

New perspectives, new strategies in venous thromboembolic disease

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Haemostasis

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Molecular basis of anticoagulation with oral anticoagulants

Charles T. Esmon

Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, University of Oklahoma Health Sciences Center, Howard Hughes Medical Institute, Oklahoma City, Oklahoma, USA

Summary

The classic orally active anticoagulant is warfarin which works by blocking the vitamin K-dependent carboxylation of coagulation factors II (prothrombin), VII, IX and X. In addition, it also inhibits activation of several anticoagulant proteins, including protein C, protein S and protein Z. For this reason, and because of important side effects associated with warfarin therapy, it is likely that agents that are more selective in terms of their inhibition of coagulation proteins will have a wider therapeutic window and exhibit less patient-to-patient variability of effect. Most developmental work on oral anticoagulants targeting enzymes of coagulation has targeted factor Xa (FXa) or thrombin, since both are part of the common pathway through which coagulation proceeds, and inhibition of either will result in anticoagulation. Indeed, clinical trials of both classes of inhibitor have shown them to be effective. However, some differences in response to FXa and thrombin inhibitors are to be

expected. The clotting time of plasma is much more sensitive to small changes in the concentration of thrombin than of FXa, suggesting that FXa inhibitors should have a wider therapeutic range. Thrombin catalyzes not only coagulant reactions but the generation of activated protein C, a cytoprotective, anti-inflammatory, and anticoagulant enzyme. In contrast, FXa has only procoagulant and proinflammatory functions. Finally, thrombin clearance from the circulation is dependent on both thrombomodulin and vascular heparin-like molecules. Thrombomodulin is down-regulated in a variety of diseases including atherosclerosis and diabetes which could conceivably add to therapeutic variability of oral direct thrombin inhibitors (DTIs).

Keywords

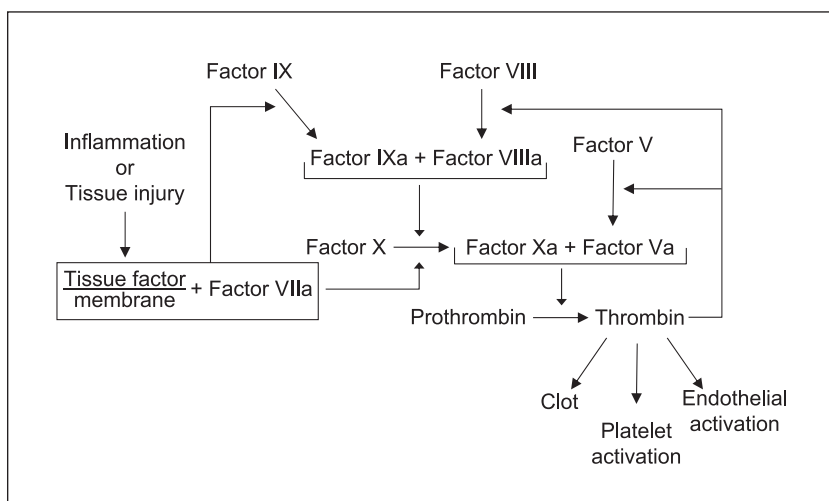
Anticoagulation, thrombosis, factor Xa, thrombin

Warfarin is the classic orally active anticoagulant. It functions as an anticoagulant by inhibiting the biosynthesis of FII (prothrombin), FVII, FIX, FX (see Fig. 1). It also inhibits activation of the natural anticoagulant proteins C, S, and Z (1). In addition, it inhibits the function of matrix Gla protein, a vessel wall protein involved in preventing blood vessel calcification (2, 3). These "off-target" activities may contribute to the adverse events seen with warfarin therapy and to narrowing of the therapeutic window. The fundamental rationale behind the development of new oral anticoagulants is straightforward: to overcome these and other limitations of warfarin such as its multiple drug and dietary interactions and genotype-based differences in its metabolism and thus its anticoagulant effect.

Heparins are the classic parenteral anticoagulants, interacting with antithrombin to catalyze inactivation of both FXa and thrombin. Inacti-

vation of either FXa or thrombin results in anticoagulation since both enzymes lie on the common pathway of the coagulation cascade and are required for thrombin generation and conversion of fibrinogen to fibrin. Unfractionated heparin (UFH) is a heterogeneous mixture of molecules containing varying numbers of monosaccharide units. In low-molecular-weight heparins (LMWHs), which are produced by depolymerization of UFH, the polysaccharide chains are on average much shorter. For a heparin molecule to catalyze inhibition of thrombin, it must bind both to antithrombin and to thrombin (FIIa); this requires at least 18 saccharide units. As mean molecular weight of a heparin preparation decreases, its relative ability to inactivate thrombin decreases. Thus, while UFH has by definition an anti-FXa:anti-FIIa ratio of 1:1, LMWHs have an anti-FXa:anti-FIIa ratio as high as 4:1 (4). On the other hand, to catalyze inhibition of FXa, a heparin molecule must bind only to antithrombin via a specific penta-

Figure 1: A model of blood coagulation. In the extrinsic pathway, coagulation is initiated by exposure of tissue factor (TF) to the blood. TF then binds activated factor VII (FVIIa). On negatively charged membrane surfaces, this complex can then activate either FIX (classically referred to as part of the intrinsic pathway) or FX. The FIXa then complexes with FVIIIa on negatively charged membrane surfaces (complexes forming on negatively charged membrane surfaces are indicated with the underline) to form a FX activation complex. FXa, whether formed as described above or directly from the TF-FVIIa complex, has the same structure and function. It forms a complex with FVa on membrane surfaces to become a potent prothrombin activation complex giving rise to thrombin. FV and FVIII circulate as inactive precursors that are rapidly proteolytically converted to active factors by thrombin.



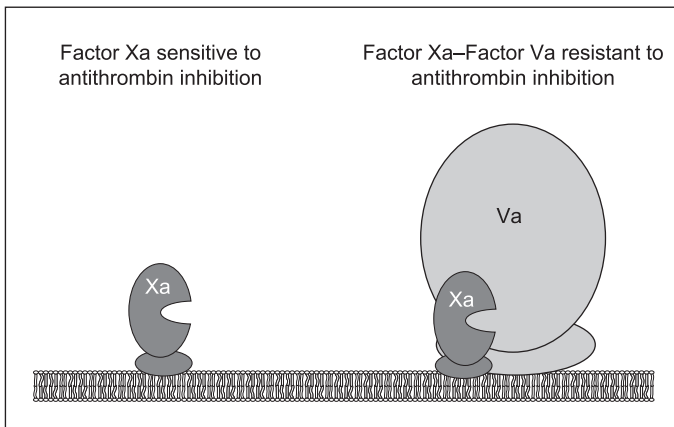


Figure 2: Differences in FXa sensitivity to inhibition by heparin. Free FXa is sensitive to inhibition by antithrombin and heparin whereas FXa in the prothrombinase complex is not. See text for details.

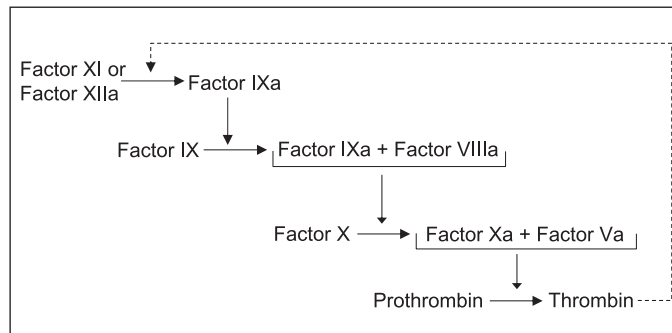


Figure 3: Thrombin activation of FXI provides feedback amplification of coagulation. Thrombin can activate FXI thus generating a TF-independent mechanism for the activation of FIX. Since FXI is not directly activated by TF, it has historically been assigned to the intrinsic coagulation pathway. FXII, which can be activated by contact with foreign substances like glass, is of unknown, but apparently limited importance in hemostasis.

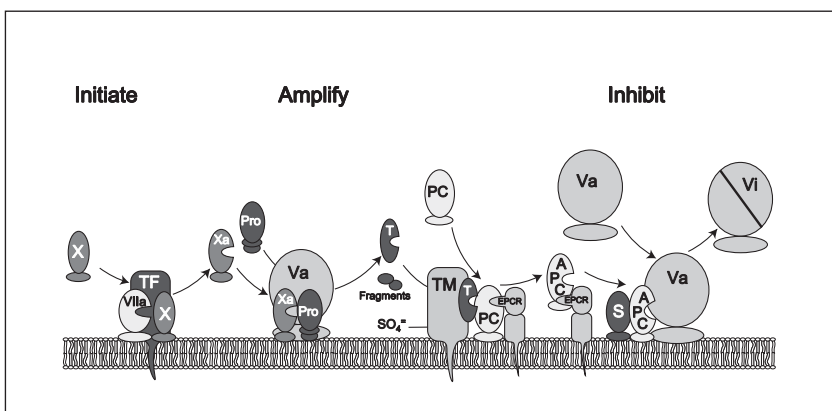


Figure 4: A model of coagulation including the protein C anticoagulant pathway. Thrombin is generated as described in Figure 1. Thrombin (T) then binds to thrombomodulin (TM), primarily on the endothelial cell surface, at which time thrombin's clotting and cellular activation activities are lost, but the ability to activate protein C is enhanced approximately 1,000-fold. Thrombin bound to thrombomodulin is rapidly inactivated by either antithrombin or protein C inhibitor. Both inactive thrombin-inhibitor complexes then dissociate from thrombomodulin. Protein C activation is enhanced *in vivo* approximately 20-fold by the endothelial cell protein C receptor (EPCR). Activated protein C (APC) can remain bound to EPCR where it can signal cells eliciting cytoprotective and anti-inflammatory responses. When APC dissociates from EPCR, it can bind protein S, forming an effective factor FVa and FVIIIa (not shown) inactivation complex.

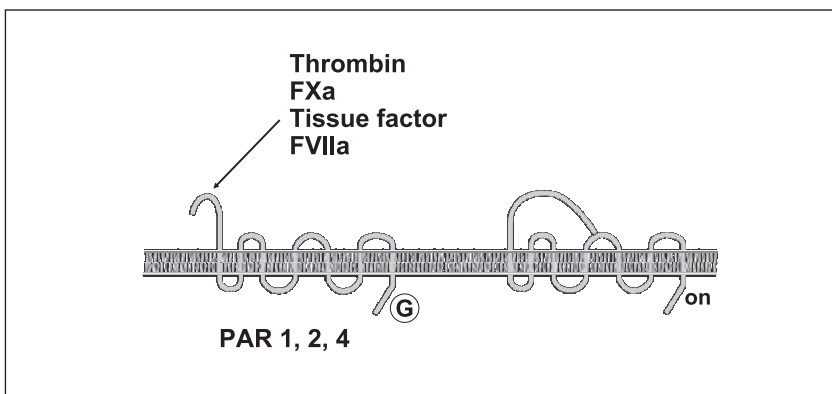


Figure 5: Coagulation factors including thrombin, FXa, and FVIIa bound to TF activate protease-activated receptors (PARs). Upon binding, the coagulation factor cleaves a peptide bond creating a new N terminus and revealing a previously cryptic ligand which then activates the receptor of which it is part. Signaling elicits a variety of responses including loss of endothelial barrier function, expression of adhesion molecules, and generation of inflammatory molecules.

saccharide sequence. Fondaparinux consists exclusively of this pentasaccharide sequence and its activity therefore is limited to FXa inhibition (5). Other parenteral anticoagulants include direct thrombin inhibitors (DTIs) such as argatroban, bivalirudin, and desirudin (6). These agents inhibit thrombin exclusively and have no effect on FXa.

