ml (0.1 mg/ml solution) of lepirudin can be used. As patients with acute HIT are at high risk for new thromboembolic compli-

cations, therapeutic levels of anticoagulation should be achieved before surgery and maintained after surgery, at least until platelet counts are normalized.

Haemodialysis

The first anticoagulant used for haemodialysis was hirudin, as performed by Haas in Germany in 1924. Because native hirudin preparations were impure and the supply of leeches was problematic, hirudin was later supplanted by heparin for haemodialysis anticoagulation.

Management of patients with renal failure requires careful dosing and frequent monitoring. Patients with transient renal failure are especially difficult to manage with hirudin, because frequent and substantial dose changes are needed. Hirudin can be given either by continuous iv infusion, typically starting at 0.005 mg/kg/h (with adjustments according to the aPTT) or by intermittent iv boluses of 0.005–0.01 mg/kg (64, 65). The latter regimen may be safer as it seems to cause less bleeding complications.

Drawbacks of hirudins

Dependency of pharmacokinetic from renal function

The very strong dependence of the pharmacokinetics of hirudin upon renal function is the most important drawback of the drug. This makes it very difficult to predict the appropriate dose, especially in elderly patients or patients with severe comorbidities. Major changes in drug dosing can be seen even in patients with transient renal impairment, which often occurs in critically ill patients.

Bleeding

In the prospective studies in patients with ACS (37, 38, 40) as well as in the HAT studies, bleeding was the most important adverse effect of lepirudin treatment. In the HAT studies, there were no major differences in bleeding rates between younger (<65 years of age) and older (≥ 65 years) patients ($p = 0.520$), or between female and male patients ($p = 0.150$). However, renal impairment was associated with an increased rate of bleeding, when comparing patients with serum creatinine values above and below 90 μM ($p < 0.001$) (48). In keeping with these prospective studies, Tardy et al. (66) reported in retrospective studies a rate of major bleeding similar to that seen in the HAT studies (20.4% in the French cohort and 17.6% in HAT 1–3), with moderate to severe impairment of renal function; prolonged treatment with lepirudin, as well as a mean dose exceeding 0.07 mg/kg/h, being independent risk factors for bleeding.

Lack of antidote

As with all DTIs, no specific antidote is available. In a patient with minor bleeding and normal renal function, stopping the drug usually suffices, since the drug concentration drops quickly. However, when bleeding is life-threatening or the patient has renal failure, cessation alone may not be adequate. Haemodialysis or haemofiltration can reduce plasma levels of lepirudin (54). However, only some filters are effective, e.g. polysulfone F80 (Fresenius, Germany) (67, 68). Variable efficacy of

filters in removing lepirudin could explain conflicting results (13). Clinical data are limited, and haemofiltration is not always a practical option in emergency situations.

Immunogenicity

The non-human protein hirudin can induce antibody formation in humans by both iv therapeutic-dose and sc prophylactic-dose use (47). Antibodies induced by lepirudin show 100% cross-reactivity to desirudin and *vice versa*. Although most data on antibodies are derived from studies with lepirudin, it is very likely that they apply also for desirudin-induced antibodies. About 40% of the hirudin-induced antibodies also react with bivalirudin, which shares several amino acids with hirudin. Antihirudin antibodies have been detected in 44 to 74% of patients treated with lepirudin (69–71). Of 196 HIT patients treated with lepirudin for five or more days, 44% developed antihirudin antibodies of the IgG class (71). Antibody formation occurred as early as day 4 and peaked at days 8–9 (71). Reexposed patients developed antibodies in about 70% of cases (48).

Antilepirudin antibodies were not associated with a decrease in efficacy (as measured by thrombin-antithrombin complexes) in the majority of patients. In fact, they usually result in increased efficacy of lepirudin as they extend the drug's half-life (72), most likely by reduced renal filtration of lepirudin-antilepirudin complexes. Only in about 2–3% of patients with antilepirudin antibodies an inhibitory effect is seen (73, 74). The biological effects of antilepirudin antibodies on anticoagulation can be easily compensated by changes in the lepirudin dose. Thus, ongoing daily aPTT measurements are recommended during lepirudin treatment, even when stable anticoagulation has been observed during the first five days.

