Direct Thrombin Inhibitors

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ABBREVIATIONS: ACT = activated clotting time; AT = antithrombin; DTI = direct thrombin inhibitor; EC = endothelial cell; ECT = ecarin clotting time; HIT = heparin-induced thrombocytopenia; INR = international normalized ratio; LMWH = low molecular weight heparin; PCI = percutaneous cardiac interventions; PT = prothrombin time; PTT = partial thromboplastin time; TAFI = thrombin activated fibrinolysis inhibitor; TF = tissue factor; UFH = unfractionated heparin.

INDEX TERMS: coagulation; thrombin inhibitors.

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DIRECT THROMBIN INHIBITORS

The direct thrombin inhibitors (DTIs) argatroban, hirudin, and ximelagatran form the newest class of anticoagulants. Unlike heparin, which depends on antithrombin (AT) to inhibit thrombin activity, and unlike Coumadin®, a vitamin K antagonist that attenuates thrombin production by reducing the activity of coagulation factors II, VII, IX, and X, the DTIs neutralize thrombin directly by occupying its catalytic or its fibrinogen binding sites or both.

In clinical trials most DTIs match or exceed the clinical efficacy of heparin and Coumadin while creating no additional hemorrhagic risk. They require little laboratory monitoring, and when monitoring is necessary, the partial thromboplastin time (PTT) or the ecarin clotting time (ECT, snake venom derived) are used.[1] The activated clotting time (ACT) may also be used during heart surgery or percutaneous cardiac interventions (PCI).

The DTIs provide crucial alternatives for patients with heparin-induced thrombocytopenia with thrombosis (HIT) and are beginning to replace heparin and Coumadin in other prophylactic and therapeutic applications.[2]

Thrombin

Injuries to veins or arteries expose subendothelial collagen and tissue factor and release von Willebrand factor. The collagen and von Willebrand factor combine to activate platelets, releasing platelet-stored coagulation factors, while tissue factor activates the coagulation mechanism to generate the protease thrombin from prothrombin (Figure 1).[3] Thrombin activates additional platelets, activates factor V, VIII, and XI, cleaves fibrinogen to form fibrin polymer, triggers the protein C control protein pathway, activates thrombin activated fibrinolysis inhibitor (TAFI), triggers inflammation, and stimulates cellular proliferation (Figure 2).[4] Thrombin binds to fibrin polymer in clots and binds to the subendothelium but continues to activate platelets and factors V, VIII, and XI, causing thrombus growth.[5] Likewise, platelet-bound factor Xa, complexed with cofactor Va, actively generates thrombin.

Limitations of heparin

Heparin has been used as an effective anticoagulant for over 50 years. As described in the companion Focus Section article, *Unfractionated Heparin and Other Antithrombin Mediated Anticoagulants* by Brian K Adler, heparin is a catalyst

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that binds and activates plasma AT. The heparin-AT complex inactivates all the serine proteases of the coagulation system except factor VII (Figure 3). Heparin is available in the unfractionated form (UFH, standard heparin), the fractionated low molecular weight form (LMWH), the now discontinued synthetic heparinoid, and, since 2002, in the pentasaccharide form. In all four forms heparin acts through AT to neutralize serine proteases, however UFH acts on all the proteases including thrombin while the others act primarily on factor Xa.

UFH activity differs by formulation, even by lot, and binds several plasma and cellular proteins, the concentrations of which vary among patients. The half-life and anticoagulant response are unpredictable, thus UFH must be monitored carefully using the PTT or chromogenic anti-Xa heparin assay to maintain a safe therapeutic level. LMWH and pentasaccharides have more predictable kinetics, however

Figure 1. Upon injury, tissue factor (TF) is exposed and activates factor VII. Factor VIIa activates factor X, Xa complex with Va then activates II to form thrombin (IIa). VIIa also activates IX. Thrombin activates factor XI, XIa activates X, enhancing the common thrombin activation pathway. Factor XII is activated in vitro and in turn activates factor XI. Platelets provide many of the coagulation factors and their phospholipid assembly points.

