New Anticoagulant Drugs

Jeffrey I. Weitz, MD; and Jack Hirsh, MD

Abbreviations: NAPc2 = nematode anticoagulant peptide c2; PAI-1 = type 1 plasminogen activator inhibitor; PPACK = D-Phe-Pro-Arg chloromethylketone; SNAC = sodium N-(8-hydroxybenzyl)aminocaproate; TAPI = thrombin activatable fibrinolysis inhibitor; TAP = tick anticoagulant peptide; TFPI = tissue factor pathway inhibitor; t-PA = tissue-type plasminogen activator

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Arterial and venous thromboses are major causes of morbidity and mortality. Whereas arterial thrombosis is the most common cause of myocardial infarction, stroke, and limb gangrene, venous thrombosis leads to pulmonary embolism, which can be fatal, and to postphlebitic syndrome. Because arterial thrombi consist of platelet aggregates held together by small amounts of fibrin, strategies to inhibit arterial thrombogenesis focus mainly on drugs that block platelet function, but they often include anticoagulants to prevent fibrin deposition. In contrast, anticoagulants are the drugs of choice for prevention of cardioembolic events. Anticoagulants also are used for prevention and treatment of venous thrombosis, because venous thrombi are comprised mainly of fibrin and RBCs.

Focusing on new anticoagulant drugs for the prevention and treatment of arterial and venous thrombosis, this chapter (a) reviews arterial and venous thrombogenesis; (b) highlights the pathways that regulate clotting; (c) outlines new anticoagulant strategies; and (d) provides clinical perspective as to the new strategies that are most likely to be clinically effective.

THROMBOSIS

Arterial thrombosis usually is initiated by spontaneous or mechanical rupture of atherosclerotic plaque, a process that exposes thrombogenic material in the lipid-rich core of the plaque to the blood. Typically, thrombi that form at sites of plaque disruption extend both into the plaque and into the vessel lumen (Fig 1). If the intraluminal thrombus is nonocclusive, and if blood flow remains rapid, the thrombus may emulsify downstream, or it may organize and become incorporated into the vessel wall. With more extensive intraluminal thrombosis, however, blood flow diminishes, and shear increases. Higher shear promotes further platelet and fibrin deposition, resulting in the formation of an occlusive thrombus that can obstruct blood flow to organs such as the heart or brain, or to the extremities.

Whereas arterial thrombi are predominantly composed of platelets, venous thrombi consist mainly of fibrin and RBCs. Venous thrombi develop under low-flow conditions and usually originate in the muscular veins of the calf or in the valve cusps pockets of the deep calf veins. Coagulation at these sites is initiated by vascular trauma and is augmented by venous stasis. Damage to the vessel wall is a particularly important predisposing factor to venous thrombosis after major hip or knee surgery.

Initiation of coagulation in veins or arteries is triggered by tissue factor (Fig 2), a cellular receptor for activated factor VII (factor VIIa) and factor VII. Most nonvascular cells express tissue factor in a constitutive fashion, whereas de novo tissue factor synthesis can be induced in monocytes. Injury to the arterial or venous wall exposes nonvascular, tissue-factor-expressing cells to blood. Lipid-laden macrophages in the core of atherosclerotic plaques are particularly rich in tissue factor, thereby explaining the propensity for thrombus formation at sites of plaque disruption. Factor VIIa, found in small amounts in normal plasma, binds to exposed tissue factor. Once bound to tissue factor, factor VIIa can catalyze the activation of factor VII, which also binds to exposed tissue factor. The factor VIIa/tissue factor complex then activates factor IX and factor X, leading to the generation of factor IXa and factor Xa, respectively. Factor IXa binds to factor VIIIa on membrane surfaces to form intrinsic tenase, the complex that activates factor X. By feedback activation of factor VII, factor Xa amplifies the initiation of clotting.

Factor Xa propagates coagulation by binding to factor Va on membrane surfaces to form the prothrombinase complex. Factor Xa within this complex activates prothrombin to thrombin, which then dissociates from the membrane surface and converts fibrinogen to fibrin monomer. Fibrin monomers polymerize to form the fibrin mesh that is stabilized and crosslinked by factor XIIIa, a thrombin-activated transglutaminase. Thrombin amplifies its own generation by feedback activation of factor V and factor VIII, cofactors in the prothrombinase and intrinsic tenase complexes, respectively. Thrombin also can activate factor XI, thereby leading to further factor Xa generation.

REGULATION OF COAGULATION

Coagulation is regulated at several levels. Key inhibitors include heparin/antithrombin, tissue factor pathway inhibitor, and activated protein C. In addition, the fibrinolytic system degrades fibrin. Because many new anticoagulant strategies are aimed at enhancing endogenous anticoagulant or fibrinolytic mechanisms, it is relevant to review these pathways.

