The Direct Thrombin Inhibitors

Their Role and Use
For
Rational Anticoagulation

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Malignancy related thrombosis; cobalamin metabolism; clinical and laboratory aspects of prostate, bladder and breast cancer.

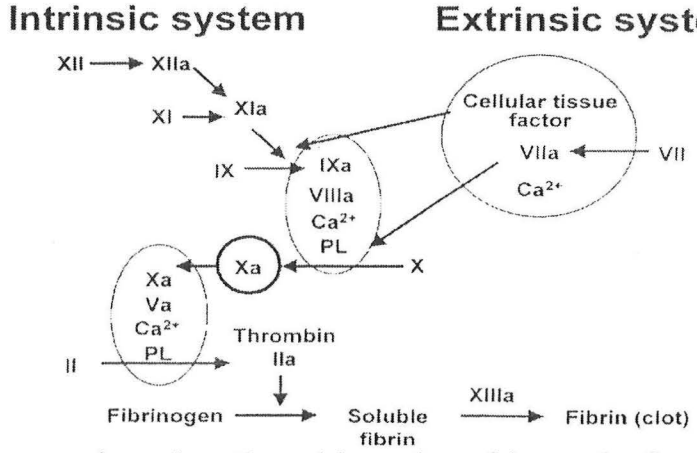
Anticoagulant therapy entered the clinical arena following the isolation of a sulfated glycosaminoglycan from canine liver, thereby termed "heparin", by Howell in 1923 (1); and, it was employed in treatment of thromboembolism in 1939 (2). Shortly thereafter, bishydroxycoumarin was defined as a Vitamin K antagonist and its potential for oral therapy of thromboembolic disease led to the development of other structurally related antagonists for clinical use (3, 4, 5). These agents have more recently been utilized in prophylaxis for thromboembolic disease in both the surgical and non-surgical setting (6). In spite of this time honored approach, it has long been evident that these agents commonly fail in thromboembolic therapy, suffer from a variety of side effects, multiple drug-drug interactions, and are inadequate in prophylaxis.

I. Role of Thrombin in Thrombogenesis:

The significant clinical burden of thrombotic disease has led to an explosion of interest in the development of agents with the potential of effectively interfering with thrombogenesis. Virtually every site in the coagulation schema has seen a focus of interest.

It is, however, thrombin that is the most important focal point in thrombogenesis. It has been termed the "master enzyme" of coagulation (7). It is the last enzyme in the coagulation cascade and its ultimate product. It is a serine protease that originates from the circulating zymogen precursor protein, prothrombin (8, 9).

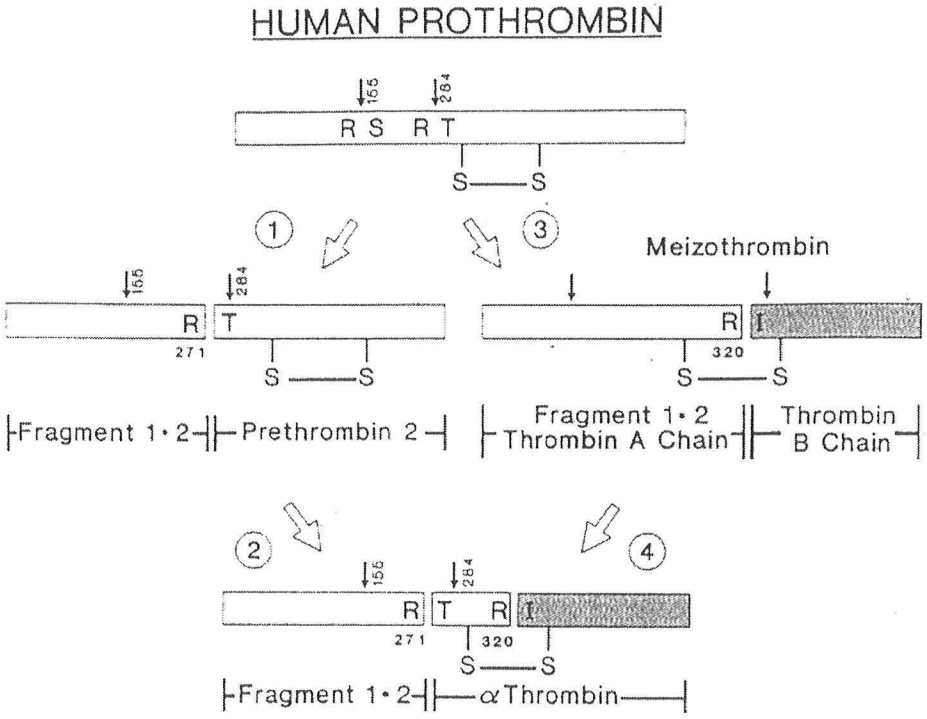
A cogent summary of the complex steps in the activation of prothrombin to thrombin has been well delineated and expanded by the Furies (5, 10), and his extensive brilliant studies by Ken Mann and coworkers (8, 11 – 20).