Lepirudin administration during prospective studies in patients with HIT was associated with a low incidence of allergic events, as well as during the much larger clinical trials in patients with ACS. Among the adverse events reported were eczema, rash, pruritus, hot flushes, fever, chills, urticaria, bronchospasm, cough, stridor, dyspnea, angioedema (face, tongue, larynx), and injection-site reactions. Any causal relationship of lepirudin to these adverse events is unclear.

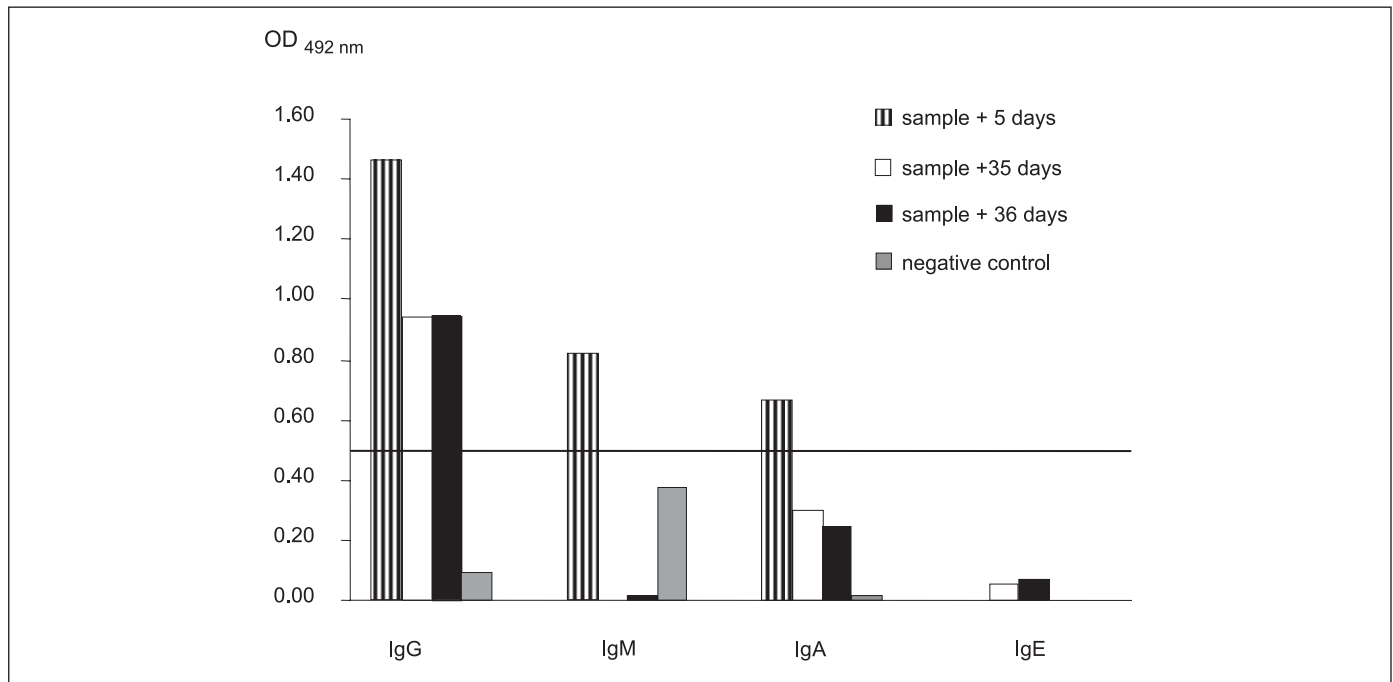


Figure 3: Anti-lepirudin antibodies in blood samples obtained 5, 35, and 36 days after clinical symptoms of anaphylactic reactions in a patient receiving lepirudin for chronic haemodialysis.