Heparin/Antithrombin

Antithrombin inhibits thrombin, factor Xa, and other activated clotting factors, but these reactions are slow in the absence of heparin. With heparin, however, the rate of inhibition is accelerated approximately 1,000-fold. Heparin binds to antithrombin via its high-affinity pentasaccharide sequence and, by altering the conformation of the reactive center loop of antithrombin, renders the protease trap more accessible to target enzymes. Although heparin is not normally found in the blood, vascular endothelium is rich in heparan sulfate. Most of the heparan sulfate is located on the abluminal surface of the endothelium and is exposed only when the vessel lining is damaged. Nevertheless, the small amounts of proteoglycan located on the luminal surface may help render intact endothelium nonthrombogenic.

Correspondence to: Jeffrey Weitz, MD, 711 Concession Street, Hamilton, Ontario L8V 1C3, Canada; e-mail: jweitz thrombosis lhscr.org
efficiently activated by thrombin in the presence of platelets.6 Initiation of coagulation by the factor VIIa/tissue factor complex. Because TFPI downregulates the majority of which is bound to endothelium.9 TFPI acts in a two-step manner (Fig 3); first, it complexes and inactivates factor Va; in the second step, the TFPI/factor Va complex inactivates factor VIIa within the factor VIIa/tissue factor complex. Because TFPI downregulates the initiation of coagulation by the factor VIIa/tissue factor complex, an alternate mechanism for propagating coagulation is necessary. This may be provided by factor XI, which is efficiently activated by thrombin in the presence of platelets.6 By activating factor IX, a key component of the intrinsic tenase complex, factor XIa induces the generation of sufficient amounts of factor Xa to propagate coagulation.

Tissue Factor Pathway Inhibitor

Inhibition of the factor VIIa/tissue factor complex is effected by tissue factor pathway inhibitor (TFPI), the majority of which is bound to endothelium.9 TFPI acts in a two-step manner (Fig 3); first, it complexes and inactivates factor Xa; in the second step, the TFPI/factor Xa complex inactivates factor VIIa within the factor VIIa/tissue factor complex. Because TFPI downregulates the initiation of coagulation by the factor VIIa/tissue factor complex, an alternate mechanism for propagating coagulation is necessary. This may be provided by factor XI, which is efficiently activated by thrombin in the presence of platelets.6 By activating factor IX, a key component of the intrinsic tenase complex, factor XIa induces the generation of sufficient amounts of factor Xa to propagate coagulation.

Protein C Pathway

In addition to inactivation by antithrombin, thrombin is also inhibited by binding to thrombomodulin, a thrombin receptor found on the endothelium (Fig 4). Once bound to thrombomodulin, thrombin undergoes a conformational change at its active site that converts it from a procoagulant enzyme into a potent activator of protein C. Activated protein C, a vitamin K-dependent protein, serves as an anticoagulant by proteolytically degrading and inactivating factor Va and factor VIIIa, thereby blocking thrombin generation.10 This reaction occurs on membrane surfaces where protein S, another vitamin K-dependent protein, serves as a cofactor.10

Fibrinolytic System

Designed to remove intravascular fibrin, thereby restoring blood flow, fibrinolysis is initiated by plasminogen activators that convert plasminogen to plasmin (Fig 5). A trypsin-like protease, plasmin degrades fibrin into soluble fibrin degradation products. Tissue-type plasminogen activator (t-PA), which is synthesized and secreted by endothelial cells, mediates intravascular plasminogen activation. Plasminogen activation is targeted to fibrin because plasminogen and t-PA bind to fibrin, and the enzymatic activity of t-PA is enhanced by fibrin.11

The fibrinolytic system is regulated at two levels (Fig 5). Plasminogen activator inhibitors, the most important of which is endothelial cell-derived type 1 plasminogen activator inhibitor (PAI-1), block t-PA, whereas α2-antiplasmin inhibits plasmin (Fig 5). Although α2-antiplasmin rapidly complexes and inactivates free plasmin, fibrin-bound plasmin is relatively protected from inactivation so that fibrinolysis can occur despite physiologic levels of this inhibitor.11

Recently, a procarboxypeptidase B that serves as a link between coagulation and fibrinolysis was identified in plasma.12 Activated by the thrombin/thrombomodulin complex, this carboxypeptidase B-like enzyme, known as thrombin activatable fibrinolysis inhibitor (TAFI), attenuates fibrinolysis by cleaving carboxyl-terminal lysine residues from fibrin.12 Removal of these lysine residues decreases plasminogen and plasmin binding to fibrin, thereby retarding the lytic process.