In brief, the conversion of prothrombin to thrombin results from the enzymatic action of factor Xa and the cofactor Va in a complex formed on membrane surfaces. This complex situated on a membrane (either platelet or endothelial) in the presence of calcium has been termed the "prothrombinase complex", since its

action is to "alter" or "activate" the substrate prothrombin. The prothrombinase complex has an unusual affinity for phospholipid (vesicles) membranes. Factor Va, of the complex binds to specific sites on the platelet surface and this binding provides a structural receptor (complex) on the platelets for binding of Factor Xa.

Two major pathways for prothrombin activation exist at the molecular level:



Prothrombin can be activated by Factor Xa producing prothrombin fragment 1•2 and some ∝-thrombin, but the latter at a very slow rate. Stepwise cleavage at Arg 271 (step 1) produces pre-thrombin fragment 1•2 (which contain the Gla domain and kringles 1 and 2, and prothrombin 2. Cleavage of pre-thrombin 2 at Arg 320 (Step 2) gives rise to prothrombin fragment 1 – 2 and ∝ thrombin (8, 13, 16, 19).

When prothrombin is activated by the prothrombinase complex, the reaction proceeds with an order of bond cleavage that is reversed when compared to that for Factor Xa. The initial cleavage is at Arg 320 producing an obligate intermediate meizothrombin, an active enzyme, but which actually lacks clotting and/or platelet-activating activity. It is cleaved at Arg 271 resulting in thrombin and prothrombin fragment 1•2. It merits emphasis that production of Factor Xa, which also may serve as an initial activation mechanism for Factor V to Va thereby triggering the prothromboninase complex formation, serves as a slow and relatively minor independent prothrombin activation mechanism. Indeed, assembly of the prothrombinase complex results in about 300,000 times greater prothrombin activation (8, 10, 11).

Recent studies have examined clotting in a sequential manner (17, 20) and have shown that, at the Initiation Phase of coagulation activation of thrombin, substrates occur at concentrations of less than 2nM thrombin (0.2%). Most thrombin (96%) is actually formed well after initial clotting occurs (20). The reason for this late overabundance of thrombin generation after (some) clot is present is still unknown, but this sequence appears very important in the issues of thrombogenesis that are today's focus.

Thrombin may be the most versatile enzyme in man. The usual focus of its function is that of clot production by virtue of its attachment to a thrombin – binding domain on fibrinogen, thereby releasing fibrinopeptides A and B in the primary initiation of fibrin generation. In fact, thrombin has multiple hemostatic effects.

Table 1

Hemostatic Effects of Thrombin

Clot Formation:

a) Cleaves Fibrinogen → Fibrin
b) Cleaves Factor XIII → Factor XIIIa

Amplification of Clot:

a) Cleaves Factor V → Factor Va
b) Cleaves Factor VIII → Factor VIIIa
c) Feedback enhancement of further prothrombin activation

Platelet Activation:

a) Stimulates platelet aggregation
b) Stimulates platelet release of storage granules
c) Increases thromboxane A_2 formation (which further activates platelets)

Physiologic coagulation "Inhibitors":

a) Activates Protein C to PCa: Thrombomodulin

However, the biologic effects of thrombin are far more complex than merely hemostatic ones (7, 9, 10, 11)

Table 2

Non-Hemostatic Effects of Thrombin

Effects on WBC's:

a: Stimulates chemotaxis
b. Triggers cytokine generation

Effects on Endothelial Cells:

a. Effects synthesis and release of:
- prostacyclin
- nitrous oxide
- t-pa
- endothelin
- tissue factor **

Effects on Other Tissue Sites:

a. Fibroblast: proliferation
b. Smooth muscle: Mitogenesis

Other Potential Effects:

a. Cancer Cells
- Affects adhesion
- Metastasis
- Cellular proliferation

Two other aspects merit note. First, the thrombin receptor, a member of the 7-transmembrane domain family has been identified in platelets, endothelial and smooth muscle cells, and fibroblasts and the receptor appears critical in the mediation of many of thrombins cellular effects (9, 21 – 24).