FXa exists in two forms: free and in complex with FVa in the prothrombinase complex (Fig. 2). In the presence of sufficient antithrombin, UFH, LMWHs, and fondaparinux effectively catalyze inactivation of free FXa but not FXa contained in the prothrombinase complex (7). Thrombin exists in two forms as well: free and clot-bound. Clot-bound thrombin re-

mains enzymatically active but is also stereochemically inaccessible to indirect thrombin inhibitors bound to antithrombin. In contrast, low-molecular-weight direct inhibitors (whether parenteral or oral) can effectively inactivate both forms of FXa (8) or thrombin (9).

In the coagulation cascade (Fig. 1), thrombin plays a variety of roles. In addition to catalyzing the conversion of fibrinogen to fibrin, it is a potent activator of FV and FVIII (1) and can feed back to activate FXI, thereby amplifying the coagulation process (Fig. 3) (10, 11). Thrombin is the most potent platelet agonist (12). Opposing some of these procoagulant functions, very low levels of thrombin, in conjunction with thrombomodulin,

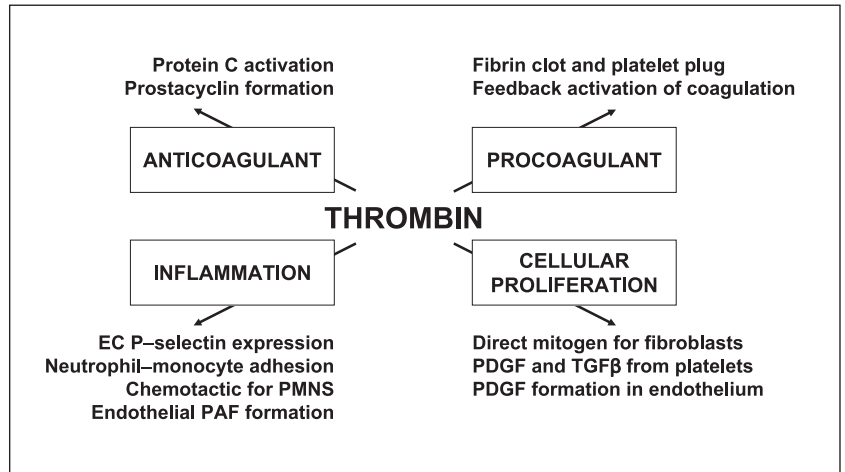


Figure 6: Thrombin has multiple physiologic functions. Thrombin can elicit mitogenic, adhesive, anticoagulant and procoagulant activity.

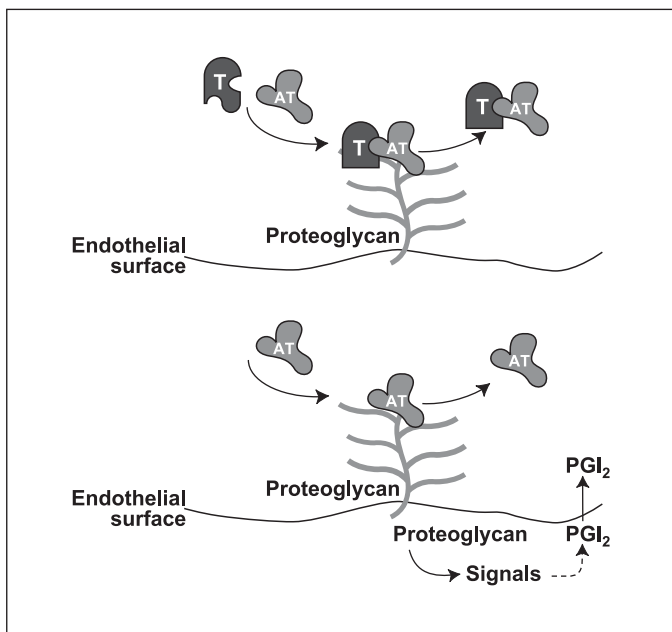


Figure 7: The heparin-antithrombin mechanism for thrombin clearance from the circulation. Heparin-like proteoglycans line the vessel wall. These can interact with thrombin (T) and antithrombin (AT) forming the template necessary for rapid thrombin inactivation. Once the thrombin-antithrombin complex forms, it rapidly releases from the vascular proteoglycan.

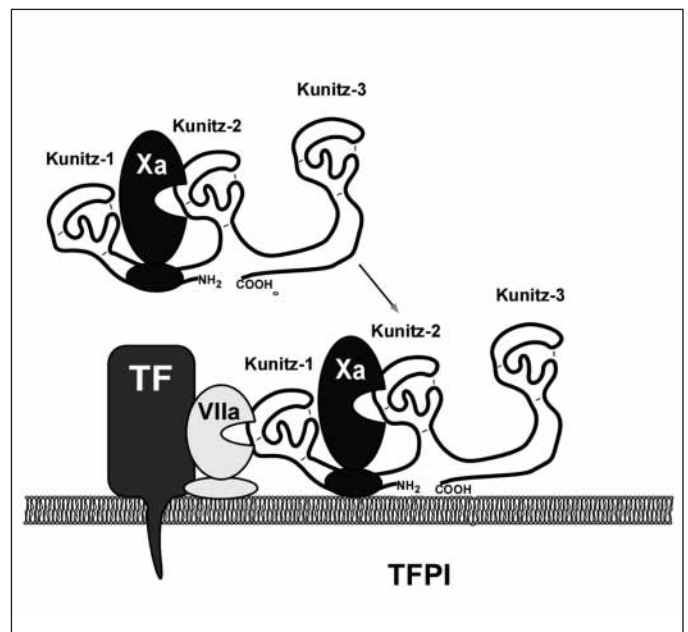


Figure 8: The tissue factor pathway inhibitor (TFPI) mechanism for inhibition of the TF-FVIIa complex. TFPI is composed of three similar domains structurally related to pancreatic trypsin inhibitor. Domain 1 binds to the TF-FVIIa complex relatively weakly and domain 2 binds tightly to FXa. Once FXa binds to TFPI it increases the concentration of the TFPI near the surface of negatively charged membranes and the increased concentration results in effective TF-VIIa inhibition.

activate protein C (1) (Fig. 4). Protein C is a critical anticoagulant with cytoprotective (13) and anti-inflammatory activities. Homozygous protein C deficiency results in neonatal death (14), indicating the importance of this pathway. In contrast to the multiplicity of thrombin's functions in hemostasis and thrombosis, FXa plays primarily, if not exclusively, a role in generating thrombin through the activation of prothrombin (Fig. 1).

In addition to their role in coagulation, thrombin and FXa are potent activators of various cell types, primarily through their interaction with G-protein-coupled protease-activated receptors (PARs). When these enzymes bind to the N-terminal exodomain of a PAR (Fig. 5), they catalyze exposure of a new N terminus by cleaving a peptide bond, thus revealing a previously hidden ligand (12). This "tethered ligand" then acti-

vates the receptor of which it is physically part and thus the coupled G proteins, eliciting a variety of cellular responses including the production of cytokines and expression of adhesion molecules (12). Whereas both thrombin and FXa activate PARs, thrombin's multiplicity of biologic functions (Fig. 6) may counterbalance its pro-inflammatory and procoagulant effects (1).

In healthy individuals, spontaneous thrombosis is an extremely rare event. In addressing the attributes of an ideal therapeutic anticoagulant, it is useful to consider the mechanisms by which coagulation is controlled physiologically. There are three major natural anticoagulant mechanisms (1): heparin-antithrombin (15), tissue factor pathway inhibitor (TFPI) and the protein C pathway (16). The heparin-antithrombin mechanism is

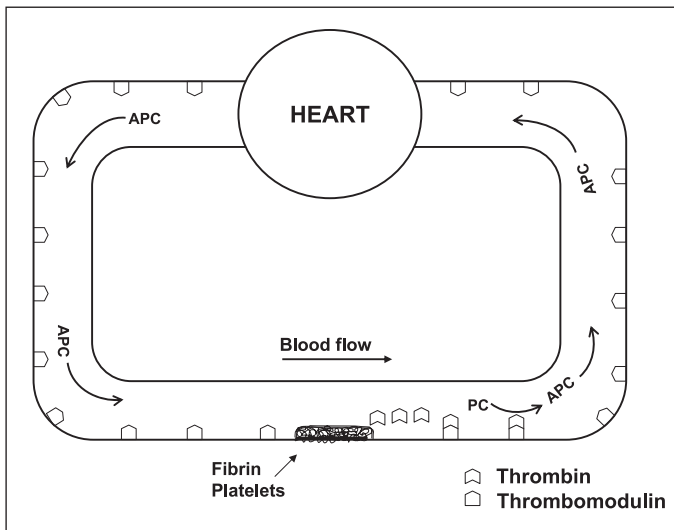


Figure 9: A schematic representation of the function and distribution of the protein C anticoagulant pathway. At the site of an injury, thrombin is generated to seal the wound. Some of the thrombin is carried downstream by the blood flow at which time it can bind to thrombomodulin and activate protein C. Unlike most coagulation enzymes, activated protein C is slowly neutralized in the circulation with a half-life of about 15 minutes. In contrast to the activated protein C, thrombin bound to thrombomodulin is inactivated in seconds, insuring a rapid cessation of further protein C activation once thrombin generation is controlled. This, like the other two natural anticoagulant mechanisms described above, constitutes a natural "time delay" in the anticoagulant response.

illustrated in Figure 7, the TFPI mechanism in Figure 8 and the protein C pathway in Figure 9. Common to these mechanisms is a short delay between the activation of coagulation and the manifestation of their inhibitory effects. Conceptually, an ideal anticoagulant might share this property of facilitating hemostasis while still blocking thrombosis.

For an anticoagulant to be useful, a dose must be identified that is sufficient to prevent thrombosis without causing excessive bleeding. For different clotting factors, the plasma concentration necessary for coagulation varies. Such is the case with thrombin and FXa, as illustrated in Figure 10. In these experiments, the concentration of thrombin and FXa were adjusted to give an initial clotting time of about 10 seconds. The samples were then diluted progressively to the same extent and the clotting times of the resultant dilutions determined. The clotting time exhibited a much more rapid response to dilution of thrombin than it did to dilution of FXa, suggesting that the therapeutic range for agents targeting thrombin would be narrower than for those targeting FXa.

Despite this prediction, this is no clinical evidence that the oral DTI dabigatran is less safe than the FXa inhibitor rivaroxaban. This disjunction between prediction and experience might reflect that fact that binding of low-molecular-weight, active-site directed inhibitors to coagulation enzymes does not prevent interaction of these enzymes with cofactors, FVa in the case of FXa and thrombomodulin in the case of thrombin. Such binding, however, prevents the inactivation of the enzyme by antithrombin, the major natural clearance mechanism for activated coagulation factors.