While strong reactivity for IgG antibodies and weak reactivity for IgM and IgA antibodies was found, there were no anti-hirudin IgE antibodies present. This indicates that anaphylaxis induced by lepirudin can be IgG mediated. The 82-year-old woman with end-stage renal insufficiency was started on chronic haemodialysis. Beginning on day 12, the haemodialysis sessions were complicated by thrombosis of the circuit, paralleled by platelet count decreases (nadir, $72 \pm 10^9/l$). HIT was confirmed serologically and subsequent dialyses were performed using lepirudin (0.04 mg/kg body weight at start of dialyses, monitored by ECT). Platelet counts normalized rapidly, but four weeks later the patient was readmitted because of bronchospasm, dyspnea, shivering attacks, thoracic pain, and hypertension during haemodialysis. These symptoms and signs had begun one week earlier, and invariably started at the commencement of the haemodialysis sessions, each event lasting approximately 6 h. Allergic

reactions to lepirudin were suspected, and anticoagulation during dialysis was switched to danaparoid-sodium (Organon, Organon, Oss, The Netherlands), without further events. Serum samples obtained five days after the last acute reaction revealed high-titre anti-lepirudin antibodies of the IgG-class, intermediate titre IgM-, low titre IgA-, but no detectable IgE antibodies in a solid-phase EIA for anti-hirudin antibodies (46, 71, 90). All other allergy tests remained negative or gave values in the normal range (total IgE, eosinophilic cationic protein, tryptase, histamine, specific IgE against latex, saccharomyces species, formaldehyde; scratch test and intracutaneous test, 15 min to 96 h: danaparoid, desirudin, bivalirudin; patch test 48 to 120 h with lepirudin, danaparoid, desirudin, and bivalirudin; also lymphocyte transformation test with bivalirudin at concentrations 5, 10, 20 and 30 $\mu g/l$ and lepirudin at concentrations 0.5, 1 and 2 $\mu g/l$ showed no increased stimulation of mononuclear cells (these assays were performed by Dr. Vetter, Department of Dermatology, University Hospital Magdeburg, Germany).

In an analysis in the year 2003, of approximately 35,000–60,000 patients treated with lepirudin, nine patients were judged to have had severe anaphylaxis in close temporal association with lepirudin use (46). All reactions occurred within minutes of iv bolus lepirudin administration, with four fatal outcomes (3 acute cardiorespiratory arrests, 1 hypotension-induced MI). In these four cases, a previous uneventful treatment course with lepirudin was identified (1–12 weeks earlier). The risk of anaphylaxis was estimated at 0.015% (5/32,500) in first-exposure and 0.16% (4/2500) in re-exposed patients (assuming 7.5% re-exposure frequency). One other case has been reported with recurrent anaphylaxis during re-exposure (75). We and others (76) demonstrated high-titer antihirudin antibodies of the IgG class, but not of the IgE class in patients with hirudin-associated anaphylaxis (Fig. 3). IgG-dependent anaphylaxis likely is Fc receptor-mediated, and is related to dose and rapidity of infusion. Thus, besides reducing bleeding risk, avoiding iv bolus administration of lepirudin should also reduce the risk of severe anaphylactic reactions. Two patients are reported with delayed reactions to hirudin. One patient developed eczematous plaques accompanied by a positive lymphocyte transformation test (77), the other had a granulomatous reaction (78). A third patient pro-

duced an Arthus-like reaction after intradermal application of lepirudin (79). Approaches to test for these reactions are reviewed in Bircher et al. (80).

Rebound hypercoagulability

Because of the relatively short half-life of lepirudin, and due to the severe hypercoagulability nature of HIT, there is the theoretical possibility that premature discontinuation of hirudin -while HIT-associated thrombin generation persists -could lead to „rebound“ thrombin generation. This is especially an issue when the drug may be held because of elevation of a global coagulation assay, such as the aPTT, in which a supratherapeutic aPTT level might not necessarily indicate a supratherapeutic drug level.

Figure 4 illustrates these concepts. This patient had HIT complicated by overt (decompensated) DIC. The patient initially did well soon after receiving the approved dosing regimen of lepirudin. However, the next three aPTT measurements – 109, 89, and 78 s -were all judged to be supratherapeutic. Thus, successive dose interruptions were made. When the patient was receiving only 0.019 mg/kg/h, the patient developed severe ischemic necrosis that ultimately resulted in amputations of four fingers and portions of both feet.