Second, two reaction systems are responsible for altenuating the expression of the activity of the prothrombinase complex in generating thrombin thereby limiting "relentless massive thrombosis":

1. Antithrombin – (heparin) system that inhibits the proteases, Factor Xa and $\propto$ thrombin
2. Thrombomodulin – Protein C system. Thus, thrombin escaping from the site of the reaction binds to thrombomodulin on the vascular cell surface and activates protein C. Activated protein C recognizes Factor Va as a substrate and cleaves both the light and heavy chains, thereby producing an incompetent form incapable of producing the binding sites needed for the production of the prothrombinase complex and blocking subsequent attachments to the membrane – binding site (8).

Another important issue relative to thrombin is that it has at least 4 distinct binding sites that function relative to substrates, inhibitors, cofactors and Na+. The Na+ binding site appears to help determine whether thrombin acts as a procoagulant by recognizing fibrinogen as a subtrate (in the presence of sodium ions) or acts as an anticoagulant by recognizing protein C as a substrate (in the absence of sodium ions) (20). The other 3 sites are exosite I, exosite II and the active site which actually recognizes a variety of different molecules and provides for the diverse functions of thrombin (25).

II. Differences between Indirect Thrombin Inhibitors and Direct Thrombin Inhibitors:

The most critical and important issue relative to attempts to alter thrombin – related thrombosis formation is the fact that during clot formation thrombin (in its near-native state) exists bound to fibrin. This fibrin-bound-thrombin remains enzymatically active and is critically protected from inactivation by classical circulating inhibitors (such as heparin). Thrombus thus serves as a reservoir of active thrombin that stimulates thrombus growth by locally activating platelets (26, 27, 28), converting proximal fibrinogen to fibrin (29) and further continued activation of Factors V and X (26, 28).

In addition, once an indirect inhibitor of thrombin, such as heparin, is stopped, there can be a re-activation of the coagulation system as the fibrin – bound – thrombin re-activates Factor X within the thrombus triggering further thrombin generation.

It also merits emphasis that thrombin generation at sites of arterial injury appears to have a very broad and extensive coagulation cascade stimulus, since it has been well documented that specific inhibitors of thrombin are far more effective than heparin at blocking injury – induced arterial thrombosis in baboons (30, 31) and in humans (32, 33).

Finally, the critical role for continued thrombin generation is aptly defined in the syndrome of Heparin Induced Thrombocytopenia (HIT) where despite stopping heparin, increased thrombin generation can be seen for as long as 21 days, with resultant risk of new thrombosis (34).

III. Current Status of Clinically Utilized Indirect Thrombin Inhibitors

Currently the two drugs commonly used in thrombotic circumstances are heparin and warfarin. Heparin is the classical indirect thrombin inhibitor. It is well acknowledged that it has only modest efficacy as an agent to limit thrombin generation and thrombogenesis.

The limitations of efficacy and some of the underlying mechanisms for such limitations have been well delineated (26):

Table 3

Limitations of Heparin Efficacy (26)

Limitation	Mechanism
Variable anticoagulant response	Heparin binds to various acute phase proteins and proteins released from activated platelets or endothelial cells
Dose-dependent clearance	Heparin binding sites on endothelium and macrophages must be saturated before heparin appears in the circulation
Reduced activity in the presence of platelets	Platelet factor 4 released from activated platelets neutralizes heparin
Unable to inactivate fibrin-bound thrombin	Heparin binds to fibrin and exosite 2 on thrombin, thereby heightening the thrombin-fibrin interaction and rendering exosite 2 on thrombin inaccessible to antithrombin-bound heparin
Unable to inactivate factor Xa within the prothrombinase complex	Factor Xa bound to the platelet surface is relatively resistant to inactivation by the heparin/antithrombin complex