Therefore, in the case of reversible DTIs the following sequence of events might take place, effectively widening the therapeutic range for these agents in circumstances where the protein C anticoagulation pathway is normal:

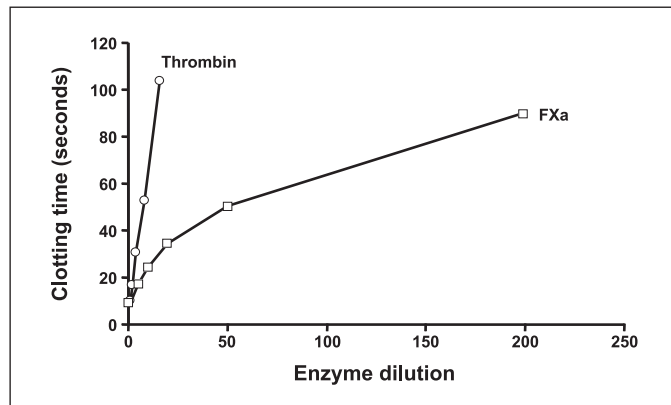


Figure 10: Coagulation times with thrombin have a much steeper concentration dependence than with FXa. Thrombin and FXa were diluted to give approximately equal coagulation times in a one-stage coagulation assay. Plasma (0.1 ml) was clotted by thrombin or FXa in a final reaction volume of 0.4 ml. For FXa, the reaction also contained 0.1 ml of 140 µg/ml phospholipid (phosphatidylethanolamine 40%, phosphatidylserine 20%, and phosphatidylcholine 40%). Coagulation was initiated in the case of FXa by the addition of 0.1 ml of 0.025 M calcium chloride. The stock solutions were then diluted progressively as indicated on the figure and the plasma clotting times were measured for each dilution.

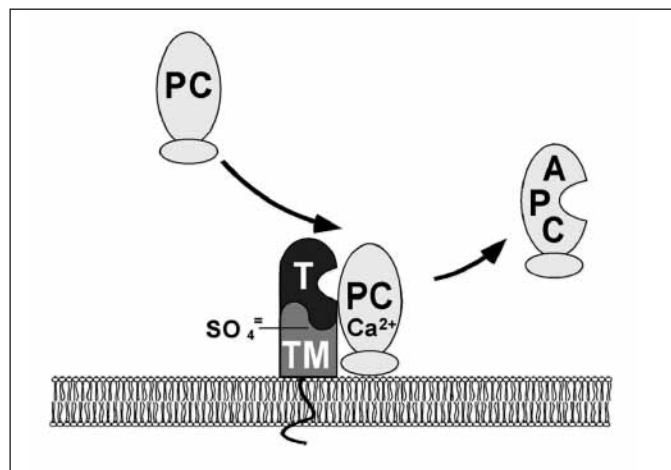


Figure 11: A simplified model of the thrombin (T)-thrombomodulin (TM) complex activating protein C (PC).

1. The inhibitor binds thrombin in the larger veins, thereby partially protecting it from inactivation by antithrombin.
2. The thrombin-DTI complex is carried to the microcirculation where, because the endothelial cell surface area exposed per ml of blood rises markedly (17), there is a dramatic increase in the concentration of thrombomodulin as well as heparin-like proteoglycans.
3. Thrombin (still bound to the DTI) binds to thrombomodulin in the microcirculation. At such time as the reversible inhibitor dissociates from thrombin, the thrombin-thrombomodulin complex activates protein C, and the uninhibited thrombin is rapidly inactivated by antithrombin in conjunction with heparin-like proteoglycans.

This model is depicted in Figures 11 and 12.

If this model is accurate, it suggests the potential for clinical complications with thrombin inhibitors in patient groups in which there is derangement of the protein C anticoagulation pathway. Both endothelial

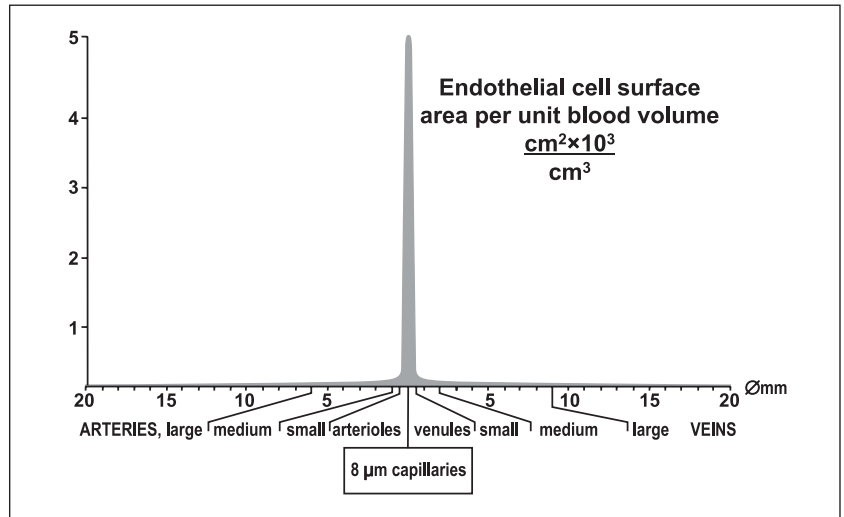


Figure 12: The relationship between blood vessel size and the endothelial cell surface area exposed per ml of blood. It is apparent that the endothelial cell surface exposed to the blood rises dramatically when blood moves from the large vessels into the microcirculation which in turn results in much higher thrombomodulin and heparin-like proteoglycan concentrations.

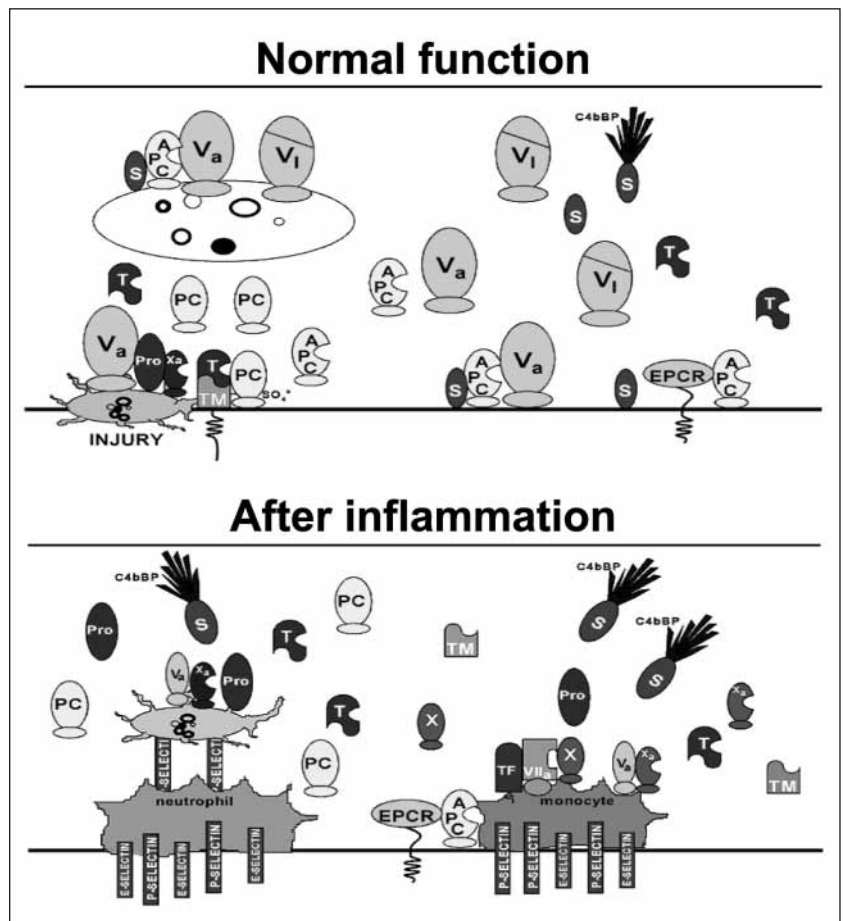


Figure 13: The impact of disease on the interaction of the blood vessels with coagulation components. When inflammation or a variety of other disease states is present, there is loss of the natural anticoagulant functions and adhesion of leukocytes to adhesion molecules, thereby promoting coagulation. This suggests that the ability to bind thrombin and modulate its function will be diminished, thus influencing the potency of direct thrombin inhibitors as described in the text.

cell protein C receptor and thrombomodulin expression are down-regulated by inflammation as seen in a variety of diseases (1) including sepsis (18), diabetes (19), inflammatory bowel disease (20), and atherosclerosis (1, 21) (Fig. 13). Paradoxically then, in diseases where thrombotic complications are prevalent, the therapeutic range for direct thrombin inhibitors can be predicted to narrow. Whether this prediction proves accurate will require further clinical investigation.

The advantages of the new oral anticoagulants directed against either thrombin or FXa are numerous. They include:

1. Defined targets that are not part of the natural defence mechanism against coagulation;
2. Minimal dietary and drug interactions; and
3. Predictable dose-response curves minimizing, if not eliminating, the need for monitoring.

For the reasons outlined above, the author feels that FXa inhibitors will ultimately prove safer and easier to use than thrombin inhibitors. The reasons for this preference are summarized below:

1. The only known functions of FXa are either procoagulant or pro-inflammatory. Thrombin has both of these activities as well as indirect anticoagulant, anti-inflammatory and anti-apoptotic activity.

2. FXa has a shallower dose-response curve than thrombin. This suggests that maintaining the appropriate dose range for FXa should be easier than for thrombin.

3. The efficacy of FXa inhibitors, unlike that of thrombin inhibitors, should not depend on the health of the vasculature.

Future clinical investigations should put these predictions to a direct test.