NEW ANTICOAGULANT STRATEGIES

Anticoagulant strategies to inhibit thrombogenesis have focused on inhibiting thrombin, preventing thrombin generation, or blocking initiation of coagulation (Fig 2). Thrombin inhibitors block thrombin activity, whereas agents that target clotting enzymes higher in the coagulation pathways prevent thrombin generation. Coagulation factors that have been targeted for inactivation include factor Xa, factor IXa, and the factor VIIa/tissue factor complex. Other approaches to attenuating thrombogenesis include enhancing endogenous anticoagulant pathways or promoting fibrinolysis. Although there are many candidate drugs to accomplish these tasks, only a small number are under development, and even fewer have progressed to clinical testing. Compounds in more advanced stages of clinical development are listed in Table 1.

Thrombin Inhibitors

Agents can inactivate thrombin indirectly, by activating naturally occurring thrombin inhibitors (namely, antithrombin or heparin cofactor II), or directly, by binding to thrombin and preventing its interaction with substrates. Of the established anticoagulants, coumarin derivatives prevent thrombin generation by reducing the concentrations of prothrombin and other vitamin K-dependent clotting factors,13 whereas unfractionated heparin and low molecular weight heparins block thrombin formation and thrombin activity by activating antithrombin, which then complexes and inhibits thrombin and factor Xa.7,14 In contrast, by activating heparin cofactor II, a selective inhibitor of thrombin, dermatan sulfate only inhibits thrombin activity.15
**Indirect Thrombin Inhibitors**

Unfractionated heparin and low molecular weight heparin are cornerstones for prevention and treatment of venous thrombosis and are widely used in combination with antiplatelet drugs, such as aspirin and glycoprotein IIIb/IIIa antagonists, and thrombolytic agents in patients with acute coronary ischemic syndromes. Because it produces a more predictable anticoagulant profile than heparin, low molecular weight heparin can be given without laboratory monitoring, making it a useful drug for out-of-hospital treatment. Consequently, low molecular weight heparin is gradually replacing heparin for treatment of patients with venous thrombosis and is rapidly establishing a niche for itself as a treatment of unstable angina.

Recently, delivery systems have been developed that make it possible to give heparin or low molecular weight heparin orally. These delivery systems utilize synthetic amino acids such as sodium N-(8-hydroxybenzoyl)amino caprylate (SNAC) or SNAC derivatives to facilitate heparin absorption by the gut. Although absorption is limited and somewhat variable, sufficient amounts of heparin can be delivered orally to prolong the activated partial thromboplastin time. With phase I and phase II trials completed, phase III studies comparing SNAC/heparin with low molecular weight heparin for thromboprophylaxis in patients undergoing elective hip or knee arthroplasty are now underway.

Dermatan sulfate, which acts as an anticoagulant by activating heparin cofactor II, has been compared with low-dose heparin for thromboprophylaxis in cancer patients. Because its low specific activity and poor solubility limit the amount of drug that can be given by subcutaneous injection, dermatan sulfate has not been evaluated in the treatment setting. A low molecular weight form of dermatan sulfate has been generated to improve bioavailability after subcutaneous injection, and various physical
methods have been used to enhance specific activity. Whether these maneuvers will render dermatan sulfate a clinically viable anticoagulant remains to be established.

**Direct Thrombin Inhibitors**

Direct thrombin inhibitors have potential advantages over heparin. Whereas thrombin bound to fibrin or fibrin degradation products is relatively protected from inactivation by heparin, bound thrombin is readily inhibited by direct thrombin inhibitors. Direct thrombin inhibitors include the following: (a) hirudin and bivalirudin, a semisynthetic hirudin fragment; (b) other naturally occurring thrombin inhibitors; (c) noncovalent inhibitors that react with the active-site of thrombin; (d) covalent inhibitors of thrombin’s active site; and (e) thrombin-binding DNA aptamers. Although all of these inhibitors bind directly to thrombin, their sites of interaction are different.

**Hirudin and Its Derivatives**

Hirudin is a 65-amino acid polypeptide originally isolated from the salivary glands of the medicinal leech, *Hirudo medicinalis*, and it is now available through recombinant DNA technology. Unlike native hirudin, the recombinant forms are not sulfated at Tyr 63, and they exhibit at least a 10-fold reduced affinity for thrombin. Hirudin is a potent and specific inhibitor of thrombin that forms a stoichiometric, slowly reversible complex with the enzyme. Analysis of the crystal structure of the thrombin/hirudin complex demonstrates the extensive contact that hirudin makes with thrombin, with its globular amino-terminal domain interacting with the active site of thrombin and the carboxyterminal domain to exosite 1 on the enzyme.

The almost irreversible nature of this complex may be considered a potential weakness of this drug, as no antidote is available should bleeding occur. Hirudin is predominantly cleared by the kidneys and undergoes little hepatic metabolism. It has a plasma half-life of 40 min after IV administration, and approximately 120 min after subcutaneous injection.