In addition to these significant limitations in efficacy, a variety of side effects complicate heparin therapy (35)

Table 4

Side Effects of Heparin Therapy (35)

Potentially severe	(Generally) Mild
Bleeding	Heparin-associated osteoporosis
Acute heparin "anaphylaxis"	Skin reactions:
	urticaria
	erythematous papules
	skin necrosis
Heparin-induced thrombocytopenia	Abnormal liver function tests
	Eosinophilia
	Hyperkalemia
	Hypoaldosteronism
	Priapism
	Alopecia

Although the true incidence of heparin-induced thrombocytopenia – thrombosis is not clear, it represents the most serious and potentially lethal complication of heparin therapy. Because of the dire seriousness of this complication, its occurrence was one

of the major stimulus to the pursuit and development of direct thrombin inhibitors. I will briefly re-iterate some of the salient features (35, 36). The clinical expression and biologic significance has commonly been defined in terms of two clinical forms:

Type I, is a non-immune, non-ideosyncratic form, the mechanism for which is not clear and which is of little true biologic significance.

Table 5

Heparin-Induced Thrombocytopenia Type I (Non-immune; non-idiosyncratic)

Episode of thrombocytopenia occurs early in exposure: Generally in first few days in naive and in first hours in previously exposed
Mild thrombocytopenia: 10–30% decrease in platelet numbers
Clinical manifestations: None
Mechanism: Heparin-induced platelet aggregation
True incidence: Uncertain; but common
Biologic issues: Episode transient; counts normalize even with continued heparin
Therapy: None
Relationship to HIT type II: Unclear, but probably none

By contrast, Type II is an immune idiosyncratic form with major biologic implications and clinical sequalae that include thrombosis and death (35, 36).

Table 6

Heparin-Induced Thrombocytopenia Type II (Immune – Idiosyncratic)

Common diagnostic criteria

1. Thrombocytopenia: Decrease of 50% or more from baseline platelet number
2. Absence of other cause
3. Confirmation by a heparin-associated antibody assay
4. Return to normal platelet numbers when heparin is stopped

Table 7

Clinical features of heparin-induced thrombocythemia type II

1. Usual onset at day 3–14 (median day 10)
2. Nadir platelet count: Usually 30,000–60,000, but may be as low as 5000. The most appropriate definition is a 50% decrease in platelet number from the baseline value
3. Risk factors:
   a. occurs with all methods of administration:
      - most common: continuous infusion of unfractionated heparin
      - seen with heparin flushes (500 U/day) and heparin-coated catheters (3 U/hr)
      - higher with IV than subcutaneous administration
      - greater: bovine > porcine > LMWH heparins
   b. can occur within hours in previously treated patients
   c. increased incidence with recent surgery (primarily venous problems)
   d. increased incidence with preexisting cardiovascular disease (primarily arterial)
4. Absent risks:
   a. equal in men and women
   b. age not a factor
   c. no relation to inherited deficiency or other defects of clotting factors

The commercial development of low molecular weight heparin's (LMWH) have resulted in reduced risk of some of the problems of heparin therapy (37). Thus, the clinical incidence of HIT with low molecular weight heparin's appear to be lower than with unfractionated heparin despite the generation of immno-quintiable antibodies. In several clinical trials (n=2000 patients) there was a significant difference in the generation of the anti-heparin-PF4 antibodies between the heparin and LMWH groups ($p < 0.05$); and, the antibodies with LMWH did not show functionality (28, 29) (38, 39).

Nevertheless, LMW heparin's have not resolved the risk of HIT and thrombosis and are certainly not free of problems as evidenced by the January 9, 2002 alert from Aventis against its use in patients with prosthetic heart valves because of thrombosis, a risk particularly relevant in pregnant women where both fetal and maternal deaths have been recorded. Similarly, new risks have been seen in pregnancy where cerebral and limb anomalies, hypospadius, and fibrotic dysplasia and cardiac defects have been identified.