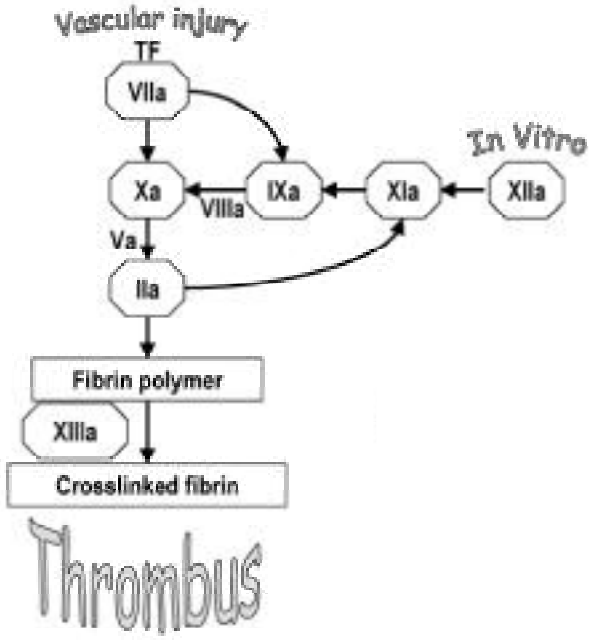


Figure 2. Thrombin is a robust protease with multiple properties. Besides digesting fibrinogen to promote fibrin polymerization, thrombin activates platelets and factors XIII, XI, VIII, and V to enhance coagulation. Thrombin binds thrombomodulin to activate the protein C coagulation control pathway, activates thrombin activatable fibrinolysis inhibitor (TAFI), and activates endothelial cell (EC) mitogens and inflammatory proteins such as platelet activating factor (PAF).

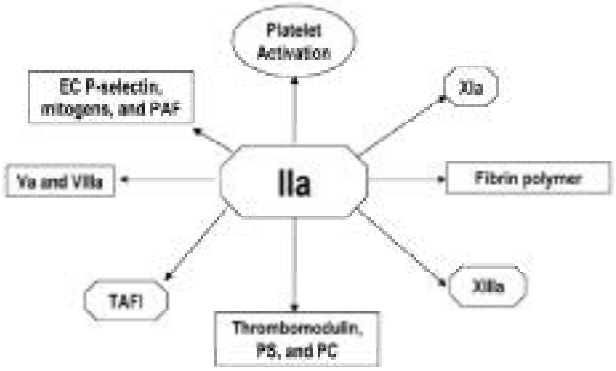
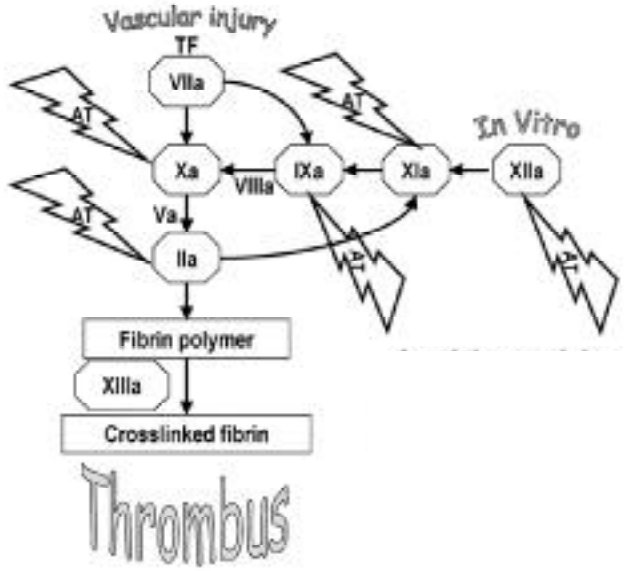


Figure 3. Heparin catalyzes the activation of antithrombin (AT), which binds and neutralizes all the coagulation pathway serine proteases except factor VIIa. Unfractionated heparin (UFH) has its greatest effect upon thrombin (IIa), low molecular weight heparin (LMWH) on Xa.