Hirudin has been used successfully to treat patients with arterial and venous thrombotic complications of heparin-induced thrombocytopenia. It also has been used effectively as an alternative to heparin during cardiopulmonary bypass in a small number of patients with heparin-induced thrombocytopenia. Based on these data, hirudin has been licensed for the treatment of heparin-induced thrombocytopenia in North America.

Hirudin has been shown to be superior to low-dose
subcutaneous heparin or low molecular weight heparin for thromboprophylaxis in patients undergoing elective hip arthroplasty, and it does not increase the risk of bleeding in this high-risk setting.36,37 In patients with unstable angina and non-ST-elevation myocardial infarction, hirudin appears to be more effective than heparin.38,39 Although hirudin increases the risk of major bleeding in these patients, there is no increase in life-threatening bleeds. Hirudin is now under consideration for licensing in patients with unstable angina and non-ST-elevation myocardial infarction.

Bivalirudin is a semisynthetic, bivalent thrombin inhibitor comprised of a dodecapeptide analog of the carboxy-terminal of hirudin, which binds to exosite 1 on thrombin, linked to an active-site directed moiety, D-Phe-Pro-Arg-Pro, by four glycine residues.40 Unlike hirudin, bivalirudin produces only transient inhibition of the active site of thrombin because, once bound to thrombin, the Arg-Pro bond on the amino-terminal extension of bivalirudin is cleaved, converting bivalirudin into a lower-affinity inhibitor.41 The shorter half-life of bivalirudin may render bivalirudin safer than hirudin. Based on enhanced safety relative to heparin in patients undergoing coronary angioplasty in phase III trials,42,43 bivalirudin is under consideration as an alternative to heparin for this indication. Only a fraction of bivalirudin is renally excreted, suggesting that hepatic metabolism and proteolysis at other sites contribute to its clearance.44

Other Natural Thrombin Inhibitors: Other natural thrombin inhibitors that interact with thrombin via its active site and/or exosites have been described. These include bothrojaracin, a bivalent thrombin inhibitor isolated from the venom of Bothrops jararaca, which, in addition to binding to exosite 1, also binds to exosite 2, the heparin-binding domain on thrombin. Bothrojaracin does not interact with the active site of thrombin.45 Other naturally occurring thrombin inhibitors are rhodin, triabin, and dipetalin, agents isolated from various hematophagous insects.46–48 Like hirudin, rhodin binds the active site and exosite 1 (46), whereas triabin binds to exosite 1 (47), and dipetalin interacts solely with the active site of thrombin.49 To our knowledge, none of these agents has been investigated for clinical use.

Noncovalent Inhibitors: Small molecules have been developed that bind noncovalently to the active site of thrombin and act as competitive inhibitors.49 Argatroban, a carboxylic acid derivative, is the prototype for this class of selective thrombin inhibitors.50 Argatroban has been used as an alternative to heparin in patients with heparin-induced thrombocytopenia and has recently been approved for this indication. Other agents include napsagatran, inogatran, melagatran, L-372,236, and L-372,460. Perhaps the most promising of these is H376/95, a prodrug form of melagatran51,52 and L-372,460, because they are orally bioavailable. H376/95, an uncharged lipophilic drug with little intrinsic activity against thrombin, is well absorbed from the GI tract and undergoes rapid biotransformation to melagatran.52 The drug produces a predictable anticoagulant response, so that little or no laboratory monitoring appears to be necessary. Phase II studies with H376/95 for prevention and treatment of venous thrombosis have been completed, and phase III trials for these indications are now underway.

Noncovalent thrombin inhibitors appear to be effective in laboratory animal models of arterial or venous thrombosis,54–56 but only limited data are available in humans.57,58 Although there is no proven antidote for these agents, the anticoagulant effect of napsagatran can be neutralized by S205A-thrombin, a recombinant thrombin variant that has its active site serine replaced by alanine.59

### Table 1—Status of New Anticoagulant Drugs*

<table>
<thead>
<tr>
<th>Target Drug</th>
<th>Route</th>
<th>Status</th>
<th>Indication</th>
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<tr>
<td>VIIa/TF</td>
<td>TFPI</td>
<td>Intravenous</td>
<td>Phase III</td>
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<tr>
<td></td>
<td>NAPc2</td>
<td>Subcutaneous</td>
<td>Phase II</td>
</tr>
<tr>
<td>Va/VIIIa</td>
<td>APC</td>
<td>Intravenous</td>
<td>Phase III</td>
</tr>
<tr>
<td></td>
<td>Pentasaccharide</td>
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<td>Phase III</td>
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<td>Intravenous</td>
<td>Phase II</td>
</tr>
<tr>
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<td>SNAC/heparin</td>
<td>Oral</td>
<td>Phase II</td>
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<td></td>
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*APC = activated protein C.
S205A-thrombin binds napsagatran with affinity similar to that of native thrombin, but it has no intrinsic enzymatic activity. Its inhibitory effect, however, is short-lived.