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Correspondence to:
 Charles T. Esmon
 Cardiovascular Biology Research Program
 Oklahoma Medical Research Foundation
 Howard Hughes Medical Institute
 Departments of Pathology and Biochemistry & Molecular Cell Biology
 University of Oklahoma Health Sciences Center
 825 N.E. 13th Street
 Oklahoma City, OK 73104, USA
 Tel.: +1 405 271 6474, Fax: +1 405 271 3137
 E-mail: charles-esmon@omrf.org

New approaches to oral anticoagulation: Direct thrombin inhibitors

Jeffrey I. Weitz

Departments of Medicine and Biochemistry, McMaster University and Henderson Research Centre, Hamilton, Ontario, Canada

Summary

Thrombin is responsible for the conversion of fibrinogen to fibrin – the essential step in thrombus formation. Thrombin is also a potent platelet agonist, and even small amounts of thrombin can amplify coagulation by feedback activation of factors V and VIII. Because of its central roles in coagulation and platelet activation, thrombin is an attractive target for new anticoagulants. All anticoagulants exert their effects by directly or indirectly reducing thrombin generation and/or inhibiting thrombin activity. Vitamin K antagonists, low-molecular-weight heparins (LMWHs), and unfractionated heparin attenuate thrombin generation or indirectly inhibit thrombin activity. None of these agents inhibits clot-bound thrombin. Direct thrombin inhibitors (DTIs) inhibit thrombin by binding to exosite 1 (which acts as a dock for substrates such as fibrinogen) and/or the active site of thrombin. Because of their antithrombin-independent mode of action, DTIs inactivate both free and clot-bound thrombin. Further, unlike heparin, they do not bind to plasma proteins, resulting in a more predictable anticoagulant effect, and are not neutralized by platelet factor 4, thus posing no risk of heparin-induced thrombocytopenia. The clinical potential of DTIs is highlighted by the results of trials of ximelagatran, an oral DTI. Ximelagatran was at least as effective as LMWH or warfarin for preventing venous thromboembolism (VTE) and as effective as warfarin for preventing stroke and systemic embolism in patients with atrial fibrillation (AF). Unfortunately, ximelagatran was withdrawn worldwide because of potential liver toxicity. Dabigatran etexilate is a new oral DTI prodrug that is rapidly absorbed and converted to dabigatran. Dabig-

atran is a potent, competitive, and reversible inhibitor of thrombin. The plasma half-life of dabigatran is 12–17 hours allowing for once-daily dosing, and elimination is primarily via the kidneys. The clinical potential of dabigatran etexilate is being investigated in the RE-VOLUTION™ clinical trial program, involving more than 38,000 patients worldwide. Results from the RE-MODEL™ and RE-NOVATE™ trials showed that dabigatran etexilate (at once-daily doses of 220 mg or 150 mg) is as effective as the European regimen of enoxaparin at reducing the risk of VTE after hip or knee replacement surgery, and has a similar safety profile. Based on these findings, dabigatran etexilate has been approved in Europe and Canada for thromboprophylaxis after hip or knee replacement surgery. Ongoing RE-VOLUTION trials are assessing the efficacy and safety of dabigatran etexilate for secondary VTE prevention, acute VTE treatment, secondary prevention of cardiac events in patients with acute coronary syndrome, and stroke prevention in patients with AF. With many of these studies well underway, it is clear that dabigatran etexilate is not associated with hepatic toxicity. How dabigatran etexilate will compare with warfarin remains to be determined. However, the promising results with dabigatran etexilate for VTE prophylaxis bring us one step closer to finding a replacement for warfarin.

Keywords

Oral direct thrombin inhibitors, dabigatran, thrombosis, anticoagulation

Introduction

For the past 65 years, vitamin K antagonists (VKAs) have been the only available oral anticoagulants. Although effective, these agents have limitations that render them difficult to administer effectively (1). These limitations have prompted a search for new oral anticoagulants. Whereas VKA exert their anticoagulant effects by reducing the synthesis of functional levels of factors (F) II, VII, IX, and X, these new oral anticoagulants are more targeted and inhibit only a single clotting enzyme. Most of the novel oral anticoagulants under development target either thrombin or FXa.

Focusing on new oral anticoagulants that target thrombin, this paper (a) describes the rationale for choosing thrombin as a target; (b) reviews the data supporting thrombin as a target; (c) outlines the evolution of DTIs; (d) lists potential advantages of DTIs over indirect inhibitors; (e) reviews the pharmacology and clinical trial data with dabigatran etexilate, a new oral thrombin inhibitor; and (f) provides perspective as to how emerging data with the new oral anticoagulants will change the face of oral anticoagulant therapy.

Why target thrombin?

Thrombin is an obvious choice as a target for new anticoagulants because of its central role in coagulation. The final effector in blood coagulation, thrombin not only converts fibrinogen to fibrin, but also renders the fibrin network resistant to lysis through its capacity to activate FXIII and thrombin-activatable fibrinolysis inhibitor (TAFI). Activated FXIII renders fibrin resistant to degradation by cross-linking adjacent fibrin monomers and by cross-linking α_2 -antiplasmin, the major inhibitor of plasmin, to fibrin, thus rendering the clot less susceptible to lysis by plasmin (2). TAFI also attenuates fibrinolysis when activated by the thrombin-thrombomodulin complex. A latent carboxypeptidase B-like enzyme, activated TAFI attenuates fibrinolysis by cleaving C-terminal lysine residues from fibrin, thereby removing sites to which plasmin binds (3).

In addition to converting fibrinogen to fibrin, thrombin amplifies its own generation by activating FV and FVIII, key cofactors for prothrombinase and intrinsic tenase, respectively. Thrombin also can activate FXI, which, by activating FIX, generates the enzyme component of the intrinsic tenase complex. Because of these multiple feedback reactions, inhibition of thrombin not only attenuates fibrin formation, but also blocks thrombin generation.

Characteristics	Hirudin	Bivalirudin	Argatroban	Ximelagatran	Dabigatran etexilate
Route of administration	Parenteral	Parenteral	Parenteral	Oral	Oral
Sites of interaction with thrombin	Active site & exosite 1	Active site & exosite 1	Active site	Active site	Active site
Half-life	60–120 minutes	25 minutes	45 minutes	2–5 hours	12–17 hours
Clearance	Kidney	Kidney, liver, other sites	Liver	Kidney	Kidney

Table 1: Characteristics of DTIs.

Beyond its role in coagulation, thrombin is a potent platelet agonist (4). Thrombin generated at sites of vascular injury recruits and activates platelets, and the resultant platelet aggregates are then incorporated into the thrombus. This process further augments thrombin generation because activated platelets provide an anionic phospholipid surface on which clotting factor complexes assemble. Formation of such complexes augments coagulation by increasing their catalytic efficiency by several orders of magnitude.

With its central role in thrombosis, thrombin is a good target for new anticoagulants. However, when choosing thrombin as a target, the critical question is whether thrombin can be inhibited long-term without compromising hemostasis. The answer to this question comes from the results of clinical trials with DTIs.

Evidence supporting thrombin as a target for new oral anticoagulants

There has been an evolution of DTIs as we have moved from irreversible to reversible inhibitors and from parenteral to orally active agents (5, 6). The properties of these drugs are summarized in Table 1. Although all of the drugs directly bind thrombin in a 1:1 stoichiometric fashion, they differ in their sites of interaction with the enzyme. Thus, hirudin (and its synthetic recombinant forms, desirudin and lepirudin) and bivalirudin are bivalent inhibitors that bind to both the active site of thrombin and to exosite 1, its substrate-binding domain. In contrast, argatroban, melagatran, and dabigatran are univalent inhibitors that bind solely to thrombin's active site. Hirudin forms an essentially irreversible complex with thrombin, whereas the other agents bind thrombin in a reversible fashion. Reversible DTIs are potentially safer than irreversible inhibitors because thrombin activity is not permanently blocked.

Table 2: Potential advantages of DTIs over indirect inhibitors.

Advantage	Mechanism
Retain activity in presence of platelet-rich thrombi	Do not bind PF4 or von Willebrand factor
Better suppression of thrombus growth	Inhibit fibrin-bound thrombin as well as free thrombin
Predictable anticoagulant response	Do not bind plasma proteins
No risk of heparin-induced thrombocytopenia	Do not bind PF4

Ximelagatran was the first oral DTI. Given in fixed doses without coagulation monitoring, ximelagatran underwent extensive phase III evaluation. In a series of randomized controlled trials, ximelagatran was shown to be (a) as efficacious as warfarin or low-molecular-weight heparin (LMWH) for thromboprophylaxis after hip or knee replacement surgery; (b) as efficacious as warfarin for stroke prevention in patients with AF; and (c) as efficacious as LMWH followed by warfarin for treatment of acute VTE (7). In all of these indications, rates of bleeding with ximelagatran were similar to those observed with conventional anticoagulant therapy (7). Unfortunately, ximelagatran was withdrawn from the market because of the risk of hepatic toxicity with prolonged exposure to the drug (8). Nonetheless, the results of the ximelagatran program provide an important "proof of principle": not only is thrombin a reasonable target for new oral anticoagulants, but long-term thrombin inhibition can be effected safely (at least from the hemorrhagic standpoint) with an oral medication that is given in fixed doses without coagulation monitoring.

Potential advantages of DTIs

Direct thrombin inhibitors (DTIs) have potential advantages over indirect inhibitors of thrombin, such as heparin (Table 2). The activity of heparin is compromised in the vicinity of platelet-rich thrombi because heparin is neutralized by platelet factor 4 (PF4) and high-molecular-weight multimers of von Willebrand factor released from activated platelets. In contrast, these platelet-derived proteins have no effect on the activity of DTIs. Consequently, DTIs have the potential to better suppress thrombus growth. Adding to this benefit is the capacity of DTIs to not only inactivate free thrombin, but to inhibit thrombin bound to fibrin. By contrast, fibrin-bound thrombin is protected from inactivation by the heparin-antithrombin complex.

DTIs produce a more predictable anticoagulant response than heparin because they do not bind plasma proteins other than thrombin. Because of this predictability, the newer DTIs can safely be given with little or no coagulation monitoring. Finally, because DTIs do not bind PF4, there is no risk of heparin-induced thrombocytopenia (HIT). In fact, DTIs are often used for treatment of patients with HIT.

Dabigatran etexilate

Dabigatran etexilate is a new oral DTI. This section describes its pharmacology and reviews the clinical trial data with this agent.

Pharmacology

A prodrug, dabigatran etexilate has an oral bioavailability of 6% (9). Once absorbed, dabigatran is rapidly converted by esterases to dabigatran, a potent and reversible inhibitor of the active-site thrombin. Plasma levels of dabigatran peak two hours after drug administration and dabigatran has a half-life of 12–17 hours, which permits once- or twice-daily administration. Dabigatran is predominantly cleared via the kidneys with 80% of the drug excreted unchanged. Dabigatran's pharmacokinetic and pharmacodynamic characteristics are predictable (9), and no clinically relevant interactions have been observed with atorvastatin (10) or with the non-steroidal anti-inflammatory drug diclofenac (11). Therefore, combination therapy with these agents is feasible. However, the P-glycoprotein inhibitor quinidine is contraindicated.

Clinical trials with dabigatran etexilate

Dabigatran is undergoing extensive phase III evaluation in a clinical trial program known as RE-VOLUTION™. This program will involve over 38,000 patients world-wide. Phase III clinical trials in patients undergoing total hip or knee replacement surgery (THR and TKR, respectively) have been completed. Trials in patients with established VTE, AF, and acute coronary syndrome (ACS) are underway.

Prevention of VTE after major orthopedic surgery

Dabigatran etexilate has been assessed in a phase II dose-ranging study in patients undergoing THR or TKR (12). In this study, a dose-dependent reduction in the incidence of VTE was observed, with a significantly lower rate of deep venous thrombosis (DVT) noted for dabigatran etexilate 225 mg twice-daily and 300 mg once-daily relative to enoxaparin. The incidence of major bleeding was significantly lower with dabigatran etexilate 50 mg twice-daily than with enoxaparin, but increased with higher doses of dabigatran etexilate.

