Direct Thrombin Inhibitors for Anticoagulation

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OBJECTIVE: To review the progress in developing direct thrombin inhibitors (DTIs) for anticoagulation within the context of existing anticoagulation therapies.

DATA SOURCES: Searches of MEDLINE (1993–June 2003) were conducted.

STUDY SELECTION AND DATA EXTRACTION: We examined English-language articles, human studies, and relevant animal studies, and obtained additional citations from the references of these articles.

DATA SYNTHESIS: Because of its pivotal role in hemostasis, thrombin is a key therapeutic target in the treatment and prevention of thromboembolic disorders. Conventional anticoagulant therapies, such as warfarin, unfractionated heparin, and low-molecular-weight heparin, exert their pharmacologic action by indirect thrombin inhibition. Although these agents are effective, each has limitations, prompting a search for more effective, specific, better-tolerated, and convenient anticoagulants. The efficacy and safety of factor Xa inhibitors are being investigated. Furthermore, the development of DTIs such as recombinant hirudin (lepirudin), bivalirudin, and argatroban continues. Challenges in the development of DTIs include establishing a binding affinity for thrombin that is not associated with excessive bleeding, attaining high thrombin specificity, achieving inhibition of both unbound and clot-bound thrombin, and producing an effective, fixed-dose oral anticoagulant to improve the practicality of anticoagulation therapy. Ximelagatran, an oral DTI designed to meet these standards, is currently in Phase III clinical trials.

CONCLUSIONS: Significant progress has been made in developing DTIs. The recent emergence of orally administered DTIs may simplify the prevention and treatment of thrombosis.

KEY WORDS: anticoagulants, direct thrombin inhibitors, thrombosis.

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Coagulation and platelet aggregation are the body's primary defenses against bleeding from vascular injury. Coordinated responses by coagulation enzymes and platelets form an insoluble clot. However, inappropriate thrombosis results in significant morbidity and mortality from disorders including venous thrombosis, pulmonary embolism, myocardial infarction (MI), and stroke. Anticoagulation therapies indicated for the prevention and/or treatment of these conditions modify the coagulation system. Thrombin is the key effector enzyme responsible for the final step in thrombus formation,[1] and most anticoagulant medications inhibit thrombin generation or activity.

The coagulation system is closely regulated, and thrombin generation is limited under basal conditions.[2] However, after the coagulation system is triggered, the amount of thrombin present at the injury site increases rapidly. The extrinsic pathway of the coagulation system is triggered by the exposure of tissue factor released from injured tissues to blood. Following binding with factor VIIa, the tissue factor/factor VIIa complex converts factor X to factor Xa (Figure 1).[2] In the presence of calcium ions and a phospholipid surface (provided by activated platelets), factor Xa

which converts prothrombin to thrombin. Thrombin catalyzes the proteolytic cleavage of fibrinogen to form fibrin monomers, which subsequently polymerize. Stabilization of the resultant fibrin polymers is facilitated by the thrombin-mediated activation of factor XIIIa, forming an insoluble gel, which, together with aggregated platelets, comprises the hemostatic plug.

Thrombin amplifies its own generation, resulting in a burst of thrombin activity. Once a small amount of thrombin is present, more is produced via the intrinsic pathway by thrombin activation of factors XI and VIII, leading to formation of the tenase complex. Thrombin also stimulates the extrinsic pathway by activating factor V and accelerating formation of the prothrombinase complex (Figure 1).[1] It also potently activates platelets,[3] thereby creating the phospholipid surface on which these reactions occur.

Once present, thrombin has several antifibrinolytic functions, including activation of factor XIII, which crosslinks fibrin strands and contributes to crosslinking of α-2 antiplasmin (the principal inhibitor of plasmin) to fibrin, and activation of thrombin-activatable fibrinolysis inhibitor. Additionally, thrombin participates in negative feedback loops to maintain hemostasis by activating protein C, which inactivates factors Va and VIIIa.[4]