**Covalent Inhibitors:** D-Phe-Pro-Arg chloromethyl ketone (PPACK) is the prototype of a class of synthetic tripeptides that form covalent complexes with thrombin.60 PPACK irreversibly inhibits thrombin by alkylating the active center histidine residue. A major limitation of PPACK is its lack of selectivity. Boronic acid derivatives, such as DUP714, are more selective than PPACK,61 but severe hypotension can occur because these agents also inhibit complement factor 1.62

The most promising covalent inhibitor is efegatran (D-MePhe-Pro-ArgH), an arginine aldehyde that forms a slowly reversible covalent complex with thrombin.63,64 It has a short half-life after IV administration65,66 and appears to be orally bioavailable. Two other arginine derivatives with oral bioavailability are S1832667,68 and CVS-1123.69,70

**DNA Aptamers:** Double-stranded DNA aptamers that bind thrombin have been identified.71 A single-stranded, 15-nucleotide DNA aptamer that binds exosite 1 on thrombin with high affinity72 is a potent anticoagulant *in vitro* and has antithrombotic activity in laboratory animals. However, with a half-life of minutes, its clinical utility is limited. Recently, DNA aptamers that bind to exosite 2 on thrombin have also been identified.73 Because there is allosteric linkage between the two exosites on thrombin, DNA aptamer binding to exosite 2 can influence ligand binding to exosite 1.74 Exosite 2-directed aptamers have yet to be tested in laboratory animals, but like other DNA aptamers, they are likely to have short half-lives *in vivo*.

**Other Thrombin Inhibitors:** Other classes of direct thrombin inhibitors are under development. BCH-2763 is a bivalent thrombin inhibitor that interacts with the catalytic site and exosite 1 of thrombin. In rat models of arterial and venous thrombosis, BCH-2763 appears to be more potent than heparin and other direct thrombin inhibitors.75-77 Nonpeptidic agents, such as the 4-amino-pyridine-derived inhibitors, also have been described.78

**Factor IXa Inhibitors**

Factor IXa is essential for amplification of coagulation,79 an observation highlighted by the bleeding that occurs in patients with severe hemophilia B.80 Strategies to block factor IXa include active-site-blocked factor IXa and monoclonal antibody against factor IXa/Xa.

**Active-site Blocked Factor IXa:** By competing with factor IXa for incorporation into the intrinsic tenase complex that assembles on the surface of the activated platelets, active site-blocked factor IXa (factor IXa) inhibits clot formation *in vitro* and blocks coronary artery thrombosis in a canine model.81 These observations support the concept that agents that inhibit factor IXa will modulate thrombosis.

**Antibodies Against Factor IX/IXa:** Monoclonal antibodies against factor IX/IXa have been described.82-84 One antibody blocks factor X activation by factor IXa,82 while the other binds to the Gla-domains of factor IX and inhibits factor IX activation in addition to blocking factor IXa activity.83,84 A chimeric humanized derivative of the latter antibody has antithrombotic activity in a rat arterial thrombosis model.83,84

**Factor Xa Inhibitors**

Both direct and indirect factor Xa inhibitors are under development. Direct factor Xa inhibitors, which bind directly to factor Xa and block its activity, include natural inhibitors, such as tick anticoagulant peptide (TAP),85 antistasin86 and lefaxin,87 and synthetic drugs, such as DX-9065a,88 YM-60828,89 and SK 549.90,91 In contrast to heparin and low molecular weight heparins,74 which block factor Xa in an antithrombin-dependent fashion and have limited ability to inhibit platelet-bound factor Xa, direct inhibitors of factor Xa inactivate factor Xa bound to phospholipid surfaces, as well as free factor Xa.85,89 Synthetic pentasaccharide, an analog of the pentasaccharide sequence of heparin that mediates the interaction of heparin with antithrombin,83 is a new indirect factor Xa inhibitor that is currently being evaluated for prevention and treatment of venous thrombosis in phase III trials.

**Direct Factor Xa Inhibitors**

**Natural Inhibitors:** Isolated from hematophagous organisms, natural inhibitors of factor Xa include TAP, antistasin, and lefaxin.

1. TAP. Originally isolated from the soft tick, Ornithodoros moubata, and now available in a recombinant form, TAP is a 60 amino acid polypeptide that forms a stoichiometric complex with factor Xa.85 TAP is a potent and specific inhibitor of factor Xa. Like the interaction of hirudin with thrombin, TAP appears to bind to factor Xa in a two-step fashion.85 An initial low-affinity interaction involves a site distinct from the catalytic site of the enzymes, which may be analogous to exosite 1 of thrombin. This is followed by a high-affinity interaction with the active site, resulting in formation of a stable enzyme-inhibitor complex.