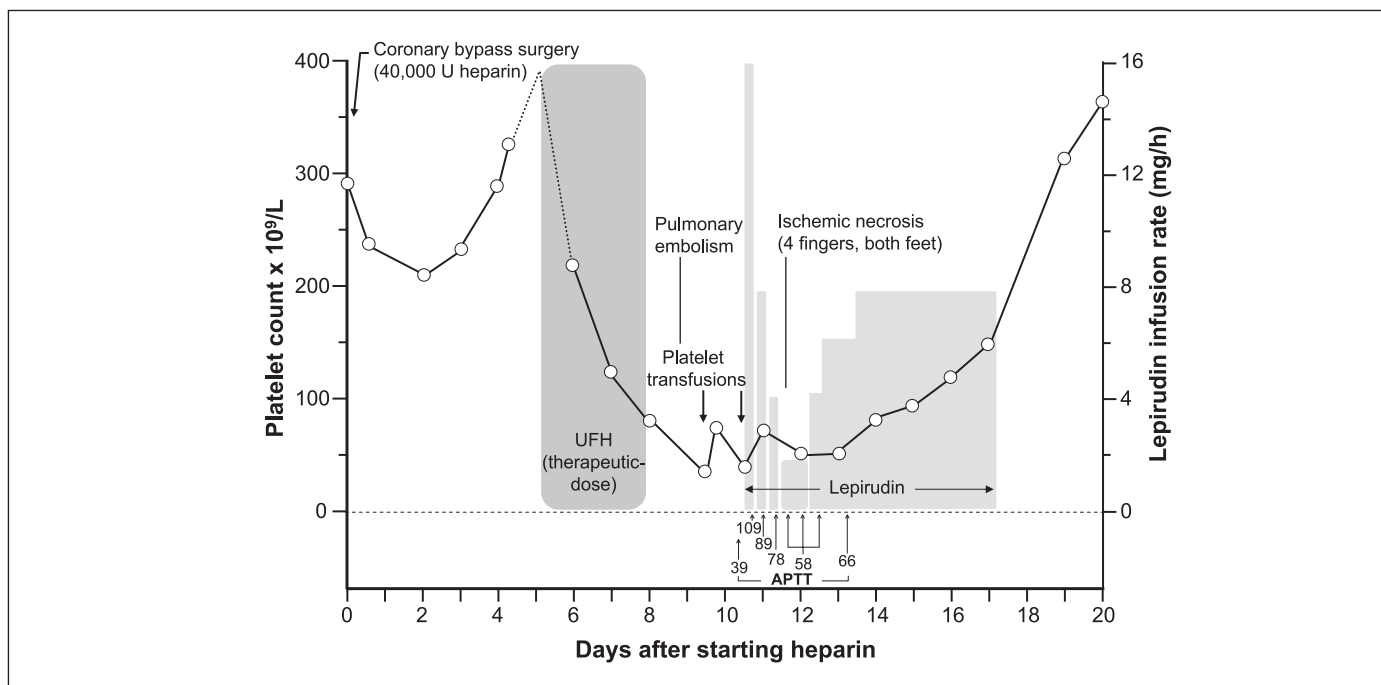


Figure 4: Multiple limb necrosis during lepirudin anticoagulation of HIT complicated by overt (decompensated) DIC.

A 56-year-old male with hypertension, diabetes, and obesity (107 kg) underwent quadruple coronary artery bypass surgery. On postoperative day 6 he received iv therapeutic-dose unfractionated heparin (UFH) because of atrial fibrillation and dyspnea. In addition, over the next three days, intermittent acrocyanosis of the fingers and toes was observed. On postoperative day 9, pulmonary embolism was diagnosed (confirmed by high-probability ventilation-perfusion scan). At this time, the platelet count was $40 \pm 10^9/l$, the INR was 2.1 (normal range, 0.9–1.2), the aPTT was 39 s (normal range, 22–33 s); the fibrinogen was 3.6 g/l (normal range, 1.5–4.5 g/l), and the fibrin split products were increased ($>80 \mu\text{g/ml}$; normal range, $<10 \mu\text{g/ml}$). The serum urea was mildly in-