The efficacy and safety of dabigatran etexilate have been evaluated in three double-blind, randomized phase III trials. In all three, dabigatran etexilate (at doses of 150 or 220 mg once-daily, starting with a half-dose on the day of surgery) was compared with subcutaneous enoxaparin. The primary efficacy endpoint was total VTE (a composite of venographic or symptomatic DVT and/or symptomatic pulmonary embolism [PE]) and all-cause mortality. In the RE-MODEL™ trial (study duration 6–10 days), both doses of dabigatran etexilate (started 1–4 hours after surgery) were non-inferior to enoxaparin (40 mg once-daily, with the first dose given the evening prior to surgery) for the primary efficacy endpoint in patients undergoing TKR, and had a similar safety profile. The incidence of major VTE (the composite of proximal DVT and PE) was also similar for both doses of dabigatran etexilate and enoxaparin (13). Similarly, in the

RE-NOVATE™ trial, which compared extended prophylaxis (28–35 days) with dabigatran etexilate or enoxaparin in patients undergoing THR, both doses of dabigatran etexilate were again non-inferior to enoxaparin (40 mg once-daily) for the primary efficacy endpoint and had a similar safety profile (14). The incidence of major VTE was similar for both doses of dabigatran etexilate and enoxaparin. By contrast, in the RE-MOBILIZE™ trial (15) of patients undergoing TKR, both doses of dabigatran etexilate failed to achieve non-inferiority compared with enoxaparin for the primary efficacy endpoint.

The design of RE-MOBILIZE differed from that of RE-MODEL in several ways. First, the initial dose of dabigatran etexilate was given 6–12 hours instead of 1–4 hours after surgery. Second, the dose of enoxaparin was higher: a 30 mg twice-daily (North American) regimen was used instead of the 40 mg once-daily regimen. Third, the treatment duration was 12–15 days instead of 7–10 days. Therefore, the difference between the results of the RE-MODEL and RE-MOBILIZE trials may reflect the higher daily dose of enoxaparin used as a comparator or the delayed start of dabigatran etexilate in RE-MOBILIZE.

A pre-planned, pooled analysis of these three trials showed that major VTE and VTE-related death occurred in 3.3% of patients in the enoxaparin group, versus 3.0% of patients in the higher-dose dabigatran etexilate group and 3.8% in the lower-dose dabigatran etexilate group. Major bleeding events occurred with similar frequency in all groups (16). Furthermore, no significant differences among the groups in the incidence of liver enzyme elevations or acute coronary events were observed across these phase III trials. Based on these findings, dabigatran etexilate was approved for the prevention of VTE after THR or TKR in the European Union and Canada in 2008. The 220 mg once-daily dose is recommended for most patients, and the 150 mg once-daily dose is reserved for elderly patients and those with moderate renal impairment (creatinine clearance of 30–50 ml/min). The efficacy and safety results with the higher-dose regimen are summarized in Tables 3 and 4, respectively.

Stroke prevention in patients with AF

In a phase II trial (PETRO) in patients with AF, three doses of dabigatran etexilate (50, 150, or 300 mg twice-daily), with or without aspirin (ASA; 81 or 325 mg once-daily), were compared with warfarin (dose-adjusted to achieve a target international normalized ratio [INR] of 2–3) (17). Excess bleeding was observed in patients receiving the highest dose of dabigatran etexilate in conjunction with ASA, leading to the discontinuation of ASA in these patients. Building on the results of the PETRO trial, dose changes were made for the open-label extension PETRO-Ex study. The 50 mg twice-daily and the 150 mg once-daily groups were discontinued because of stroke rates of 8.4% and 8.1%, respectively, and pa-

Table 3: Primary efficacy outcomes with the higher-dose dabigatran etexilate regimen.

Trial	Setting	Enoxaparin regimen	Dabigatran regimen	DVT/PE/Death (%)	RR (95% CI)
RE-MODEL (N = 2,076)	TKR	40 mg OD 8 days	110/220 mg OD 8 days	38 vs. 36	0.97 (0.82–1.13)
RE-MOBILIZE (N = 1,896)	TKR	30 mg BID 13 days	110/220 mg OD 13 days	26 vs. 31	1.23 (1.03–1.47)
RE-NOVATE (N = 3,494)	THR	40 mg OD 33 days	110/220 mg OD 33 days	7 vs. 6	0.90 (0.63–1.29)

TKR=total knee replacement; THR=total hip replacement; OD=once daily; BID=twice daily; DVT=deep vein thrombosis; PE=pulmonary embolism; RR=relative risk; CI=confidence interval.

Table 4: Rates of major bleeding with enoxaparin or higher-dose dabigatran etexilate.

Trial	Setting	Major bleeding (%)	
		Enoxaparin	Dabigatran
RE-MODEL	TKR	1.3	1.5
RE-MOBILIZE	TKR	1.4	0.6
RE-NOVATE	THR	1.6	2.0

tients were transferred to the 150 mg twice-daily or 300 mg once-daily group. Later, it was observed that the stroke rate in patients receiving the dabigatran etexilate 300 mg once-daily regimen was similar to that with the placebo (18). Based on these data, the ongoing phase III RE-LY™ study is comparing dabigatran etexilate doses of 110 or 150 mg twice-daily with warfarin, dose-adjusted to achieve a target INR of 2–3, for the prevention of stroke in patients with AF (19). The trial has completed enrollment of more than 18,000 patients and results will be reported in 2009.

Treatment of VTE

Dabigatran etexilate is also being investigated in phase III trials for the treatment of acute symptomatic VTE (RE-COVER™) and in the long-term secondary prevention of recurrent VTE in patients who have completed a course of conventional anticoagulant treatment with a VKA for 6–18 months (RE-MEDY™), or 3–6 months (RE-SOLVE™) (20–22). The RE-COVER trial has been completed, and results will be reported in 2009. A second trial with the same study design has been initiated.

Other indications

A dose-finding phase II study in patients with ACS (RE-DEEM) is currently ongoing (23). This trial will determine whether the addition of dabigatran etexilate to dual antiplatelet therapy with ASA and clopidogrel reduces the risk of recurrent ischemia in ACS patients.

Conclusions and future developments

Emerging data support the efficacy and safety of oral DTIs. Dabigatran etexilate is already being used in place of LMWH to streamline thromboprophylaxis in patients undergoing major orthopedic surgery. However, the greatest unmet need in anticoagulation therapy is to find a replacement for warfarin. The results of the RE-LY and RE-COVER trials will be available in 2009. These trials will establish the risk-to-benefit profile of dabigatran etexilate relative to warfarin for long-term administration. What we know so far is that dabigatran etexilate has no hepatic toxicity, a finding that indicates that this complication does not represent a class effect. Both dosage regimens of dabigatran etexilate were taken to completion in the RE-LY trial, which bodes well for a positive outcome.

In parallel with the clinical development of dabigatran etexilate, a number of oral FXa inhibitors are also being investigated for the same indications. Of these agents, rivaroxaban and apixaban are in the most advanced stages of development and rivaroxaban also has been licensed in the European Union and Canada for thromboprophylaxis after THR or TKR. Head-to-head trials comparing oral DTIs with FXa inhibitors are unlikely to be performed in the near future. Instead, clinicians will have to make a choice when prescribing new oral anticoagulants. That choice will not

only depend on the strength of the clinical trial data, but also on patient characteristics that may render one agent better than another.

For the past 65 years, VKAs have been the only available oral anti-coagulants. With recent advances, we now have dabigatran etexilate and rivaroxaban as two new alternatives. More agents are likely to follow and the approved list of indications for these two new drugs will soon expand. Oral anticoagulation has taken a huge step forward. As the results of ongoing clinical trials unfold over the coming months and years, we will soon know the full extent of this advance.

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Correspondence to:
Dr. Jeffrey Weitz
Henderson Research Centre
711 Concession Street
Hamilton, Ontario, L8V 1C3, Canada
Tel.: +1 905 574 8550, Fax: +1 905 575 2646
E-mail: jweitz@thrombosis.hhscr.org

New approaches to anticoagulation: Oral factor Xa inhibitors

Alexander G. G. Turpie

Department of Medicine, Hamilton Health Sciences Corporation, Ontario, Canada

Summary

Long-available anticoagulants including unfractionated and low-molecular-weight heparins (UFH and LMWHs, respectively) and vitamin K antagonists (VKAs), as well as the more recently introduced synthetic pentasaccharide, fondaparinux, have demonstrated efficacy in preventing thromboembolic events. However, all of them have a number of practical limitations. UFH, LMWHs, and fondaparinux must be administered parenterally, making them less attractive for long-term prophylaxis, and heparins are associated with immune-mediated thrombocytopenia. Until recently, VKAs such as warfarin were the only approved oral anticoagulants, but they have a narrow therapeutic window and require routine laboratory monitoring of the international normalized ratio. In addition, VKAs have multiple food and drug interactions. Patients scheduled for operation need to stop VKA treatment and may require "bridging" anticoagulant therapy with UFH or LMWH before and after surgery. Heparins and VKAs have multiple targets in the coagulation cascade and are indirect inhibitors of thrombin. The various limitations of these anticoagulants have provided the impetus for the development of new drugs for the prevention and treatment of both venous (VTE) and arterial thromboembolism. Such development has focused on small molecules that target a single factor which is part of the final common pathway in the coagulation cascade. Numerous agents that specifically target factor IIa (thrombin) or factor Xa (FXa), are currently in development. Dabigatran, an orally adminis-

tered direct thrombin inhibitor, was recently launched in Europe and Canada for VTE prevention in patients undergoing elective total hip and knee replacement (THR and TKR, respectively). Another promising group of novel anticoagulants are the direct FXa inhibitors. These bind directly to FXa, inhibiting its procoagulant activity. Proof-of-concept for targeted FXa inhibition was provided by fondaparinux, the parenteral indirect inhibitor. FXa is an ideal target for an anticoagulant because it is positioned at the start of the common pathway of coagulation. Direct FXa inhibitors currently being evaluated in clinical trials are YM150, DU-176b, betrixaban, apixaban, and rivaroxaban. YM150, DU-176b, and betrixaban are at an earlier stage of development than apixaban and rivaroxaban, and are being investigated in phase II studies for VTE prevention in orthopedic surgery. Major orthopedic surgery is often used as a development model because it provides relatively fast validation of efficacy and safety, in an established indication requiring prophylaxis for only 2–5 weeks. Such validation can then provide support for development in other indications. Apixaban and rivaroxaban are the most advanced in development, and rivaroxaban was recently approved in Canada and the EU for preventing VTE in patients undergoing elective THR or TKR.

Keywords

Oral factor Xa inhibitors, apixaban, rivaroxaban, thrombosis, anticoagulation

Introduction

Anticoagulants are recommended for the prevention of VTE (comprising deep venous thrombosis [DVT] and pulmonary embolism [PE]) in patients undergoing major general or orthopedic surgery and in medically ill hospitalized patients. Anticoagulants are also indicated for the treatment and secondary prevention of VTE, stroke prevention in patients with atrial fibrillation (AF), and secondary prevention of major ischemic outcomes in patients with acute coronary syndromes (ACS).

Venous thrombosis, which occurs in veins where blood flow is stagnant or very slow, can develop anywhere in the venous system, but arises mainly in the deep veins of the leg (1). This can result in PE or post-thrombotic syndrome. Venous thrombi consist mainly of fibrin and red blood cells.

Disruption of atherosclerotic plaques in arteries is known to initiate thrombus formation leading to thrombotic and thromboembolic events (2). The most common sites for arterial thrombosis are the coronary arteries, the carotid, vertebral, or intracerebral arteries, and the peripheral arteries – usually in the leg. The arterial thrombus consists of aggregated platelets bound by small amounts of fibrin (3). Strategies to inhibit arterial thrombosis focus mainly on drugs that block platelet function, but often include anticoagulants to prevent fibrin deposition (2, 4).