2. Antistasin. Initially isolated from the salivary glands of the Mexican leech, Haementeria officinalis, antistasin is a 119 amino acid polypeptide.86 Both native and recombinant forms of antistasin are tight-binding, slowly reversible inhibitors of factor Xa.82 Like TAP, antistasin is highly selective for factor Xa.

3. Lefaxin. Isolated from the saliva of the Hemanteria depressa leech, lefaxin is a 30-kd polypeptide.87 Although the gene encoding this protein has yet to be cloned, limited sequence analysis shows no homology between lefaxin and other natural inhibitors of factor Xa.

**Synthetic Factor Xa Inhibitors:** As nonpeptidic, low molecular weight, reversible inhibitors of factor Xa, DX-9065a,88 YM-60828,89 SF 303, and SK 54980,91 are effective
in thrombosis models in laboratory animals. IV DX9065a is currently undergoing phase II testing in patients with unstable angina. YM-50829, a more potent analog of DX-9065a, has been reported to have oral bioavailability in squirrel monkeys, whereas SK 549 exhibits oral bioavailability in rabbits.

Indirect factor Xa inhibitors: With higher affinity for antithrombin than the naturally occurring pentasaccharide, synthetic pentasaccharide has greater inhibitory activity against factor Xa than heparin or low molecular weight heparin. Because it is too short to bridge antithrombin to thrombin, synthetic pentasaccharide enhances the rate of factor Xa inactivation by antithrombin, but it has no effect on the rate of thrombin inhibition. The drug is given subcutaneously on a once-daily basis. Based on promising results in phase II studies, phase III trials comparing synthetic pentasaccharide with low molecular weight heparin for venous thrombosis prevention and treatment are underway.

Inhibitors of the Factor VIIa/Tissue Factor Pathway

Given that coagulation is initiated by the factor VIIa/tissue factor complex, strategies to block this pathway have received much recent attention. These include the development of inhibitors that (a) target tissue factor, (b) inhibit factor VIIa, or (c) target the factor VIIa/tissue factor complex.

Tissue Factor Inhibitors: A major stimulus for the development of tissue factor inhibitors comes from the observation that inhibitory antibodies against tissue factor block the coagulopathy induced by Escherichia coli infusion into baboons. Recently, a soluble tissue factor variant that has reduced cofactor activity for factor VIIa-mediated activation of factor X has been expressed. This mutant had antithrombotic activity in a rabbit arterial thrombosis model. Peptide analogs of various regions of tissue factor inhibit the cofactor activity of tissue factor in vitro by competing with intact tissue factor for factor VIIa binding. These peptides have the potential to serve as prototypes for synthetic small molecule inhibitors.

Factor VIIa Inhibitors: Active-site-blocked factor VIIa (factor VIIai) competes with factor VIIa for tissue factor binding. Factor VIIai attenuates the coagulopathy and improves survival in a baboon sepsis model. Recently, a soluble tissue factor variant that has reduced cofactor activity for factor VIIa-mediated activation of factor X has been expressed. This mutant had antithrombotic activity in a rabbit arterial thrombosis model. Peptide analogs of various regions of tissue factor inhibit the cofactor activity of tissue factor in vitro by competing with intact tissue factor for factor VIIa binding. These peptides have the potential to serve as prototypes for synthetic small molecule inhibitors.

Factor VIIIa/Tissue Factor Inhibitors: Agents that inhibit the factor VIIIa/tissue factor complex include the naturally occurring anticoagulant, TFPI, as well as a family of nematode anticoagulant proteins, of which nematode anticoagulant peptide c2 (NAPc2) is the best characterized. More recently, synthetic inhibitors also have been identified.

1. TFPI. A factor Xa-dependent inhibitor of factor VIIa (Fig 3), only small amounts of TFPI circulate in blood in the free state or are stored in platelets. Most of the TFPI circulates in association with lipoproteins or is bound to the endothelium. Full-length TFPI is released from the endothelium when heparin or low molecular weight heparin is given, presumably because these agents displace TFPI bound to endothelial glycosaminoglycans. When given IV, TFPI is rapidly cleaved into truncated forms by an unknown protease, and it has a short half-life. In pigs, TFPI attenuates injury-induced neointimal hyperplasia and inhibits smooth muscle cell migration in vitro. TFPI attenuates the coagulopathy and improves survival in a sepsis model in baboons or rabbits. Based on these results, TFPI is now undergoing phase III testing in patients with sepsis.

2. NAPc2. Small proteins have been isolated from Ancyllostoma caninum that contain Ascaris-type protease motifs. Some of these proteins directly inhibit factor Xa, whereas others, like NAPc2, bind to a noncatalytic site on factor X or factor Xa and inhibit factor VIIa within the factor VIIa/tissue factor complex. Because it binds to factor X, as well as factor Xa, NAPc2 has a half-life of almost 50 h after subcutaneous injection. Functionally, however, NAPc2 behaves like TFPI, and attenuates sepsis-induced coagulopathy in laboratory animals. NAPc2 is currently undergoing phase II testing for prevention of venous thrombosis in patients undergoing elective knee arthroplasty.