The occurrence of HIT-T represents such a threatening clinical problem that therapeutic goals have been well established:

- **Stop The Heparin**
- **Inhibit Further Thrombin Generation**

And these goals helped stimulate the development of anticoagulant alternatives to heparin and the generation of direct thrombin inhibitors. (34-36; 40-42)

Devious approaches to a more direct effect on thrombin have focused on activating (or supplementing) naturally occurring thrombin inhibitors (ie antithrombin or heparin cofactor II). The most classical anticoagulant utilized to reduce thrombin generation has been the coumarin derivatives which function by reducing the concentrations of prothrombin and the other Vitamin K-dependent clotting factors. This complex mechanism of action as well as the extensive serious problems of absorption, drug-drug interactions, and biologic variation represent serious therapeutic limitations to their use (43, 44).

IV. Rationale and Function of Direct Thrombin Inhibitors

A. Approaches to Inhibition of Thrombin Generation

Historically, the recognition of naturally occurring inhibitors (of what we subsequently learned to be thrombin) of coagulation came from the study of blood sucking animals. During evolutionary development, hematophagia developed in several classes of animals (45). Such a form of nutrition for the blood sucking parasites required the development of substances to counteract blood clotting in the host (ie their dinner) (46, 47). The most studied hematophagus animal was the medicinal leech, Hirudo medicinalis. The isolation of the active moiety Hirudin was carried out in the late 1800's, and it was the first parenteral anticoagulant to be used. Indeed, in 1909, it was used to treat eclampsia (48). In 1926, it was used in hemodialysis in patients (49). However, problems of availability and purification led to its disuse when heparin became available.

Molecular cloning and recombinant technology that exploited point mutations and n-terminal modifications has led to the development and availability of recombinant forms hiruden; one of which Lepiridin (Refludan®) has become an available clinically applicable direct thrombin inhibitor.

The serious deficits of heparin, particularly in our new understanding of the hemostatic and biologic aspects related to the management of arterial thrombotic disease (50, 52) has led to an explosion of pharmaceutical interest in the development of agents capable of altering thrombin generation. This has included exploration of inhibitors from a variety of hemophogocytic leaches and bugs.

One recent alternative approach is synthetic heparin pentasaccharide, Fondaparinux which functions to inhibit Factor Xa binding to antithrombin, thereby producing a secondary inhibitor of thrombin generation. It has excellent bioavailability by the

subcutaneous route, rapid onset of action, and a prolonged half-life (14 – 20 hours). In the once daily dose of 2.5mg it has been shown to have a 50% relative risk reduction of venous thromboembolic events in orthopedic surgery, when compared to LMW heparin (53, 54). Unfortunately, its functionality critically depends upon endogenous antithrombin levels (55), it is not a direct thrombin inhibitor (working rather via Factor Xa), and it requires normal renal function since there is no metabolism prior to renal excretion. In addition, monitoring cannot be done with the a PTT, and no antidote exists should bleeding occur (53).

B. The "Why" of Direct Thrombin Inhibition:

As previously reviewed, thrombin has such a central role in thrombogenesis, that it is the ideal "target site" for therapeutic manipulation.

Table 8

The Rationale for Using Thrombin as the Therapeutic Target in Thrombogenesis

1. Thrombin has a central role in thrombogenesis.
2. It amplifies its own generation.
3. It activates Factors V, VIII and XI, providing positive feedback to enhance (further) thrombin formation.
4. It activates platelets: It is a potent agonist
5. It converts fibrinogen to clottable fibrin.
6. It activates Factor XIII cross-linking of fibrin to form a stable clot.
7. It enhances resistance of thrombi to fibrinolysis

With these important mechanisms, the rationale for the exploitation of a direct thrombin inhibitor is well defined.

Table 8a

The Functional Basis for Role of Direct Thrombin Inhibitors

1. Ability to inhibit Fibrin-Bound thrombin and thereby delimit thrombus growth.

2. Binds thrombin at its active site thereby inhibiting downstream events.

3. Inhibits platelet factor 4 activation (an agonist that also inactivates heparin)

4. Provide more predictable anticoagulant responses, because:
   - Not bound to plasma proteins
   - No drug – drug interactions

However, it is important to note that:
"Not all direct thrombin inhibitors are the same"

V. Status and Use of Currently Available Direct Thrombin Inhibitors

Presently four direct inhibitors of thrombin generation are available for clinical use. In spite of the explosion of knowledge relative to structure and function of these drugs it merits emphasis that proof of efficacy has required extensive studies and documentation, generally related to the so-called "gold standards" – heparin and/or warfarin. Such data presently requires formidable evaluation in large clinical trials commonly focused in circumstances of heparin-induced thrombocytopenia, prophylaxis of venous thromboembolic events post surgery (especially hip and knee replacements), and in atrial fibrillation or coronary occlusive events.