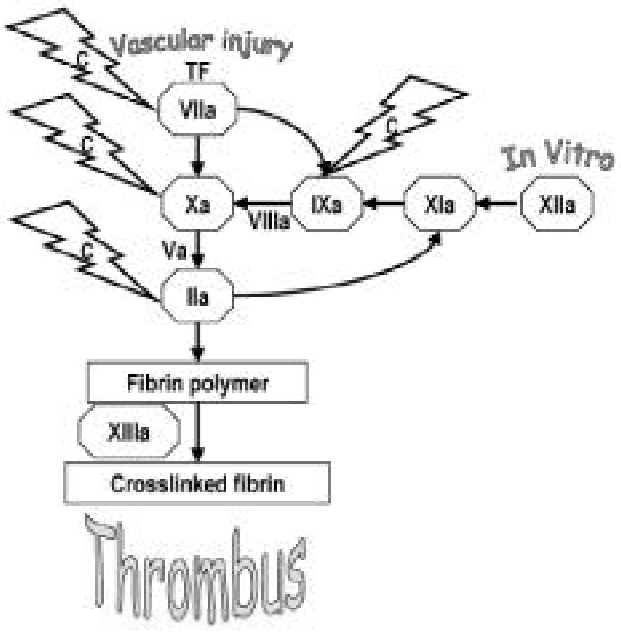


they, like unfractionated heparin, are variably neutralized by platelet factor 4 released during clot formation. Further, heparin-AT complexes are sterically hindered from binding platelet-bound factor Xa or fibrin-bound thrombin, allowing these to continue thrombus propagation.[6]

Limitations of Coumadin

As described in the companion Focus Section article by David McGlasson, *Oral Anticoagulants*, Coumadin is a vitamin K antagonist that interferes with the production of active clotting factors II, VII, IX, and X, a process that requires two to seven days to reach a stable therapeutic level (Figure 4). Coumadin is metabolized by the microsomal enzymes of the liver including cytochrome P450. At least 100 drugs interfere with the activity of this enzyme, rendering the effect of Coumadin unpredictable.[7] Further, high-prevalence mutations (30% to 40%) of the P450 gene reduce its Coumadin-metabolizing activity, rendering the patient sensitive to small doses. Coumadin is dangerous, accounting for 1.1 major hemorrhages and 0.25 deaths per 100 patient years.[8] Hemorrhage risks rise to 50% in patients older than 65 who have a history of recent gastrointestinal bleeding, cerebrovascular accident, myocardial infarction, renal disease, anemia, or diabetes.[9] When the Coumadin dosage fails to achieve a therapeutic international normalized ratio (INR) through non-compliance, mismanagement, or interference, the risk of rethrombosis is high. Consequently, Coumadin therapy must be monitored with monthly prothrombin time assays to maintain a therapeutic INR between 2 and 3.

Figure 4. Coumadin interferes with normal vitamin K-dependent production of coagulation factors II, VII, IX and X. During Coumadin therapy, inactive decarboxy-II, VII, IX, and X are produced, reducing thrombin generation.

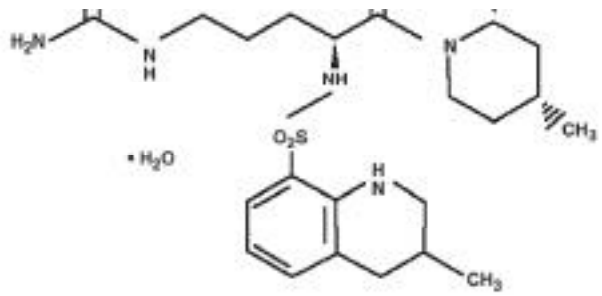


Argatroban (Novastan®)

The DTIs employ neither the AT nor the cytochrome P450 systems.[10] Argatroban, trade name Novostan (Glaxo-Smith Kline), is an l-arginine derivative that competes directly and reversibly for the active serine site of thrombin (Figure 5). Sterically unhindered because its molecular weight is 527 daltons, Argatroban neutralizes the active serine catalytic site of both free and bound thrombin (Figure 6).

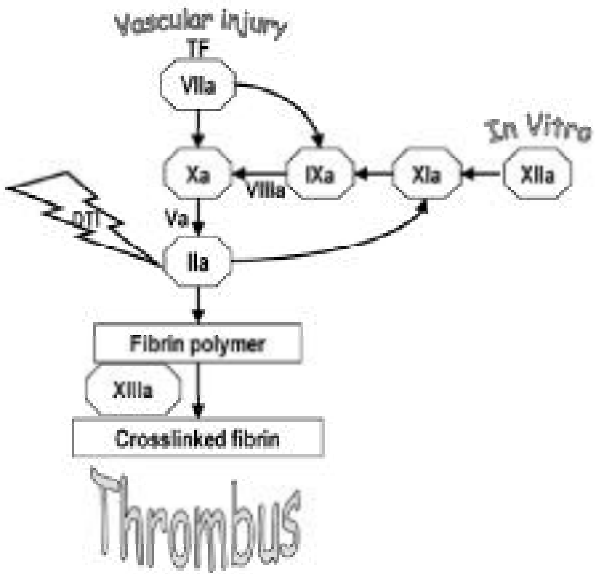
Argatroban is approved for both treatment and prophylaxis in patients with HIT and may be used during PCI such as angioplasty. Argatroban is administered intravenously at 2 mg/kg/h and its dosage is monitored using the PTT (Table 1). If heparin is in use, it must be discontinued long enough for an accurate baseline PTT, which must be obtained prior to initiating argatroban therapy. The therapeutic range for argatroban is 1.5 to 3 times the baseline PTT or the mean of the PTT reference interval (MRI), performed on citrate plasma collected two hours after initiation of therapy. If the dosage is changed in response to the PTT a new PTT is required two hours later to insure the therapeutic range is reached. The PTT is linear up to 40 mg/kg/min. Monitoring is particularly necessary for patients with liver disease, as the liver is the primary metabolic and excretory site. In liver disease, the dosage may be as low as 0.5 mg/kg/h. When used during PCI, Argatroban dosage may be monitored using the ACT with a target of 300 to 450 seconds.[11]