3. Synthetic inhibitors. Several synthetic compounds that inhibit factor VIIa within the factor VIIa/tissue factor complex have been identified. In addition, a novel Kunitz-type inhibitor of factor VIIa, discovered using phage display techniques, had modest efficacy in a rabbit thrombosis model. These observations set the stage for orally active small molecules that inhibit the factor VIIa/tissue factor complex.

Enhancement of Endogenous Anticoagulant Activity

Strategies aimed at enhancing endogenous anticoagulant activity have focused on the protein C anticoagulant pathway (Fig 4). Activated protein C, a naturally occurring anticoagulant, is generated when the thrombin-thrombomodulin complex activates protein C. By proteolytically degrading and inactivating factor Va and VIIIa, activated protein C blocks thrombin-induced autocatalysis. Strategies aimed at enhancing the protein C anticoagulant pathway include administration of (a) protein C or activated protein C concentrates, (b) soluble thrombomodulin, (c) thrombin derivatives that preferentially activate protein C, or (d) small molecules that bind to thrombin and induce
allosteric changes similar to those evoked by the interaction of thrombin with thrombomodulin.

1. **Protein C Derivatives.** IV activated protein C shows promise in the treatment of patients with sepsis-induced coagulopathy,109 and is currently undergoing phase III testing for this indication. Both plasma-derived and recombinant forms of protein C are available. Recombinant activated protein C can be produced by replacing the thrombin-cleaved activation peptide in protein C with a sequence in the insulin receptor precursor. Cells expressing this protein secrete activated protein C because their endogenous insulin receptor processing enzyme effects the necessary activation step.109 Alternatively, the functional activity of recombinant protein C can be enhanced by altering the extent of glycosylation110 or by generating protein C mutants that are more readily activated by thrombin111,112 or have longer half-lives.113

2. **Soluble Thrombomodulin.** Like membrane-bound thrombomodulin, soluble thrombomodulin complexes thrombin and induces a conformational change in the active site of the enzyme that abolishes its procoagulant activity and converts it into a potent activator of protein C.114 Now available by recombinant DNA technology,115 soluble thrombomodulin is an effective antithrombotic agent in a variety of animal models.116,117

In mammalian expression systems, soluble thrombomodulin is produced with or without attached chondroitin sulfate.118 These two forms of soluble thrombomodulin are similar to those isolated from human urine,119 suggesting that they are naturally occurring variants. Chondroitin sulfate-containing forms of soluble thrombomodulin have higher affinity for thrombin and are more potent cofactors for thrombin-mediated protein C activation.115 Using site-directed mutagenesis to increase chondroitin sulfate attachment, greater expression of the active form of thrombomodulin can be achieved.120

3. **Thrombin Variants.** Thrombin can be mutated to dissociate its procoagulant and anticoagulant substrate specificity. Most promising of the thrombin variants generated by site-directed mutagenesis are those that have the Glu residue at position 229 replaced by an Ala or Lys residue.121,122 When infused into animals, these thrombin derivatives have anticoagulant activity because they activate protein C and have minimal procoagulant activity.123

4. **Allosteric Modulators of Thrombin.** Soluble thrombomodulin or thrombin derivatives capable of activating protein C are not orally active, nor are they likely to have antithrombotic activity greater than that produced by protein C or activated protein C administration. A promising approach is the development of small molecules that bind to thrombin and induce conformational changes in its active site similar to those evoked by the interaction of thrombin with thrombomodulin. Small ligands capable of allosterically modulating the substrate specificity of thrombin have recently been identified.124 To explore the utility of this approach in vivo, however, more potent agents will need to be developed.

**Modulation of Endogenous Fibrinolytic Activity**

Although traditional antithrombotic strategies have been aimed at inhibiting platelet function or blocking coagulation, a better understanding of physiologic fibrinolysis has identified potential methods to enhance endogenous fibrinolytic activity. These include (a) inhibition of PAI-1, (b) blocking carboxypeptidase B (TAFI), or (c) inhibition of activated factor XIII (factor XIIIa).

1. **PAI-1 Inhibitors.** PAI-1 is the major physiologic inhibitor of t-PA and urinary-type plasminogen activator. Consequently, inhibition of PAI-1 results in increased endogenous fibrinolytic activity. PAI-1 activity can be reduced by (i) decreasing PAI-1 gene expression, or (ii) by reducing the activity of PAI-1. Lipid-lowering drugs, such as niacin and fibrates, decrease PAI-1 synthesis in vitro.125,126 These agents are not specific for PAI-1, however, and they also affect the synthesis of other proteins.