Traditional Anticoagulant Therapies

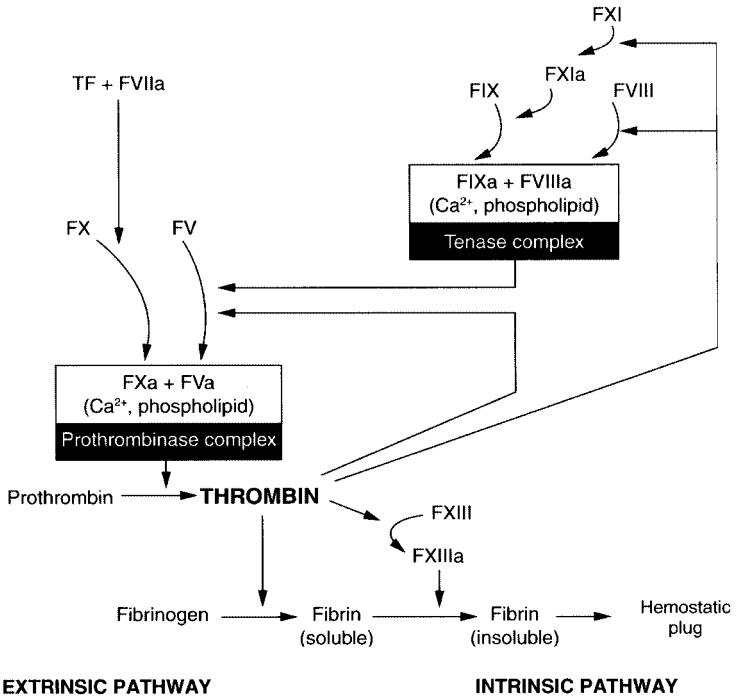
Traditional anticoagulants are indirect thrombin inhibitors: warfarin, unfractionated heparin (UFH), and low-molecular-weight heparins (LMWHs). The Food and Drug Administration–approved indications for these medications are described in Appendix I.[5]

WARFARIN

Warfarin interferes with vitamin K–dependent carboxylation of several coagulation factors including prothrombin (factor II), VII, IX, and X, as well as the anticoagulant proteins C and S.[6] Warfarin's full anticoagulant effect may not be achieved until >5 days after treatment initiation or any change in dose because depletion of the active coagulation factors is gradual. Therefore, additional anticoagulant therapy, typically UFH or an LMWH, is required during the initiation of therapy and periods of significant underanticoagulation.[7]

Patient response to warfarin varies considerably. The average daily dose to maintain patients within the appropriate therapeutic range is 4–5 mg, but can range from <1 to >20 mg/day. Age, hepatic function, underlying disease states, and patient-specific metabolic characteristics (ie, cytochrome P450 isoenzymes) influence dosing requirements.[8] Interactions with dietary vitamin K, numerous medications including herbal and other natural products, and lifestyle issues also influence patient response to warfarin.

Warfarin treatment is associated with a significant risk for bleeding, with a cumulative incidence at 48 months ranging from 3% in low-risk patients to 53% in highest-risk patients.[9] A portion of this risk is due to the narrow therapeutic window associated with warfarin, which requires that therapy be carefully maintained within an appropriate therapeutic range, monitored using the international normalized ratio (INR). The need for frequent testing and dose adjustments detracts from warfarin's ease of use in clinical practice.[7,8] Warfarin activity can be reversed by administration of vitamin K or infusion of coagulation factors.



HEPARINS

UFH is a heterogeneous mixture of glycosaminoglycans. Because of the size heterogeneity of heparin preparations (3000–30 000 Da; mean 15 000), only one-third of the heparin molecules in a given preparation exhibit anticoagulant activity.[10] UFH exerts its anticoagulant effect via antithrombin. Heparin binds to and produces a conformational change in antithrombin, converting it into a rapid inhibitor of thrombin (factor IIA) and factor Xa.

UFH has a short half-life (~1 h) and must be administered either subcutaneously or via continuous infusion.[10] It is heavily sulfated and therefore has a high negative-charge density that promotes nonspecific binding to several plasma and cellular proteins. This results in decreased bioavailability, substantial interpatient variability in anticoagulant response, and an increased potential for bleeding and thrombotic complications due to heparin–platelet interactions. Therefore, when given in therapeutic doses, UFH requires frequent laboratory monitoring

by activated partial thromboplastin time (aPTT). Heparin requires frequent dosage adjustments, limiting its ease of use in clinical practice.