Established anticoagulants

Established anticoagulants include UFH and LMWHs, VKAs, and the synthetic pentasaccharide fondaparinux. They have proven efficacy for the prevention of thromboembolic events; however, they have a number of practical limitations (Table 1). UFH, LMWHs, and fondaparinux are administered parenterally, making them less attractive for long-term prophylactic use, and heparins are associated with immune-mediated thrombocytopenia. VKAs, which include warfarin, have a narrow therapeutic window and require routine laboratory monitoring of the international normalized ratio with intermittent adjustments in dose. In addition, VKAs have multiple food and drug interactions (5). Surgical patients need to stop VKA treatment and start substitution or "bridging" anticoagulant therapy with UFH or LMWHs before and after surgery. Heparins and VKAs have multiple targets in the coagulation cascade and are indirect inhibitors of thrombin.

These practical limitations provided the impetus for the development of new drugs for the prevention and treatment of both venous and arterial thromboembolism. Such development has focussed on small molecules that target only one coagulation factor in the final common pathway of the coagulation cascade. Ximelagatran, a direct thrombin inhibitor (DTI) was the first oral anticoagulant since warfarin to receive marketing approval (only in the EU). In spite of favorable results in clinical trials of orthopedic thromboprophylaxis, ximelagatran was withdrawn in 2006 because of hepatotoxicity. Nevertheless, ximelagatran provided

Table 1: Properties of established and new oral anticoagulants.*

Property	Vitamin K antagonists	UFH	LMWH	Fondaparinux	Dabigatran	Apixaban	Rivaroxaban
Target	Vitamin K-dependent clotting factors	Multiple	Multiple	Indirect factor Xa	Direct factor IIa	Direct factor Xa	Direct factor Xa
Administration	Oral	Subcutaneous	Subcutaneous	Subcutaneous	Oral	Oral	Oral
Prodrug	No	No	No	No	Yes	No	No
Dosing [†]	Once daily INR-adjusted	2–3 times daily	1–2 times daily	Once daily	1–2 times daily	Twice daily	Once daily
Onset of action	72–96 hours	20–60 minutes	2 hours	2 hours	2 hours	3 hours	2–4 hours
Bioavailability by route of administration	100%	Variable	80–90%	100%	6%	50%	>80%
Half-life	40 hours	Dose dependent 1–1.5 hours <i>in vitro</i>	4–7 hours	17 hours	12–17 hours	9–14 hours	5–9 hours (11–13 hours in the elderly)
Antidote	Vitamin K produces slow reversal	Protamine sulphate	Partially neutralized by protamine sulphate	None	None	None	None
Mode of elimination	Renal	Renal; reticulo-endothelial	Renal	Renal	Renal (80%); hepatic (20%)	Renal (25%); hepatic (75%)	Renal (66%); hepatic (34%)
Monitoring	Yes	Yes	Yes	No	No	No	No
Drug interactions	Multiple	Other anticoagulants, antiplatelets and thrombolytics	None relevant known	None relevant known	Proton pump inhibitors	CYP3A4 inhibitors	CYP3A4 inhibitors
*Compiled with data from Stangier, et al. 2007 (38), Gross and Weitz 2008 (39), Haas 2008 (40), Kubitzka, et al. 2005 (41), Weinz, et al. 2004 (42); [†] may vary with indication. INR=international normalized ratio; LMWH=low-molecular-weight-heparin; CYP=cytochrome P 450.							

proof-of-concept for the use of a single-target oral anticoagulant. Numerous new oral agents specifically targeting FIIa (thrombin) or FXa are in development, and two were recently approved in the EU and Canada for the prevention of VTE following major orthopedic surgery.

Oral direct FXa inhibitors

At present, there is one orally administered DTI available on the market, dabigatran, which was launched recently in Europe and Canada for VTE prevention in patients undergoing elective total hip and knee replacement (THR and TKR, respectively) surgery. It is under investigation for VTE treatment, as well as prevention of cardioembolic events in patients with chronic AF. Other oral DTIs such as AZD0837 are in development. Clinical trials of dabigatran are discussed by Dr. Weitz elsewhere in this supplement.

Another promising group of novel anticoagulants are the direct FXa inhibitors. These bind directly to FXa, inhibiting its procoagulant activity. Proof-of-concept for targeted FXa inhibition, albeit indirect, was provided by fondaparinux, also licensed for VTE prevention, although this agent requires parenteral administration (6).

FXa is an ideal target for an anticoagulant because it is positioned at the start of the common pathway of coagulation. Direct FXa inhibitors currently being evaluated in clinical trials are YM150, DU-176b, betrixaban, apixaban, and rivaroxaban. YM150, DU-176b, and betrixaban are at earlier stages of development than apixaban and rivaroxaban (7). Major orthopedic surgery is often used as a development model because it allows the relatively fast validation of efficacy and safety, in an established indication requiring prophylaxis for only 2–5 weeks. Such validation can then provide support for development in other indications (8).

Apixaban

Pharmacology

Apixaban is an oral, highly selective, direct inhibitor of free and prothrombinase-bound FXa (9). Properties of apixaban are summarized in Table 1.

Clinical trials

Three large phase III studies will define the safety and efficacy of oral apixaban in patients undergoing elective TKR (ADVANCE-1 and -2) and THR (ADVANCE-3). In ADVANCE-1 (10), apixaban 2.5 mg orally twice daily was compared to enoxaparin 30 mg subcutaneously (SC) twice daily (the

Table 2: Comparison of phase III RECORD trials.*

	Total hip replacement surgery				Total knee replacement surgery	
	RECORD1		RECORD2		RECORD3	
	Enoxaparin	Rivaroxaban	Enoxaparin	Rivaroxaban	Enoxaparin	Rivaroxaban
Total VTE, n (%)	58/1,558 (3.7%)	18/1,595 (1.1%)	81/869 (9.3%)	17/864 (2.0%)	166/878 (18.9%)	79/824 (9.6%)
P-value	<0.001		<0.0001		<0.001	
Symptomatic VTE, n (%)	11/2,206 (0.5%)	6/2,193 (0.3%)	3/1,212 (0.2%)	15/1,207 (1.2%)	24/1,217 (0.2%)	8/1,201 (0.7%)
P-value	0.222		0.004		0.005	
Incidence of major bleeding, n (%)	2 (0.1%)	6 (0.3%)	1 (<0.1%)	1 (<0.1%)	6 (0.5%)	7 (0.6%)
P-value	0.18		n/a		0.77	
Total VTE = total venous thromboembolism, the composite of any deep vein thrombosis, nonfatal pulmonary embolism and all-cause mortality; n/a=not applicable. * RECORD4 has been completed and is being prepared for publication.						

North American regimen) for prevention of VTE in 3,195 patients undergoing TKR. Each agent was begun 12–24 hours post-operatively and continued until mandatory bilateral venography was performed at 12 ± 2 days. The primary efficacy outcome was a composite of DVT by venography, symptomatic, objectively confirmed DVT or PE, and death from any cause. The rate of the primary efficacy endpoint was similar in the two treatment groups: 9.0% for apixaban versus 8.9% for enoxaparin ($p=0.064$). Prespecified statistical criteria for demonstrating the non-inferiority of apixaban were not met, however. It has been suggested that because the rate of VTE in the enoxaparin group was lower than observed in previous, similar trials, the study lacked power to demonstrate non-inferiority. There was no difference between the two arms in the incidence of major bleeding according to International Society on Thrombosis and Haemostasis (ISTH) (11) criteria (0.7% for apixaban versus 1.4% for enoxaparin; $p=0.053$). The composite rate of clinically relevant non-major and major bleeding was lower in the apixaban group (2.9% versus 4.3% in the enoxaparin group; $p=0.034$). In the ADVANCE–2 and –3 trials, apixaban 2.5 mg orally twice daily is being compared to enoxaparin 40 mg SC once daily in patients undergoing major orthopedic surgery.

In the phase II, dose-finding APPRAISE trial (12), 1,246 patients with onset of an ACS within 7 days of randomization and at least one additional risk factor such as age ≥ 65 years, diabetes mellitus, or prior myocardial infarction (MI) within the preceding 12 months, were randomized to apixaban 2.5 mg orally twice daily ($n=317$); apixaban 10 mg orally once daily ($n=318$); or placebo ($n=611$). Two higher doses of apixaban (10 mg orally twice daily and 20 mg orally once daily) were initially studied as well, but at interim analysis these arms were terminated because of an excess incidence of major and minor bleeding.

Patients were treated according to physician discretion with aspirin, clopidogrel, or both, with approximately 75% receiving dual antiplatelet therapy. The primary safety outcome was a composite of major bleeding (defined, as in ADVANCE–1, according to ISTH criteria) and clinically relevant non-major bleeding. The secondary efficacy outcome was a composite of cardiovascular (CV) death, MI, severe recurrent ischemia, or ischemic stroke. The mean duration of follow-up was six months.

The incidence of the primary composite safety endpoint was 3.0% in the placebo group ($n=599$), 7.9% in the apixaban 10 mg once daily group

($n=315$; hazard ratio [HR] 2.45; 95% confidence interval [CI], 1.31–4.61), and 5.7% in the apixaban 2.5 mg twice daily group ($n=315$; HR 1.78; 95% CI, 0.91–3.38). Among clopidogrel-treated patients, incidence of the composite safety endpoint was 7.0% for apixaban 2.5 mg twice daily ($n=230$) and 9.1% for apixaban 10 mg once daily ($n=241$) versus 3.1% for placebo ($n=453$).

The secondary endpoints of CV death, MI, severe recurrent ischemia, or ischemic stroke occurred in 8.7%, 7.6%, and 6.0% of those in the placebo, apixaban 2.5 mg twice daily, and apixaban 10 mg once daily groups, respectively (HR 0.73; 95% CI, 0.44–1.19, $p=0.21$ for apixaban 2.5 mg twice daily versus placebo; HR 0.61; 95% CI, 0.35–1.04; $p=0.07$ for apixaban 10 mg once daily versus placebo).

Thus in APPRAISE–1, addition of apixaban to single or dual antiplatelet therapy following ACS resulted in a dose-dependent increase in bleeding, as well as a trend towards reduction in the incidence of clinically relevant ischemic events. A phase III trial of apixaban in ACS, APPRAISE–2, is underway (13).

The apixaban clinical trial program is also investigating apixaban in other clinical contexts (14–19), including VTE prevention in medically ill patients (ADOPT), VTE treatment (AMPLIFY and AMPLIFY–EXT) and stroke prevention in AF (ARISTOTLE and AVERROES). An ongoing phase II, randomized, double-blind, placebo-controlled, multicenter clinical trial (ADVOCATE) is assessing the safety, tolerability, and effectiveness of oral apixaban in preventing thrombotic events in patients with advanced or metastatic cancer administered chemotherapy for >90 days.

Rivaroxaban

Pharmacology

Rivaroxaban is an oral, direct FXa inhibitor that not only effectively inhibits free FXa activity but also prothrombinase activity and clot-associated FXa activity (20). Rivaroxaban has predictable dose-dependent pharmacokinetics and pharmacodynamics. The properties of rivaroxaban are summarized in Table 1.

