College of American Pathologists Conference XXXI on Laboratory Monitoring of Anticoagulant Therapy: Future Directions

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OVERVIEW

Edwin G. Bovill, MD

Heparin and warfarin have constituted the dominant modes of anticoagulation for more than 50 years. Consequently, laboratory monitoring strategies for anticoagulation monitoring have focused almost exclusively on these 2 agents. This situation is about to change! We are on the verge of spectacular growth and diversification of available therapeutic agents as a result of the past decade's explosive growth in understanding molecular and cellular mechanisms of hemostasis and thrombosis. These new agents are targeted to modulate different biochemical pathways leading to thrombosis and include a wide assortment of highly selective antiproteases, numerous novel platelet inhibitors, and a number of novel variants of traditional agents, like low-molecular-weight heparin. On the thrombolytic side, there have been numerous new and highly targeted agents developed following the success of tissue plasminogen activator and streptokinase in the lysis of coronary thrombi in acute myocardial infarction. Many of these drugs have entered phase I and phase II clinical trials, and some are just now entering clinical practice. Unfortunately, a number of these new agents cannot be monitored with the tradi-

tional coagulation screening tests. The most obvious examples are antiplatelet drugs, for which there is no readily available assay system; platelet aggregation is cumbersome, slow, and expensive, and the bleeding time is too imprecise and crude a measure.

This section of the symposium was designed to give the practicing pathologist a window on the future of anticoagulation/antithrombotic therapy and the laboratory monitoring challenges involved. Kenneth Mann, PhD, is the chairman of biochemistry at the University of Vermont College of Medicine and is an internationally acknowledged scientific leader in unraveling the mysteries of the mechanisms of hemostasis and thrombosis. His section reviews the current state of the art of understanding the biochemistry of coagulation. Two leading figures from 2 different cutting-edge biotechnology pharmaceutical companies address antiplatelet and antiprotease agents. David Phillips, PhD, was one of the founders of COR Therapeutics, Inc, and he discusses their groundbreaking work in antiplatelet therapeutics. George Vlasuk, PhD, is a major scientific contributor to the development of novel, highly specific antiprotease agents at Corvas International Inc. These two scientists from industry give a lucid review of the expectations for the near future in antithrombotic therapy. Richard Becker, MD, is a cardiologist and professor of medicine at the University of Massachusetts. Dr Becker has been actively involved for years with many studies of new antithrombotic and thrombolytic agents. He presents a thoughtful discussion of these newly available agents from a clinician's perspective. The authors have produced lucid figures that help organize and summarize this new information for the practicing pathologist.

THROMBOSIS: DIAGNOSIS AND PROPHYLAXIS 1997

Kenneth G. Mann, PhD

My laboratory's research in the biochemistry of coagulation was initiated approximately 30 years ago. At that time, the nature of thrombin was enigmatic; fibrinogen was reasonably well understood, but the remainder of the vitamin K–dependent proteins, the inhibitors, and all of the cofactors that we now know to participate in the coagulation reactions were identified only as "factors" using complex nonspecific and marginally quantitative plasma assays. The coagulation process was thought to be a consequence of 2 reaction pathways, one initiated by the interaction of plasmic constituents with a foreign surface (the contact pathway), and the other associated with the presentation of tissue factor as a consequence of a mechanical injury to the vasculature. These reaction mechanisms were deduced through the use of plasma samples from individuals congenitally deficient in 1 or more of the constituents of each reaction pathway through the use of 2 important plasma-based assays, namely, the activated partial thromboplastin time, in which plasma clotting is initiated by a foreign surface, and the prothrombin time, in which a tissue homogenate was added to plasma at concentrations sufficiently high to induce the clotting of plasma in an interval of 10 to 15 seconds. These 2 bioassays combined with the thrombin time, in which thrombin is directly added to plasma to evaluate the final step of clot formation, are the assays still most commonly available to the clinical laboratory for the evaluation of the continuity of the coagulation system and for the evaluation of anticoagulants administered for prophylaxis of thrombosis.