Although heparin can be reversed by protamine, its use is complicated by the potential for bleeding. In addition, heparin-induced thrombocytopenia (HIT), which may occur in up to 5% of exposed patients, causes significant venous and arterial thrombosis and is associated with a high mortality rate.[10] Osteoporosis can also develop in long-term users.

The development of LMWHs in the late 1970s resulted from the discovery that shorter heparin chains (3800–5000 Da) sufficiently enhance the anti-factor Xa activity of antithrombin. LMWHs are derived by the chemical or enzymatic depolymerization of UFH and inactivate thrombin to a lesser extent than UFH because smaller molecular fragments cannot bind thrombin and antithrombin simultaneously. Therefore, LMWHs inhibit factor Xa more than they inhibit thrombin.[10] They have better bioavailability and a longer half-life than UFH and are administered subcutaneously either once or twice daily, which is convenient in clinical practice. LMWHs display reduced binding to plasma and cellular proteins and have a more predictable dose response than UFH. Thus, monitoring anticoagulation intensity and dose adjustments are generally unnecessary. Protamine cannot be used for adequate reversal because it only partially reverses LMWH. HIT type II occurs much less often with LMWH than with UFH. However, LMWH crossreacts with antibodies against UFH and should not be given as an alternative anticoagulant in patients with new-onset HIT or a history of HIT.

Recent research has investigated the feasibility of delivering UFH or LMWH orally via a delivery agent, such as sodium *N*-[8(-2-hydroxybenzoyl) amino] caprylate (SNAC)[11] or conjugates of deoxycholic acid (DOCA).[12] SNAC-heparin recently failed to show better efficacy than enoxaparin in a Phase III trial of 2264 patients.[13] Poor adherence in the SNAC-heparin group due to the poor taste of the liquid preparation may have affected these results. Current research is directed at developing improved oral formulations.

Targeted Anticoagulation

Potential targets within the coagulation system are being explored to develop anticoagulant therapies with improved effectiveness, safety, and ease of use. The anticoagulants in development generally seek specific molecular targets for a predictable anticoagulant effect. This mechanism may avoid the need for monitoring and dose adjustment. Significant progress has recently been made in the development of factor Xa inhibitors and direct thrombin inhibitors (DTIs), both of which ultimately prevent thrombin production.

FACTOR XA INHIBITION

Fondaparinux is a synthetic version of the pentasaccharide sequence that binds to antithrombin and modifies its conformation, inhibiting factor Xa.[14] Fondaparinux is administered by subcutaneous injection and does not require anticoagulation monitoring due to its predictable pharmacokinetic profile and stable dose response. It has a long half-life (17–21 h), allowing once-daily administration,[14-16] and is renally excreted. It has no known antidote. Due to the lack of hepatic metabolism, fondaparinux is not responsible for drug interactions mediated by cytochrome P450.[17] Unlike the heparins, fondaparinux does not affect platelet function, nor does it inhibit platelet aggregation stimulated by various agonists.[16,18,19] In vitro studies suggest that fondaparinux does not react with heparin platelet factor 4 antibodies, thus potentially eliminating the risk of HIT.[18,19] This observation has not been confirmed in vivo; additional studies are needed to elucidate the potential of using fondaparinux in patients with HIT.

Fondaparinux was approved in the US for the prevention of venous thromboembolism (VTE) following hip and knee replacement and hip fracture surgeries (Appendix I). Ongoing studies may support its use for additional indications.[20,21]

Additional factor Xa inhibitors are in development. Idraparinux, a compound closely related to fondaparinux that can be given by injection once weekly, has completed Phase II trials in patients with proximal deep vein thrombosis (DVT).[21] Direct factor Xa inhibitors for parenteral delivery in early development include FXV673, RPR130737,[22] ZK807834 (CI-1031),[23] M55113,[24] SF303, and SK509.[25] Oral direct factor Xa inhibitors, such as DX-9065A,[26] DPC423,[27] and JTV-803,[28] are also being investigated.