Clinical trials

An extensive phase II program indicated that rivaroxaban can be given to patients without dose modification for age, weight, gender, or mild-to-

moderate renal impairment (6, 21–23). The phase III RECORD program (see Table 2) evaluated the safety and efficacy of oral rivaroxaban in patients undergoing elective THR (RECORD1 and 2) and TKR (RECORD3 and 4) (24–27). In both RECORD1 and 2, patients undergoing THR were given rivaroxaban for 31–39 days. Enoxaparin was given for 31–39 days in RECORD1 (23) or 10–14 days in RECORD2 (24). In RECORD3 (25) and RECORD4 (26), patients undergoing TKR received prophylaxis for 10–14 days.

Results from these studies showed that the incidence of total VTE (the composite of any DVT, non-fatal PE, and all-cause mortality) occurred in significantly fewer patients receiving the rivaroxaban regimens compared with those receiving the enoxaparin regimens with similar rates of major bleeding. When compared in RECORD4 to the North American regimen of enoxaparin (30 mg twice daily starting 12–24 hours after wound closure), rivaroxaban was more effective in preventing VTE after TKR, without significantly increasing the risk of bleeding.

Rivaroxaban was also evaluated in ATLAS-TIMI 46, a phase II dose-finding study in which it was compared to placebo in ACS (28). Patients were stratified based on whether they were to receive single or dual antiplatelet therapy, that is with aspirin alone or with aspirin plus clopidogrel.

A total of 3,491 patients were randomized, of whom 761 received aspirin alone and 2,730 aspirin and clopidogrel. Patients were randomized in 1:1:1 fashion to placebo (n=1,160) or once-daily (n=1,166) or twice-daily (n=1,156) rivaroxaban, with total daily doses escalated from 5 mg to 10 mg to 20 mg. About one-half of the patients presented with ST-elevation MI.

The primary efficacy endpoint was a composite of death, MI, stroke, or severe recurrent ischemia requiring revascularization while the primary safety endpoint was clinically significant bleeding, defined as a composite of TIMI major bleeding, TIMI minor bleeding and any reported bleeding event requiring medical attention.

Rivaroxaban was associated with a 21% reduction in the relative risk of the primary efficacy endpoint ($p=0.1$) and a significant 31% reduction in the relative risk of the secondary endpoint of death, MI, or stroke ($p=0.028$), with a consistent trend for efficacy across doses. Rates of clinically significant bleeding were: 3.3% for placebo; 6.1% for rivaroxaban 5 mg; 10.9% for rivaroxaban 10 mg; 12.7 % for rivaroxaban 15 mg; and 15.3% for rivaroxaban 20 mg ($p < 0.001$ for dose response). For all rivaroxaban doses studied, rates of bleeding were higher in patients receiving dual antiplatelet therapy than for those receiving aspirin alone. Most bleeding in both strata was classified as that requiring medical attention. There was no evidence of drug-induced hepatotoxicity.

Based on the results of ATLAS-TIMI 46, doses of 2.5 mg and 5 mg twice daily have been selected for evaluation in the phase III study in ACS (29). The phase II and phase III programs also include studies evaluating rivaroxaban for VTE treatment (EINSTEIN DVT [30] and EINSTEIN PE), long-term prevention of recurrent symptomatic VTE (EINSTEIN EXT) (31), VTE prevention in medically ill patients (MAGELLAN) (32), and the prevention of stroke in patients with AF (ROCKET AF) (33).

Rivaroxaban has recently received approval in Canada and the EU for the prevention of VTE in patients undergoing elective THR or TKR surgery.

These selective oral inhibitors of specific coagulation factors represent a new class of antithrombotic agents with potential practical advantages relative to established agents. Unlike warfarin, their predictable dose-response allows them to be administered at fixed doses without the need for coagulation monitoring. Unlike parenteral anticoagulants, they can be used conveniently for venous thromboprophylaxis both in-

hospital and following discharge. In clinical settings requiring long-term anticoagulation, that such agents do not require monitoring and lack warfarin's drug and food interactions might improve both guideline adherence and patient compliance (34, 35).

Pharmacoeconomic analyses suggest that anticoagulation is most cost-effective in populations at highest risk for thrombotic events (36). Preliminary economic analyses of the new oral agents suggest that savings might be realized in comparison to parenteral agents requiring self-injection or home nursing visits following discharge (37). Furthermore, if the new oral anticoagulants are safer or associated with greater rates of adherence than VKAs and if there are, in consequence, fewer bleeding or thromboembolic events, cost savings might be realized.

Conclusion

After many years of limited therapeutic options for both initial and extended antithrombotic therapy, new oral anticoagulants have the potential to streamline the prevention and treatment of VTE. Several oral direct thrombin and direct FXa inhibitors are in advanced clinical development, or are newly approved. These anticoagulants combine the convenience of oral dosing with predictable pharmacokinetic and pharmacodynamic properties, offering the possibility of treating patients without monitoring or dose-adjustment, and might also offer cost benefits both in terms of patient management and reductions of potential thromboembolic disorders.

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Correspondence to:
 Alexander G. G. Turpie
 Department of Medicine
 Hamilton Health Sciences—General Division
 237 Barton Street East, Hamilton
 Ontario L8L 2X2, Canada
 Tel.: +1 905 929 4385, Fax: +1 905 521 1551
 E-mail: turpie@mcmaster.ca

Bleeding in trials of anticoagulants: A clinical perspective

Michael Rud Lassen

Spine Clinic, Clinical Trial Unit, Hørsholm Hospital, Hørsholm, Denmark

Summary

Significant progress has been made over the past decade in the development of new anticoagulants, as we have moved from unfractionated heparin (UFH), low-molecular-weight heparins (LMWHs), and warfarin to more target-specific inhibitors like direct and indirect factor Xa (FXa) inhibitors and direct thrombin inhibitors (DTIs). Warfarin and its congeners were for decades the sole available oral anticoagulants. Fondaparinux, a specific FXa inhibitor approved for preventing venous thromboembolism (VTE) after elective total joint replacement and hip fracture surgery is, like UFH and LMWHs, a parenteral agent. Many of the target-specific anticoagulants in late clinical development are oral agents. Three of them, apixaban, dabigatran etexilate, and rivaroxaban, have been or are being investigated in phase III trials in major orthopedic surgery. Each has been through intensive dose-finding studies to

define the optimal dose regimen. To translate successfully into clinical practice the enormous amount of information yielded by these trials requires careful critical analysis. While regulatory agencies focus on the benefit:risk ratio, it remains to be established how such ratios are to be applied in the clinic. With particular focus on how bleeding is defined and measured, this article will consider recently completed clinical trials of new oral anticoagulants and of one parenteral agent to highlight the challenges of interpreting trial data and translating them to patient care.

Keywords

Bleeding definitions, clinical trials, anticoagulants

Introduction

The past decade has seen active development of numerous new anticoagulants intended to meet unmet needs in antithrombotic therapy. With the temporary exception of ximelagatran – introduced and then withdrawn from the market in Europe – warfarin, with all its limitations, has been the only available oral anticoagulant for 60 years. UFH and LMWHs, DTIs such as desirudin and bivalirudin, and fondaparinux are all parenteral agents.

Following careful dose-finding studies, a number of new, direct, single-target oral anticoagulants have been or are being studied in phase III clinical trials for thromboprophylaxis in major orthopedic surgery as well as in other indications. Two of these agents, dabigatran etexilate and rivaroxaban, have been approved in Europe and Canada for thromboprophylaxis in the setting of total joint arthroplasty.

Transforming the enormous amount of information from clinical trials of these agents into clinical practice requires careful and critical analysis by clinicians. While regulatory agencies consider benefit:risk ratios that reflect data on efficacy and safety of a given agent in a trial population, the clinician must ask how and whether these data apply to patients seen on a daily basis.

The significance of bleeding

Bleeding is *the* critical safety measure when evaluating new anticoagulants for prevention or treatment of thrombosis. Surgeons in particular have an aversion to bleeding that may exceed any objective measure of its clinical import, although increasingly bleeding is thought to be a predictor of mortality that may occur well after the bleeding itself. In the OASIS-5 trial comparing fondaparinux to enoxaparin for the relatively prolonged and generally non-invasive management of non-ST-elevation acute coronary syndrome (ACS) (1), although fondaparinux was non-inferior to enoxaparin at nine days in terms of the incidence of a compos-

ite efficacy endpoint of death, reinfarction, or refractory ischemia, there was a small (0.6%–0.7%) absolute but statistically significant increase in the incidence of death at 30 and 180 days ($p=0.02$ and $p=0.05$, respectively) which was associated with an excess of major bleeding in the enoxaparin group. In a subsequent analysis of OASIS-5, the investigators found that major bleeding was associated with an approximately four-fold increased hazard of death, myocardial infarction, or stroke during the first 30 days and an approximately three-fold increased hazard during 180 days of follow-up (2). Eikelboom et al., in a meta-analysis of data from the OASIS Registry (3), OASIS-2 (4), and CURE (5), found that in ACS patients without persistent ST-elevation, there was a strong correlation between major bleeding and death at 30 days (6). However in the ACUITY trial (7), in which bivalirudin, bivalirudin plus a glycoprotein (GP) IIb/IIIa receptor antagonist, and UFH plus a GP IIb/IIIa receptor antagonist were compared in 13,819 patients with generally low-to-moderate 1/risk ACS (according to the TIMI risk score [8]) undergoing percutaneous coronary intervention, the lower incidence of bleeding in the bivalirudin alone group did not translate into a mortality benefit at 1 year (9). It is thus difficult to determine whether bleeding itself is a cause of adverse outcomes, a marker of other conditions adversely affecting prognosis, or the proximate cause of changes in therapy (discontinuation of antiplatelet agents, for example) that may themselves adversely affect outcome.

Evaluating the safety and efficacy of anticoagulants

New anticoagulants, whether oral or injectable, are usually evaluated in two classic models: total hip and total knee arthroplasty. Each of these procedures carries a high risk of postoperative thromboembolism and the surgical setting is very useful for identifying bleeding (10). Although this model is widely used in anticoagulant development, one must ask whether or how it allows comparison of results across clinical trials.

In terms of efficacy, a standardized assessment – mandatory bilateral venography at a specified time point or upon development of symptoms

Table 1: Definitions of major bleeding used in four phase III trials of investigational anticoagulants (with sites of bleeding adjudication) compared to the ISTH definition of major bleeding. Hb=hemoglobin; EMEA=European Medicines Agency.