A. Recombinant Hirduin: Lepirudin; Refludan®

Recombinant hirudin (56) entered the clinical arena largely because of the dramatic clinical sequalae of heparin-induced thrombocytopenia and its evident therapeutic efficacy in randomized prospective trials led to approval for use in the European Community in 1997, by the US-FDA in 1998, and Canada in 1999 (57, 58, 59).

Lepiruden has been examined in prospective trials of HIT and HIT with associated thromboembolic complications (i.e. HIT-T) and shown to be effective and safe (58, 59), thereby establishing its initial clinical role.

Table 9

Biologic Features of Lepirudin (recombinant Hirudin) {Refludan®}

- Derived from yeast cells
- It is a bivalent thrombin inhibitor, simultaneously binding to the active (catalytic) site of thrombin and the fibrinogen (accessory) binding site. (The NH_2 terminal region aminoacids bind to the thrombin site cleft, while the core of the hirudin molecule closes off the active site pocket. COOH – terminal tail interacts with the fibrinogin anion binding site blocking thrombin – catalyzed cleavage).

- Inhibition constant for thrombin in picomolar range (k_I = 20 fM)
- Forms non-covalent, irreversible 1:1 complex with thrombin, thereby inhibiting all of the biological activities of thrombin.

- Plasma Pharmacokinetics (59, 60)

  - Two compartment model (IV): Initial T1/2 8-12 min Terminal elimination 0.8 – 1.7 hrs.
  - Subcutaneous: Peak concentration at 3 – 4 hrs.
  - Not transported into CNS or breast milk
  - Profile does not change with repeated doses; however, approximately 50% of patients Rx for > 5 days develop antibodies to Leperudin; and, in 2% of these the antibodies appear to "enhance" function so dose reduction (by 50 – 60%) needed; Mechanism not clear but may be reduced renal clearance of Lepirudin – immunoglobulin complexes (61, 62).

Drug clearance is by kidneys (>90%) (Since renal blood flow decreases during anesthesia; half-life is prolonged to 3 – 5 hour and dose reduction (30 – 50%) during surgery.

Dosage: Continuous IV: 0.2 mg/kg/hr

or

Subcutaneous: 0.5mg/kg B.I.D.

(Bolus given with only severe thromboembolic complications to avoid overdosage)

Adjust dose downward in renal failure:

- Decrease by 50% for creatinine 1.6 – 2.0
- By 70 – 75% for Cr 2.1 – 3.0
- By 85 – 80% for Cr 3.0 – 6.0

(Therapeutic blood levels: 0.5 to 1.5 mg/ml)

Monitoring: The best method not yet established. Thrombin time is not linear and is too sensitive. Prothrombin time is too insensitive. A partial thromboplastin time can be used in the lower hirudin level range where standard curve is linear. High (toxic) levels cannot be determined with a PTT.

Best current method: Ecarin clotting time (ECT)

- Developed as a rapid "point of service" assay: A snake venom enzyme (a metalloprotease of Echis carinatus) specifically cleaves prothrombin (at arginine 320/isoleeucine bond) generating meizothrombin, which is inhibited by Lepiruden with the same kinetics as the active site of thrombin; thereby allowing a clot monitoring method (63 – 65). *This is especially relevant in cardiopulmonary bypass surgery (66, 67).

Bleeding complications: It is irreversibly bound, no antidote exists for bleeding. *For Lepiruden (and all other direct thrombin inhibitors) elevation of the therapeutic level to five-fold causes life threatening bleeding). Therapy for bleeding has included Hemofiltration onto cellulose; trials of recombinant Factor VIIa (i.e. Novo Seven)

Following the evident efficacy in HIT, the clinical use of Lepiruden has been extensively explored in circumstances of coronary artery disease with and without infarction (60, 68, 69), and in venous thromboembolism, both in surgical prophylaxis and therapy (69 – 72). Efficacy is evident, but irreversible thrombin binding has led to interest in the even newer direct inhibitors of thrombin generations. Nevertheless, hirudin was the historical, biologic and clinical agent that provided the platform for such development.