DIRECT THROMBIN INHIBITION

As thrombin is the central effector of coagulation and amplifies its own production (Figure 1), it is a natural target for pharmacologic intervention. Several DTIs are in clinical use (Appendix I). Acronyms for clinical trials are defined in Appendix II.

DTIs target sites on the thrombin molecule responsible for substrate recognition and/or cleavage (Figure 2).[29] The substrate recognition site (exosite 1) acts as a docking station, binding thrombin to fibrinogen prior to its enzymatic actions. The catalytic site (active site) is responsible for the enzymatic actions of thrombin, including activation of platelets and cleavage of fibrinogen for thrombus formation.[30] By blocking either the active site alone or both the active site and exosite 1, DTIs specifically inhibit thrombin activity.

Conversely, heparin-activated antithrombin binds to the active site of thrombin, but also blocks the fibrin-binding site (Figure 1). Thus, when thrombin and fibrin are already bound, which occurs within a fibrin clot, heparin is unable to inactivate thrombin.[31] Because DTIs do not bind to the fibrin-binding site, they can bind both unbound and fibrin-bound thrombin, preventing the dual processes of thrombus initiation and propagation. Based on these pharmaco-

anticoagulants. In addition, they are not inhibited by platelet factor 4 or associated with the development of HIT.

Properties of an ideal DTI include high selectivity for thrombin, rapid onset/offset of action, predictable pharmacokinetics and pharmacodynamics, lack of drug interactions, a wide therapeutic window to prevent thrombosis while minimizing bleeding, inhibition of unbound and clot-bound thrombin, and oral administration.

Hirudins

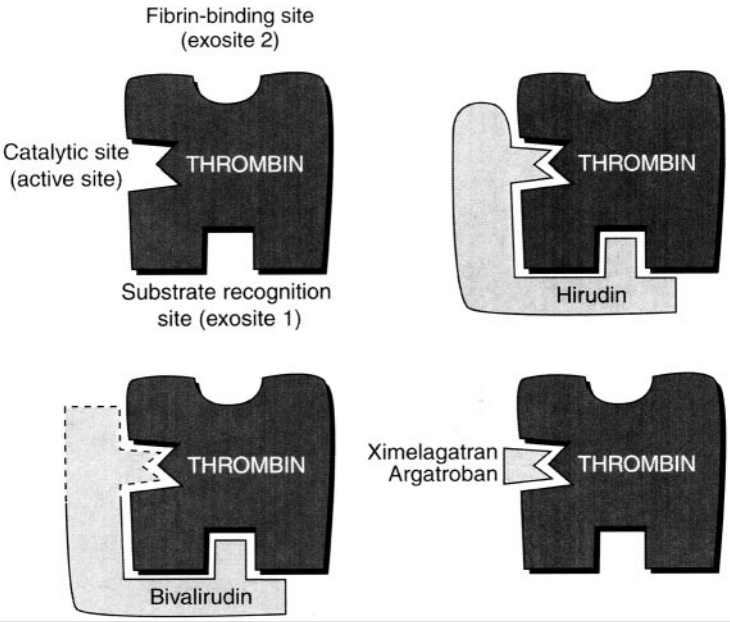
The first DTI to become available was hirudin, a 65-amino-acid polypeptide (7000 Da) originally obtained from the salivary glands of the medicinal leech (*Hirudo medicinalis*). Although hirudin is not commercially available, derivatives produced by recombinant technology have been developed. Lepirudin is available in the US (Appendix I), and desirudin has been investigated in Europe. Hirudins are potent and specific thrombin inhibitors,[32] forming a stoichiometric and very slowly reversible complex by binding to both the active site and exosite 1 of the thrombin molecule (Figure 2). As a result of this bivalent binding, hirudins are the most potent inhibitors of thrombin. The potency of inhibition is expressed as the inhibition constant, which is the dissociation constant for the inhibitor-enzyme complex. Smaller inhibition constants (little dissociation) are associated with stronger inhibition. The inhibition constant for hirudin is 0.1–2.3 pmol/L.