Peptides have been identified that block PAI-1 activity either by preventing insertion of the reactive center loop on cleavage by the target protease127 or by converting PAI-1 into a latent conformation.128 However, the effectiveness of these agents has yet to be tested in vivo. A more promising strategy is the development of small-molecule PAI-1 inhibitors, some of which exhibit antithrombotic activity in vivo.128

2. **Procarboxypeptidase B Inhibitors.** Procarboxypeptidase B or TAFI is a latent carboxypeptidase B-like enzyme that is activated by thrombin in a reaction that is enhanced in the presence of thrombomodulin.12 On activation, procarboxypeptidase B attenuates fibrinolysis, presumably by elevating carboxy-terminal lysine residues from fibrin.129 Removal of these lysine residues decreases plasminogen or plasmin binding to fibrin, thereby retarding the lytic process. Given this mechanism of action, inhibitors of procarboxypeptidase B should enhance fibrinolytic activity, a concept supported by studies in dogs and rabbits demonstrating that a potato-derived carboxypeptidase B inhibitor increases t-PA-induced thrombolysis.130,131

Recently, thrombin variants with decreased ability to activate procarboxypeptidase B, yet normal protein C-activating activity, have been identified.132 These findings raise the possibility that the antifibrinolytic activity of thrombin can be blocked without affecting its anticoagulant activity.

3. **Factor XIIIa Inhibitors.** A thrombin-activated transglutaminase, factor XIIIa crosslinks the α- and γ-chains of fibrinogen to form α-polymers and γ-dimers, respectively. Crosslinking stabilizes the fibrin polymer and renders it more refractory to degradation by plasmin.133,134 Inhibition of factor
XIIIa, therefore, has the potential to increase the susceptibility of the thrombus to lysis.

Agents that react with the active-site thiol of factor XIIIa serve as acceptor amino groups or chelate calcium will inhibit factor XIIIa. However, these compounds lack selectivity and inactivate other transglutaminases, and most have short half-lives. Tridegin, a peptide isolated from the giant Amazon leech, Haementeria ghilianii, is a specific inhibitor of factor XIIIa and enhances fibrinolysis in vitro when added before clotting of fibrinogen. Destabilase, a leech enzyme that hydrolyzes crosslinks, also provides a promising approach to reversing the consequences of factor XIIIa-mediated fibrin crosslinking.

CHALLENGES AND OPPORTUNITIES FOR NEW ANTICOAGULANT DRUGS

Further clinical testing is needed to define the role of new anticoagulants in the prevention and treatment of venous and arterial thrombosis. In addition to establishing the benefit-to-risk profiles of new agents, cost-effectiveness analyses will be critical when evaluating drugs with marginal advantages over existing agents. The challenges for the development of drugs for venous thromboembolism will be different from those for arterial thrombosis.

New Drugs for Venous Thromboembolism

Although hirudin has been shown to be superior to low-dose heparin or low molecular weight heparin for thromboprophylaxis in patients undergoing major orthopedic surgery of the lower limbs, hirudin is unlikely to gain wide acceptance for this indication unless its cost is comparable to that of low molecular weight heparin. Cost considerations may also limit the utility of synthetic pentasaccharide or NAPc2 in this setting, should these drugs prove superior to those in current use.

The success of hirudin for thromboprophylaxis in high-risk patients bodes well for orally available agents in this class. With progressive reductions in hospital stay and evidence that the risk of thrombosis remains high for several weeks after major orthopedic surgery to the lower limbs, orally available drugs that have a rapid onset of action and need little or no laboratory monitoring may prove to be more convenient than low molecular weight heparin or coumarin derivatives. Although oral delivery systems for heparin or low molecular weight heparin are promising, variable absorption may limit the utility of this approach. In contrast, a prodrug form of melagatran exhibits good bioavailability after oral administration in patients undergoing coronary angioplasty or when used as an adjunct to streptokinase in patients with acute myocardial infarction. Approval for bivalirudin as an alternative to heparin in patients undergoing coronary angioplasty is pending, but further trials are needed to determine whether bivalirudin reduces mortality when used as an adjunct to streptokinase, or whether it decreases the need for glycoprotein IIb/IIIa antagonists in patients undergoing percutaneous coronary interventions.

Inhibition of clotting enzymes higher in the coagulation cascade than thrombin may be effective, but safety will be a major consideration. Although the factor VIIa/tissue factor complex is an attractive target for inhibition because it initiates coagulation at sites of arterial injury, tissue factor is essential for hemostasis. Consequently, the safety of this approach requires careful evaluation.

Given that atherothrombotic disease develops over decades, long-term therapy is likely to be needed to prevent thrombosis at sites of plaque rupture. Drugs that enhance the activity of natural anticoagulants, such as protein C or TFPI, or that increase endogenous fibrinolytic activity are likely to be safe. The challenge will be development of orally available small molecules that accomplish these tasks.

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