B. Bivalirudin (Hirulog®)

A variety of synthetic peptides based on the hiruden structure have been developed. A dodecapeptide, initially derived from the carboxy-terminal region made up of residues 53 to 64, was developed which was an exosite-targeted peptide. It was shown to inhibit fibrinogen binding and fibrin generation, but failed to interrupt thrombin – mediated platelet dependent thrombus propagation, which critically requires inhibition of the catalytic site. It was then combined via four glycene residue (Gly) 4 bridge with a tetrapeptide with active center specificity (D-Phe-Pro-Arg-Pro) (73).

This resulted in a bi-functional antithrombin peptide, bivalirudin. This is "bivalent", because there is binding to exosite 1 (ie fibrinogen site) and to the catalytic site, thereby decreasing platelet – dependent thrombosis.

Unlike hirudin, bivalirudin produces only transient inhibition of the active site of thrombin, because once bound to thrombin, the Arg-Pro band on the aminoterminal extension is cleared; thereby, converting bivaliruden into a low affinity inhibitor (74).

Its desirable features include:

- Reversibility, thereby rendering it safe than Lepiruden; short half-life.
- Only a fraction is excreted by the kidneys. Hepatic metabolism and proteolysis contribute to actual clearance.

Currently Bivaliruden is approved as an alternate to heparin in patients undergoing angioplasty (68, 75) and in the treatment of patients with unstable angina (76).

C. Argatroban

Argatroban was discovered and developed by Professor S. Okamoto in Tokyo in the 1970's. It is a small molecule (approx. 526 Daltons) synthetically derived from L-arginine as a complex mixture of R and S isomers. Early on the Japanese recognized Argotroban to be a unique direct inhibitor of thrombin generation with reversible features. In Japan in the 1980's it was first used to treat peripheral arterial occlusive disease and its approval in Japan now extends to that indication; and, to acute ischemic cerebral thrombosis, anticoagulation (largely hemodialysis) of antithrombin deficient patients, and now heparin-induced thrombocytopenia – thrombosis (77). In the early '90's J.T. Willerson bought the rights to the drug and has directed its development in the U.S. It is now approved for use in HIT and HIT-T and was just approved for use in percutaneous coronary artery interventions.

It merits emphasis that this unique developmental activity by Okamoto and his team is the best example of the new vision in the approach to issues of thrombosis. They began this new arena by designing protease-inhibitors through the mimicry of substrates (45). By imitating the amino acid sequence around the thrombin-scissible bond in fibrinogen, inhibitors were produced that enter in a covalent or non-covalent bond with the active site of the enzyme.

Table 10

Biologic Features of Argatroban

Characteristics:

- Synthetic, small molecule derivative of arginine.
- It is a reversible direct thrombin acting by blocking the active catalytic site.
- It inhibits both soluble and clot bound thrombin.

Pharmacokinetics:

- Rapid onset of response: 30 minutes
- Linear relationship between dose and plasma concentration
- Steady state plasma concentration achieved by 1 to 3 hours following start of continuous infusion. (Bolus will reduce this time).
- Plasma protein binding 54% (20% to albumin; and 34% to $\propto$ 1 acid glycoprotein)
- Major route of elimination: feces by biliary secretion
- Kinetics not affected by age, gender, or renal dysfunction.

- Hepatic impairment influences the kinetics prolonging half-life; dose reduction is needed.

Metabolism: by the liver via hydroxylation and aromatization by CYP 3A 4/5 microsomal system. 4 resultant metabolites: some with weak but present "anticoagulant" effect.

Monitoring: excellent linear effect on a PTT;
therapeutic goal 1.5 to 2.5 of control

*Argatroban causes increase in INR; when combined with warfarin special calculations needed (78).

Dosage: Initiate at 2 μg/kg/min
At steady state of PTT (2 hours), adjust dose (not to exceed 10 μg/kg/min or a PTT of 100 seconds)

Antibodies are not formed because it is not a protein.

The efficacy of Argotraban in HIT-T has been well documented in the excellent pivotal trial that documented its value efficacy based on a composite of all-cause death, all cause amputation, or new thrombosis (79); and, subsequent correlative trials have been extensively reviewed (80).