Due to the almost irreversible nature of the bond between lepirudin and thrombin, bleeding problems have been associated with lepirudin treatment. In a meta-analysis of studies in patients with acute coronary syndrome (ACS), hirudin was associated with more major bleeding than heparin (1.7% vs 1.3%; OR 1.28; 95% CI 1.06 to 1.55).[33] Since no antidote is available to reverse the effect of hirudin or its derivatives, irreversible binding to thrombin is considered one of its major shortcomings.

Lepirudin has a short plasma half-life of approximately 40 minutes after intravenous administration and 120 minutes when given subcutaneously. Elimination of lepirudin is primarily renal; therefore, doses must be adjusted according to patients' renal function. The dose should be monitored and adjusted to an aPTT ratio of 1.5–2.5 because the bleeding risk increases above this range with no increase in efficacy.[34]

Clinical trial data have shown that lepirudin can benefit patients with HIT and thrombosis. In the HAT-1 trial of 82 patients, the combined endpoint of new thromboembolic complications, limb amputation, and death at day 35 was 25.4% in lepirudin-treated patients versus 52.1% in historical control patients given no anticoagulation or warfarin (p = 0.014). A smaller difference was seen in HAT-2 (n = 112), with 31.9% of treated patients experiencing the triple endpoint compared with the same control group (p = 0.15). No significant difference in the need for blood transfusion was observed (9.9% in HAT-1, 12.9% in HAT-2, 9.1% in the control group).[35]

The results of a meta-analysis of 6 trials using various forms of hirudin (total of 28 545 patients) also showed that hirudin significantly reduces the risk of death or MI compared with heparin in patients with ACS (OR 0.81, 95% CI 0.73 to 0.91).[33] Furthermore, the results of a clinical trial of 1587 patients showed that desirudin 15 mg given subcutaneously twice daily is more effective than enoxaparin 40 mg given subcutaneously once daily in preventing DVT in patients after total hip replacement (4.5% vs 7.5%; p = 0.01; RR = 40%) and has a similar safety profile.[36] A small trial (n = 121) addressing the efficacy of recombinant hirudin in preventing pulmonary embolism also demonstrated that significantly fewer patients given hirudin developed ventilation/perfusion abnormalities after 5 days of treatment compared with patients given intravenous heparin.[37]



Bivalirudin

The lower-affinity DTI, bivalirudin, is a synthetic 20-amino-acid peptide (2178 Da) designed on the basis of structural studies of hirudin. It binds to both the active site and exosite 1, but produces only transient inhibition of the active site of thrombin (Figure 2). The inhibition constant for thrombin is 1.9 nmol/L.[38] Reversible binding was anticipated to decrease the risk of bleeding, which has been a concern with hirudin treatment. Indeed, a meta-analysis of patients with ACS undergoing percutaneous transluminal coronary angioplasty (PTCA) showed that the risk of major bleeding due to bivalirudin is less than that of heparin (4.2% vs 9.0%, respectively; OR 0.44; 95% CI 0.34 to 0.56).[33] Again, no antidote is

Bivalirudin appears to be well tolerated.[38] However, its use is limited by the need for intravenous administration. Bivalirudin has a shorter elimination half-life (~25 min) than lepirudin and is only partially eliminated renally. Patients with moderate or severe renal impairment (creatinine clearance <60 mL/min) may need dose adjustment and monitoring of anticoagulation status because clearance of bivalirudin is reduced by approximately 20% in these patients.[39,40] The activated clotting time can be used to monitor the anticoagulant effect of bivalirudin during percutaneous coronary intervention.