Figure 5. Argatroban is an L-arginine derivative, MW 527 D, that binds the active serine site of thrombin.



The PTT loses validity when prolonged by a coagulation factor deficiency, a plasma factor inhibitor, or a lupus anticoagulant. In instances where the baseline PTT is prolonged, the ecarin clotting time (ECT) may be substituted. The ECT employs an enzyme derived from the venom of the snake *Echis carinatus*. Ecarin cleaves patient or control plasma prothrombin to yield a moderately active intermediate called *meizothrombin*. DTIs inhibit meizothrombin, whereas heparin-AT does not. The ECT reagent is unaffected by coagulation factor deficiencies, coagulation factor inhibitors, and lupus anticoagulant, and the normal range is 30.9 to 54.9 seconds.[12] An ECT therapeutic range for Argatroban therapy is being established. The ECT is available as a whole blood point-of-care assay classified as a "humanitarian use device" from Pharmanetics Inc and as a plasma-based reference assay from coagulation specialty and reference laboratories.

Figure 6. The direct thrombin inhibitors bind and inactivate thrombin independent of antithrombin and the vitamin K pathway.



Like the PTT, ACT, and ECT, the prothrombin time (PT) and thrombin time are prolonged in proportion to Argatroban dosage. The PT and thrombin time are not linear in practical ranges, the PT being insensitive and the thrombin time overly sensitive to Argatroban. Because Argatroban prolongs the PT, it is necessary when transitioning from Argatroban to Coumadin to aim for an INR of about 4 for the period in which the patient is receiving both. The following formula may also be used, assuming Argatroban at 2 µg/kg/min and thromboplastin (PT reagent) with an ISI near 1:

$$INR_W = 0.19 + 0.57\ (INR_{WA})$$

Where:

INR_W: INR for warfarin (Coumadin) alone, and
INR_{WA}: INR for warfarin + Argatroban

Example:

$INR_{WA} = 4.1$, then $INR_W = 0.19 + 0.57\ (4.1) = 2.53$

Table 1. Direct thrombin inhibitors (DTIs) characteristics

DTI	Dosage	Half-life	PTT target	Comment
Argatroban	2 mg/kg/h	39 – 51 m	1.5 – 3.0 x MRI	Approved for use during PCI, maintain ACT 300 – 450 seconds
Lepirudin	0.4 mg/kg bolus 0.1 – 0.15 mg/kg/h	20 – 30 m	1.5 – 3.0 x MRI	Reduce dosage in renal insufficiency
Bivalirudin	1 mg/kg bolus 2.5 mg/kg/h x 4h 0.2 mg/kg/h to 20 h	24 m	None	Reduce dosage in renal insufficiency
Ximelagatran	24 – 36 mg BID	2.5 – 3.5 h	None	Up to 60 mg BID for therapy

Other formulas apply for less sensitive thromboplastins, for example, with an ISI near 2 the formula is:

$$INR_W = 0.18 + 0.45\ (INR_{WA})$$

Heparin may be rapidly reversed by protamine sulfate and Coumadin may be reversed in four to six hours by intravenous vitamin K administration. There are no non-biological rapid reversal agents for the DTIs. Fresh frozen plasma, prothrombin complex concentrate, or activated factor VII, NovoSeven, may be used to counteract an overdose in threatened hemorrhage. However, DTI half-lives are short in the absence of renal or liver disease (Table 1).