The reversibility of the inhibiting action of Argatroban provides an important safety aspect in the consideration of its use. In addition, Argatroban has several other advantages, since it does not compromise other thrombin regulatory processes, which include:

1. Inhibition of factor XIIIa
2. Antagonism of protein C activation, activation of thrombin
3. Activatable fibrinolytic inhibitor (TAFI)
4. Inhibition of thrombin mediated hemostatic effects of platelets
5. Inhibition of the vasomodulatory effects of thrombin
6. The modulation of the inflammatory cytokines.

Clearly, it is an agent that will enjoy extended therapeutic utilization in the future.

D. Melagatran and Ximelagatran (Exanta®)

The newest direct thrombin inhibitor which is of particular interest is Melagatran. It has captivated the clinical world of thrombosis, because early on, potential oral bioavailability was recognized (69). The formulation of a pro-drug H376/95, ximelagatran (Exanta®) with excellent oral bioavailability has provided a truly new dimension in anti-thrombotic prophylaxis and therapy.

Table 11

Biologic Features of Melagatran (69,73)

- Synthetic low molecular weight (429.5 Da) dipeptide analogue designed to mimic fibrinopeptide A.
- It is a univalent (binding only to the active site), reversible, active site-directed thrombin inhibitor that inhibits thrombin activity and thrombin generation.
- High potency for thrombin inhibition evidenced by low inhibition constant for thrombin (0.002 μmol/l); inhibition of thrombin-induced platelet aggregation at very low concentrations.
- Low plasma protein binding (< 15%)
- Very effective inhibition of both arterial and venous thromboembolism in several animal models.
- Little or no inhibition of the fibrinolytic enzyme (system)

Pharmacokinetics and Pharmacodynamics (74)

- Rapid onset of inhibition; achieved steady state in approximately 30 minutes.
- Half-life of 2 to 4 hours; so no need to stop a long time before surgery
- Neither obesity nor liver dysfunction affect parameters.

Excreted by kidneys: dose adjust (or monitor) for Cr >1.8

Will cross placenta

Monitoring: a PTT provides effective monitor with stable steady state at 1.5 to 2.5 x normal. INR cannot be used (75)

Excellent therapeutic window; that is, achievable plasma concentration that produces beneficial anti-thrombin effect without increased risk of bleeding.

Therapeutic approach to bleeding: Drug cessation and r VIIa if needed.

Clinical efficacy of melagatran was clearly shown in a series of patients with deep vein thrombosis where phelbographically-verified regression of thrombus size was examined (74).

However, the prompt development of an oral formulation of the melagatran pro-drug H376/95, ximilagatran (Exanta) has been the truly most exciting chapter in direct anti-thrombin therapy. Prompt recognition of excellent oral bioavailability of ximelagatran with rapid conversion to the active melagatran (75) has provided the basis for its exploration and utilization in the prophylaxis of thromboembolism in surgical replacement of hips and knees, and in the approach to thromboembolic prevention in the management of atrial fibrillation or prosthetic heart valves.

Table 12

Biologic features of Ximelagatran (Exanta®)

Excellent oral bioavailability (75, 76)

Metabolism into melagatran rapid and complete with (76):

- Predictable pharmacodynamics
- Immediate onset of anticoagulation

Monitoring: Wide therapeutic index, so that monitoring is not needed, except in patients with renal failure; the a PTT is non-linear with severe renal dysfunction.

No known drug-drug interactions

Bioavailability not affected by obesity or altered liver function.

Crosses the placenta

Dosage: 24 mg B.I.D. orally

As noted earlier, thrombosis prophylaxis is a very slow and difficult model in the clinical evaluation of a new drug. Nevertheless, several excellent studies have documented efficacy and safety in the management of acute deep vein thrombosis (77), and prophylaxis in the management of patients with knee and hip replacement (78, 79). Active expanded clinical trials are currently ongoing.

Conclusions

The development and utilization of direct thrombin inhibitors have proven their safety and efficacy. It is clear that not all direct thrombin inhibitors are the same. Their current entry into the clinical arena has demonstrated that these agents are rapidly changing the landscape of anticoagulation management.

I want to express my sincere appreciation to Ms. Levia Alford for her incredible assistance in preparation for this presentation.

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