Bivalirudin is approved for use in patients undergoing PTCA (Appendix I), and its efficacy has been assessed in several studies of patients with ACS. Bivalirudin was at least as effective as high-dose heparin in preventing such ischemic complications as MI, abrupt vessel closure, rapid cardiac deterioration, or death in a study of 4312 patients undergoing angioplasty for unstable or postinfarction angina.[41] Furthermore, in this study, bivalirudin reduced postinfarction angina compared with UFH (9.1% vs 14.2%, respectively; p = 0.04), with a lower incidence of bleeding (3.8% vs 9.8%, respectively; p < 0.001). The larger, more recent HERO-2 study randomized 17 073 patients with acute ST-elevation MI to receive bivalirudin or heparin combined with thrombolytic treatment.[42] The results of this study demonstrated fewer reinfarctions within 96 hours in the bivalirudin group than in the heparin group (OR 0.70; 95% CI 0.56 to 0.87; p = 0.001). However, rates of moderate and mild bleeding were significantly higher in the bivalirudin group than in the UFH group (moderate bleeding OR 1.32; 95% CI 1.00 to 1.74; p = 0.05; mild bleeding OR 1.47; 95% CI 1.34 to 1.62; p < 0.001). Nevertheless, preliminary results of REPLACE-2 (n = 6002) indicated that bivalirudin is superior to heparin alone and at least as effective as a heparin/glycoprotein IIb/IIIa inhibitor combination when given to patients undergoing PTCA. The relative risk of the composite endpoint of death, MI, urgent revascularization, and major bleeding was reduced by 38% (p = 0.001).[43] Taken together, these results support the usefulness of bivalirudin in the treatment of patients with ACS.

Argatroban

Argatroban is a small (527 Da) DTI that binds reversibly to the active site of the thrombin molecule (Figure 2).[44] It is selective for thrombin (inhibition constant 0.04 µmol/L) and has little effect on related serine proteases.

Argatroban is intravenously administered and has an elimination half-life of 40–50 minutes. Monitoring of the aPTT is required to assess its anticoagulant activity.[45] Dose adjustments may be required to attain a steady-state aPTT of 1.5–3 times the mean normal value.[46] Argatroban is hepatically metabolized via hydroxylation and aromatization reactions, so dosing reductions and careful monitoring are recommended in patients with hepatic dysfunction.[47] The metabolized products are removed via biliary excretion half-life of argatroban and dose adjustments therefore are unnecessary in patients with renal dysfunction. Argatroban has no known antidote.

Patients with HIT who were treated with argatroban experienced more major bleeding (4.9%) than historical controls given oral anticoagulation or no treatment.[45] However, in a study of 1001 patients given either argatroban or heparin with thrombolytic therapy, the incidence of major bleeding was not significantly different (0.4% vs 1.2%, respectively).[48]

Argatroban is approved for use in patients with HIT or HIT with thrombosis (Appendix I). In a study of 160 patients with HIT, the incidence of all-cause death, all-cause amputation, or new-cause thrombosis was significantly reduced compared with control subjects after 37 days (25.6% vs 38.8%, respectively; p = 0.014).[49] In 144 patients with HIT and thrombosis, the reduction in the composite endpoint did not reach significance compared with control subjects (43.8% vs 56.5%, respectively; p = 0.13); however, the time-to-event analysis favored treatment with argatroban.

Although a small trial (n = 125) suggested that argatroban might have superior efficacy to heparin in acute MI patients treated with thrombolysis,[50] the results of a larger study (n = 1001) did not demonstrate such a difference.[48]

Ximelagatran

DTIs can be structurally modified for oral administration. Approximately 10 oral DTIs are reported to be in development[51]; however, to date, ximelagatran is the only one in Phase III clinical trials.

Ximelagatran is a small-molecule prodrug. Following oral administration, it is rapidly absorbed and converted to melagatran, the active form, achieving peak plasma concentrations in 1.6–1.9 hours. Melagatran is a potent, reversibly binding, active-site inhibitor of thrombin (inhibition constant 0.002 µmol/L) (Figure 2).[52] After ximelagatran administration, melagatran has been shown to inhibit the generation of thrombin, probably because of reduced thrombin-mediated positive feedback to the coagulation system (Figure 1).[53]

The bioavailability of melagatran following a single oral dose of ximelagatran 5–98 mg is approximately 20% and is not significantly affected when administered with food.[54] Ximelagatran displays low interindividual variability and has linear pharmacokinetic and pharmacodynamic profiles. Ximelagatran dose and AUC are linearly related. The half-life of melagatran is 3–4 hours.[55] Melagatran is predominantly excreted via the kidneys[56]; therefore, dose adjustment may be required in patients with renal dysfunction. Ximelagatran and melagatran are not metabolized by known hepatic microsomal enzymes and, to date, they appear to lack cytochrome P450 drug and food interactions.[57] There is no known antidote to the antithrombotic effect of ximelagatran.

The therapeutic window of melagatran has been shown

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