creased (28 mg/dl; normal range, 9–20 mg/dl) but the serum creatinine was normal (1.2 mg/dl; normal range, 0.8–1.5 mg/dl). Lepirudin therapy was commenced at the FDA-approved regimen, namely an initial 43 mg bolus (given iv over 20 s) plus infusion at 16 mg/h ($\sim 0.15 \text{ mg/kg/h}$). As shown in the inset, three subsequent dose interruptions (2 h each) plus three dose reductions were made (each by 50%), until the patient was receiving only 2 mg/h ($\sim 0.019 \text{ mg/kg/h}$). After the third dose reduction, progression to severe multiple limb ischemic necrosis occurred, ultimately necessitating amputations of four digits (left hand), the left mid-foot, and the right forefoot. The patient case questions the appropriateness – at least in some patients – of monitoring by aPTT in the setting of HIT-associated DIC, and also raises the issue of “rebound” hypercoagulability (see text).

This patient case illustrates that concomitant overt DIC can make the aPTT a potentially unreliable monitoring tool. In such situations, assays which are independent of prothrombin should be used to monitor DTIs (see above). Another issue raised by this case is the potential for "rebound" hypercoagulability following interruption of the lepirudin because of an "elevated" aPTT value, particularly as the drug level falls below the therapeutic range. Given the extreme nature of HIT-associated hypercoagulability, this infers that hypercoagulability "rebound" in the context of HIT treatment (or, rather, interruption of such treatment) could lead to dramatic worsening of thrombotic events. Further indirect evidence supporting a role for „rebound“ hypercoagulability in HIT includes the well-known observation that risk of thrombosis in HIT is very high soon after discontinuation of heparin because of suspected HIT (81, 82), as well as the observation that a relatively high frequency of thrombotic events during therapy with the DTI, argatroban, occurred soon after discontinuation of this DTI (83).

Precautions

Bleeding

In the presence of renal failure, lepirudin must be started at a VERY LOW DOSE of 0.005–0.01 mg/kg/h, with very close anticoagulant monitoring. Even when renal function appears normal, the potential for unrecognized "compensated" renal dysfunction exists. Several studies—including secondary analyses of the approval studies of lepirudin for treatment of HIT (84) as well as other post-marketing studies (66) (85), have concluded that the approved dosing regimen is too high in many patients. Accordingly, the current recommendations of the ACCP Consensus Conference on Antithrombotic and Thrombolytic Therapy (January 2008) (86) are to avoid in most circumstances the initial lepirudin bolus and to begin with a lower iv infusion rate (between 0.05 to 0.10 mg/kg/h) (85), even when renal function

appears to be normal. In addition, it is recommended that the aPTT be measured at 4 h intervals until it is clear that a stable plateau has been achieved; thereafter, once-daily monitoring is appropriate.

Dependence of functional monitoring tests on prothrombin levels

In most centers, anticoagulant monitoring of hirudin therapy is accomplished using the aPTT. In prospective trials, lepirudin caused minimal prolongation of the prothrombin time (PT) (or INR) once the therapeutic range had been reached. Thus, an increased PT (or INR) in a patient receiving lepirudin infers either a concomitant coagulopathic process or a significant lepirudin overdose.

However, the aPTT can be prolonged in many clinical circumstances, including liver disease, DIC, antiphospholipid syndrome, and concomitant administration of VKAs (i.e. coumarins such as warfarin, acenocoumarol, and phenprocoumon). Indeed, the use of VKAs just before or together with administration of lepirudin can lead to markedly elevated aPTT values (due to the combined effects of coumarin-induced hypoprothrombinemia and hirudin) with the potential for inappropriate lepirudin dose reductions (87) (see Fig. 4). This is a serious issue because coumarin-associated microthrombosis syndromes (coumarin-induced venous limb gangrene or "classic" skin necrosis) are important complications of VKA therapy in a patient in the acute (thrombocytopenic) phase of HIT. Accordingly, 10–20 mg vitamin K should be given in the situation that a patient is recognized as having HIT after a VKA has already been given, and therapy with a DTI is planned or underway (88, 86). If prothrombin deficiency is suspected, hirudin treatment should be monitored by an assay independent of the prothrombin levels. This can be achieved by the chromogenic ecarin test and by a hirudin quantitation assay supplemented with prothrombin (see section on *Monitoring of DTI's*).

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