ISTH ¹²	PENTAMAX ¹⁵ (fondaparinux)	RE-MOBILIZE ¹⁹ (dabigatran)	ADVANCE-1 ¹⁸ (apixaban)	RECORD4 ¹⁷ (rivaroxaban)
Major bleeds are those that result in death, are life-threatening, cause chronic sequelae, or consume major health-care resources.		Major bleeding events are defined according to EMEA criteria (22), as shown below.	Acute clinically overt bleeding is defined as new-onset, visible bleeding or signs or symptoms suggestive of bleeding with confirmatory imaging techniques which can detect the presence of blood.	Clinically overt bleeding is thought to be excessive in nature and unusual for this type of surgery.
Fatal bleeding	Fatal bleeding	Fatal bleeding	Fatal bleeding	Fatal bleeding
	Bleeding leading to re-operation	Bleeding leading to re-operation	Bleeding leading to re-operation	Bleeding requiring re-operation
Symptomatic bleeding in a critical organ, such as that which is: • Intracranial • Intraspinal • Intraocular • Retroperitoneal • Intra-articular • Pericardial • Intramuscular with compartment syndrome	Bleeding that is at least one of the following: • Retroperitoneal • Intracranial • Intraspinal • Involving any other critical organ	Bleeding that is at least one of the following: • Symptomatic retroperitoneal • Intracranial • Intraocular • Intraspinal	Bleeding that is or in at least one of the following: • Intracranial • Intraspinal • Intraocular (not conjunctival) • Pericardial • An operated joint and requires re-operation or intervention • Intramuscular with compartment syndrome • Retroperitoneal	Bleeding in at least one of the following critical sites: • Retroperitoneal • Intracranial • Intraocular • Intraspinal
		Bleeding requiring treatment cessation	Bleeding requiring treatment cessation	
Bleeding causing a fall in Hb level of 20 g/l (1.24 mM) or more, or leading to transfusion of two or more units of whole blood or red cells	Overt bleeding with a bleeding index ≥ 2 . Bleeding index = [number of units of packed red blood cells or whole blood transfused] plus [(pre-bleeding) minus (post-bleeding) Hb values, in g/dl]	Bleeding associated with ≥ 20 g/l (corresponds to 1.24 mM) fall in Hb in excess of what was expected or leading to transfusion of ≥ 2 units packed cells or whole blood in excess of what was expected	Overt bleeding with a decrease in Hb of ≥ 2 g/dl over a 24-hour period or a transfusion of ≥ 2 units of packed red blood cells	<u>Extracurricular</u> site bleeding associated with ≥ 2 g/dl fall in Hb or <u>Extracurricular</u> site bleeding leading to infusion of ≥ 2 units of whole blood or packed cells
Independent adjudication committee	Holland	Sweden	Hamilton, Ontario, Canada	Sweden/Holland

suggestive of deep venous thrombosis (DVT) with central adjudication of the venograms – has been used increasingly over the past decade. Two of the most experienced adjudication centers are in Hamilton, Ontario, Canada, and Gothenburg, Sweden. Although these centers differ in their approaches to interpreting venograms, with readers at Gothenburg identifying asymptomatic proximal or distal DVT approximately twice as frequently as readers at Hamilton in the setting of total hip replacement and

50% more frequently in the setting of total knee replacement, there has been over time and across studies a notable consistency of reading *within* each institution (11). Given this consistency, and given that definitions of symptomatic DVT and non-fatal pulmonary embolism are identical across protocols, some cross-trial comparison of efficacy is both possible and reasonable (at least when the studies are adjudicated at Hamilton or Gothenburg).

Table 2: Efficacy and safety (bleeding) in four total knee replacement studies using enoxaparin 30 bid as control group. VTE=venous thromboembolism; NA=not available; od=once daily; bid=twice daily.

	PENTAMAX ¹⁵ (fondaparinux)		RE-MOBILIZE ¹⁹ (dabigatran)		ADVANCE-1 ¹⁸ (apixaban)		RECORD4 ¹⁷ (rivaroxaban)	
	Fondaparinux [#] 2.5 mg od	Enoxaparin* 30 mg bid	Dabigatran [€] etexilate 220 mg od	Enoxaparin* 30 mg bid	Apixaban* 2.5 mg bid	Enoxaparin* 30 mg bid	Rivaroxaban 10 mg od	Enoxaparin* 30 mg bid
N for total VTE (primary efficacy endpoint)	361	363	604	643	1,157	1,130	965	1564
Total VTE, n (%)	45 (12.5)	101 (27.8)	188 (31.1)	163 (25.3)	104 (9.0)	100 (8.9)	15 (6.9)	97 (10.1)
N for major VTE	NA	NA	604	643	1,269	1,216	1,122	1,112
Major VTE, n (%)	NA	NA	20 (3.3)	15 (2.3)	26 (2.1)	20 (1.6)	13 (1.2)	22 (2.0)
N for safety	517	517	857	868	1,596	1,588	1,526	1,508
Major bleeding, n (%)	11 (2.1)	1 (0.2)	5 (0.6)	12 (1.4)	11 (0.7)	22 (1.4)	10 (0.7)	4 (0.3)
Clinically relevant major bleeding, n (%)	NA	NA	23 (2.7)	21 (2.4)	35 (2.2)	47 (3.0)	39 (2.6)	30 (2.0)
Minor bleeding, n (%)	14 (2.7)	19 (3.7)	NA	NA	39 (2.4)	40 (2.5)	NA	NA
Any bleeding, n (%)	NA	NA	NA	NA	85 (5.3)	108 (6.8)	160 (10.5)	142 (9.4)
[#] started 4–8 hours after wound closure. * started 12–24 hours after wound closure. [€] started 6–12 hours after wound closure with half dosage (110 mg).								

In contrast, bleeding definitions have varied significantly across trials. Commonly used descriptors include major or severe bleeding, overt bleeding, clinically relevant non-major bleeding, and minor bleeding. Absent precise definition, none of these descriptors is useful. Some cross-trial differences in the definition of (major) bleeding are driven by the requirements of regulatory agencies, others by investigators, and yet others by industry sponsors. Two recent phase II dose-finding trials of novel oral anticoagulants for secondary prevention following an episode of ACS, the APPRAISE study of apixaban and the ATLAS-TIMI 46 trial of rivaroxaban, used different definitions of major bleeding, those of the International Society on Thrombosis and Haemostasis (ISTH) (12) and TIMI group respectively (13), prompting Dr. Elaine Hylek to state following presentation of the ATLAS-TIMI 46 results at the 2008 Scientific Sessions of the American Heart Association:

It's incredibly important that we adopt universal reporting across trials and indications worldwide. This will facilitate a more informed assessment of the benefits and risks [of such agents] for patients and providers (14).

Defining bleeding

According to the Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the ISTH (12), a useful definition of major bleeding should be based on objective criteria; thus major bleeds are those that result in death, are life-threatening, have chronic sequelae, or result in the consumption of major health-care resources. For non-surgical patients, the ISTH defines major bleeding as that which is

1. Fatal, and/or
2. Symptomatic in a critical area or organ, such as that which is intracranial, intraspinal, intraocular, retroperitoneal, intra-articular or pericardial, or intramuscular with compartment syndrome, and/or
3. Causes a fall in hemoglobin level of 20 g/l (1.24 mM) or more, or leading to transfusion of two or more units of whole blood or red cells.

However useful the ISTH definition of major bleeding might be, it is infrequently used without modification, particularly in trials of surgical patients. Table 1 compares the ISTH definition of major bleeding to analogous definitions used in four phase III trials of investigational anticoagulants (fondaparinux, dabigatran, apixaban, or rivaroxaban) tested against the North American regimen of enoxaparin (30 mg subcutaneously twice daily). The differences among these definitions are important. For example, in the trial of fondaparinux (but none of the other trials), among criteria for major bleeding was overt bleeding with a "bleeding index" ≥ 2 (15). This index was defined as equal to the "number of units of packed red cells or whole blood transfused plus the hemoglobin values before the bleeding episode minus the hemoglobin values after the episode (in g/dl). It is not obvious how a bleeding index ≥ 2 should be compared to "bleeding causing a fall in hemoglobin level of 20 g/l (1.24 mM) or more, or leading to transfusion of two or more units of whole blood or red cells," the corresponding ISTH criterion. Indeed, four of the PENTAMAKS investigators ultimately observed that

...the clinical relevance of a bleeding index of 2 or more is uncertain because it was not reflected as a difference in fatal bleeding, critical organ bleeding, bleeding leading to another operation, wound infection, or com-

lications at the surgical site leading to prolonged hospitalization or re-hospitalization... (16).

In the RECORD4 trial of rivaroxaban (17), in contra-distinction to PENTAMAKS and the ADVANCE-1 trial of apixaban (18) and RE-MOBILIZE trial of dabigatran (19), bleeding at the surgical site was not included as a criterion for major bleeding. Surgical-site bleeding was, however, captured as a "hemorrhagic wound complication," a measure of non-major bleeding.

Adding to the difficulty of reconciling variations in definition of major bleeding is the absence in published reports of how data on bleeding were collected. From the perspective of an investigator in these trials, it is clear that meaningful differences in data collection existed. In certain trials, for example, clinical research associates combed patient records to identify bleeding events; in others, only bleeding judged by the investigator to be "excessive" was reported and adjudicated.

Moreover, the published reports do not define how bleeding events were adjudicated. In each trial, an independent adjudication committee was kept blinded as to treatment allocation; however, the committees differed in composition as did, presumably, their processes. As discussed above, significant inter-group differences in venogram interpretation have been described and quantified. These differences are apparent when comparing DVT rates in RE-MOBILIZE (adjudicated in Gothenburg) with those in ADVANCE-1 and RECORD4 (both adjudicated in Hamilton [although *bleeding* in RECORD4 was adjudicated in Sweden and Holland]) (Table 2). No analogous description and quantification of inter-institution differences in the assessment of bleeding has been reported, although it would be perfectly reasonable to assume that meaningful differences could exist.

As shown in Table 2, the benefit:risk ratio for the novel anticoagulant was acceptable in each of the four trials. However, given the heterogeneity of these trials and absent head-to-head comparison of these agents, it is not possible to draw cross-trial conclusions as to which regimen is preferred from a clinical point of view. Meta-analysis can actually impair interpretation of trial data and mislead clinicians when it is performed using "second-hand" data (from publications and presentations) and without appropriate weighting of the data for differences in collection, handling, and statistical treatment. An example of this is a putative meta-analysis of data from RECORD1-4 (20).

Selecting a dose of an anticoagulant for phase III development requires balancing efficacy and safety. Implicit in this process is an understanding that some degree of efficacy will be sacrificed to achieve an acceptable degree of safety, and that some patients will therefore experience clinically significant thromboembolic events. The benefit:risk ratio can be increased if those at greatest risk of bleeding could be identified prospectively. Borris et al. have reported that patients who bleed after surgery have significantly lower thrombin generation potential as measured by urinary F_{1+2} than a matched control group (21); at the same time, they have demonstrated that patients with clinically symptomatic VTE or venographically demonstrated asymptomatic DVT had significantly *higher* urinary F_{1+2} than a matched control group. Whether this simple screening method could improve the benefit:risk ratio of anticoagulant therapy must be evaluated in a prospective clinical management trial. None of the patients who experienced bleeding developed VTE during or after treatment with either rivaroxaban or enoxaparin.

In conclusion, evaluating bleeding across studies is difficult because of many differences in definition of outcome, different adjudication committees and methods, and differences in data collection. There is a great need for standardizing bleeding criteria in clinical trials as well as the

adjudication and data collection processes. Such standardization would make cross-trial meta-analyses more reliable and less subject to misuse. Individualized dosing of anticoagulants for thromboprophylaxis may be cumbersome but may increase both efficacy and safety.

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Correspondence to:
Michael Rud Lassen, MD
Spine Clinic
Clinical Trial Unit, Hørsholm Hospital
Usserød Kongevej 102
DK-2970 Hørsholm, Denmark
Tel.: +45 48 29 27 78, Fax: +45 48 29 26 17
E-mail: mirula@noh.regionh.dk

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