Hirudin (Lepirudin)

Hirudin is a medicinal leech (Hirudo medicinalis) saliva anticoagulant that is the prototype direct thrombin inhibitor. Synthetic hirudin, generic name Lepirudin and trademarked Refludan® by manufacturer Hoechst, mimics Hirudin and was released in 1998 for prophylaxis and therapy in patients with HIT. Lepirudin is composed of 65 or 66 amino acids and has a molecular weight of 7000 daltons. Lepirudin is bivalent; the carboxyl terminus binds the fibrinogen-binding site of thrombin while the amino terminus binds the catalytic serine site. Lepirudin inactivates free thrombin only; it is too large to reach the active sites of bound thrombin.

Lepirudin is given intravenously beginning with a 0.4 mg/kg bolus and followed by 0.1 to 0.15 mg/kg/h for two to ten days (Table 1). The dosage of Lepirudin is monitored using the PTT, maintaining a therapeutic range of 1.5 to 2.5 times the baseline PTT or the mean of the normal range, taken four hours after initial administration. The PTT is linear up to 0.6 mg/L as measured by immunoassay. Lepirudin functional activity may also be monitored using the ACT or the ECT (see the discussion of these assays in the Argatroban section). Lepirudin achieves therapeutic levels immediately upon administration and has a half-life of 20 minutes. The drug is retained in renal disease, requiring dose reduction proportional to serum creatinine elevation or creatinine clearance decrease, and daily laboratory monitoring. Lepirudin has no effect on the PT and may be coadministered with Coumadin.

At least 40% of patients develop antibodies to Lepirudin that prolong its clearance without abrogating its activity.[13] There have been seven reports of anaphylactic shock traced to secondary Lepirudin therapy; five were fatal.

Bivalirudin

The synthetic bivalirudin, trade name Angiomax™ (The Medicines Company) is a 20-amino acid analog of the carboxyl terminus of hirudin linked to a second peptide that binds the active site of thrombin, yielding a MW of 2180 daltons. Like Lepirudin, Bivalirudin's half-life is 24 minutes, but unlike Lepirudin, it binds both free and fibrin-bound thrombin. Bivalirudin is retained in renal disease, requiring dose reduction proportional to creatinine level. Bivalirudin has not been shown to induce antibody formation, and is not expected to, given its diminutive size. Bivalirudin may be monitored using the PTT or the ECT.[14]

Both Lepirudin and Bivalirudin have been used in PCI and monitored using the point-of-care ECT method using the Thrombolytic Assessment System®, Pharmanetics Inc. In this method, equal volumes of patient citrated plasma and normal plasma (to provide normal prothrombin levels) are mixed and a 30 mL aliquot is transferred to the test card. Plasma concentrations of four to five mg/mL of Lepirudin or 10 to 15 mg/mL of Bivalirudin both prolong the ECT to 400 to 450 seconds.

Melagatran and Ximelagatran

Melagatran is a dipeptide synthesized to mimic the thrombin-binding sequence at the cleavage site on the Aa chain of fibrinogen. Ximelagatran, trade name Exanta™, developed by Astra-Zeneca, is an oral formulation that converts to Melagatran after ingestion. The half-life is 2.5 to 3.5 hours and it is cleared unchanged by the kidney. The half-life makes twice-a-day dosing necessary, and dosages must be modified in renal disease with elevated serum creatinine concentrations. Melagatran inhibits both free and clot-bound thrombin.[15]

A proposed dosing regimen consists of oral Ximelagatran at 24 or 36 mg BID for an extended period. Clinical trials for prophylaxis following general surgery and orthopedic surgery have been concluded and the drug is currently being evaluated for treatment of venous thrombosis and for stroke prevention in atrial fibrillation.

Provided all safety requirements are met, ximelagatran may be released in 2004. It will be the first oral anticoagulant since Coumadin, developed over 50 years ago. Melagatran plasma levels are linear with PTT results, should monitoring be desired.

The ideal anticoagulant

The ideal anticoagulant is effective at preventing thrombosis, has a low rate of bleeding, has no long-term side effects such as osteoporosis, may be orally administered, is free of interference from drugs and diet, bypasses the P-450 cytochrome system, and has predictable kinetics. It has a wide therapeutic window, requires no laboratory monitoring, has a short half-life for acute episodes, a long half-life for prophylaxis, is safe during pregnancy, and is inexpensive. These criteria may not be readily met in our lifetime, but each succeeding generation of anticoagulant drug more closely approaches the ideal, and with market-driven cost reduction, the DTIs may come close.

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