During the past 30 years, many laboratories have contributed to the isolation of a complex array of coagulation components and an even more complex array of anticoagulants that arrest the propagation of the fibrin-platelet clot. Parallel investigations of the fibrinolysis system have led to the quantitative description of clot lysis. Other investigations have identified that coagulation serine proteases only act in concert with cofactors on membrane surfaces to provide their catalytic efficiency. These data have also shown that the generation of coagulation enzymes is a ternary process involving serine protease activation, cofactor activation or expression, and the generation of the appropriate cell membrane for assembly of the enzymatic complex. The procoagulant complexes (Figure) are essential in the process of normal coagulation.

The procoagulant process is thought to be initiated when small amounts of circulating 2-chain factor VIIa (an active but catalytically insufficient serine protease) bind to tissue factor presented as a consequence of vascular disruption or an inflammatory process. The tissue factor–factor VIIa–membrane complex activates the serine proteases factor Xa and factor IXa, leading to the generation of the intrinsic tenase (factor IXa–factor VIIIa) and prothrombinase (factor Xa–factor Va) and ultimately resulting in rapid generation of thrombin. The formation of thrombin is tightly regulated by a system of stoichiometric inhibitors, including the tissue factor pathway inhibitor and antithrombin (AT-III). In addition, through the dynamic activated protein C pathway, thrombin acts as its own regulator by complexing with vascular thrombomodulin. The resulting thrombin-thrombomodulin complex activates the plasma zymogen protein C to activated protein C. Activated protein C down-regulates coagulation by proteolytic inactivation of factor Va.

The relevance of the contact pathway is diminished by the observation that individuals who lack factor XII do not have bleeding abnormalities. Individuals who lack factor XI present a relatively mild and variable bleeding disorder, most likely as a consequence of contributions to the tissue factor coagulation system.

Tissue factor–mediated coagulation arises from a complex interplay between the kinetics of activation of coagulation components and the inhibition of these reactions by the appropriate inhibitors. It is the choreography of the dynamics of the overall ensemble of constituent inhibitors and activators in the reaction, coupled with their concentrations, that produces a visible biological response (the clot) or no response. The reaction system is a complex mosaic in which the qualitative and quantitative properties of the reaction components present in an individual's plasma, their blood cells, and their vasculature lead to overall wellness or the pathology of a coagulation defect, either hemorrhage or thrombosis. The sum of the individual parameters of concentrations and qualitative features displayed in an individual's mosaic is a consequence of a complex history involving genetics and passively and actively acquired environmental factors that lead to the risk of hemorrhagic or thrombotic disease. While hemorrhagic disorders are relatively rare, thrombosis is a common primary or secondary cause of morbidity and mortality in our society, and is a pathology that is difficult to predict prior to a crisis. As a consequence, interventive procedures for thrombosis are generally developed following a crisis and involve prophylaxis aimed at preventing subsequent events rather than initial prevention. Thrombosis is the terminal process of virtually all cardiovascular disease. The net cost in our society approaches $200 billion annually.

Three avenues of prophylaxis for thrombosis are currently available to a treating physician: (1) oral warfarin anticoagulants, which interrupt the synthesis of the vitamin K–dependent proteins; (2) intravenous heparin, which enhances the inhibitory activity of antithrombin III and enables release of tissue factor pathway inhibitor from the vascular endothelium; and (3) aspirin, which inhibits the arachidonic acid pathway of platelet activation. All of these agents have been available to the medical community for more than 50 years, and aspirin has been available even longer as an analgesic. Only more recently has aspirin been recognized as an antiplatelet agent. Warfarin-based anticoagulants have been in use since 1941 and have been monitored by the prothrombin time. Heparin has been used for an even longer period of time and is most frequently monitored by the activated partial thromboplastin time. Aspirin is not usually monitored.

It is noteworthy that the therapies for thrombosis pre-
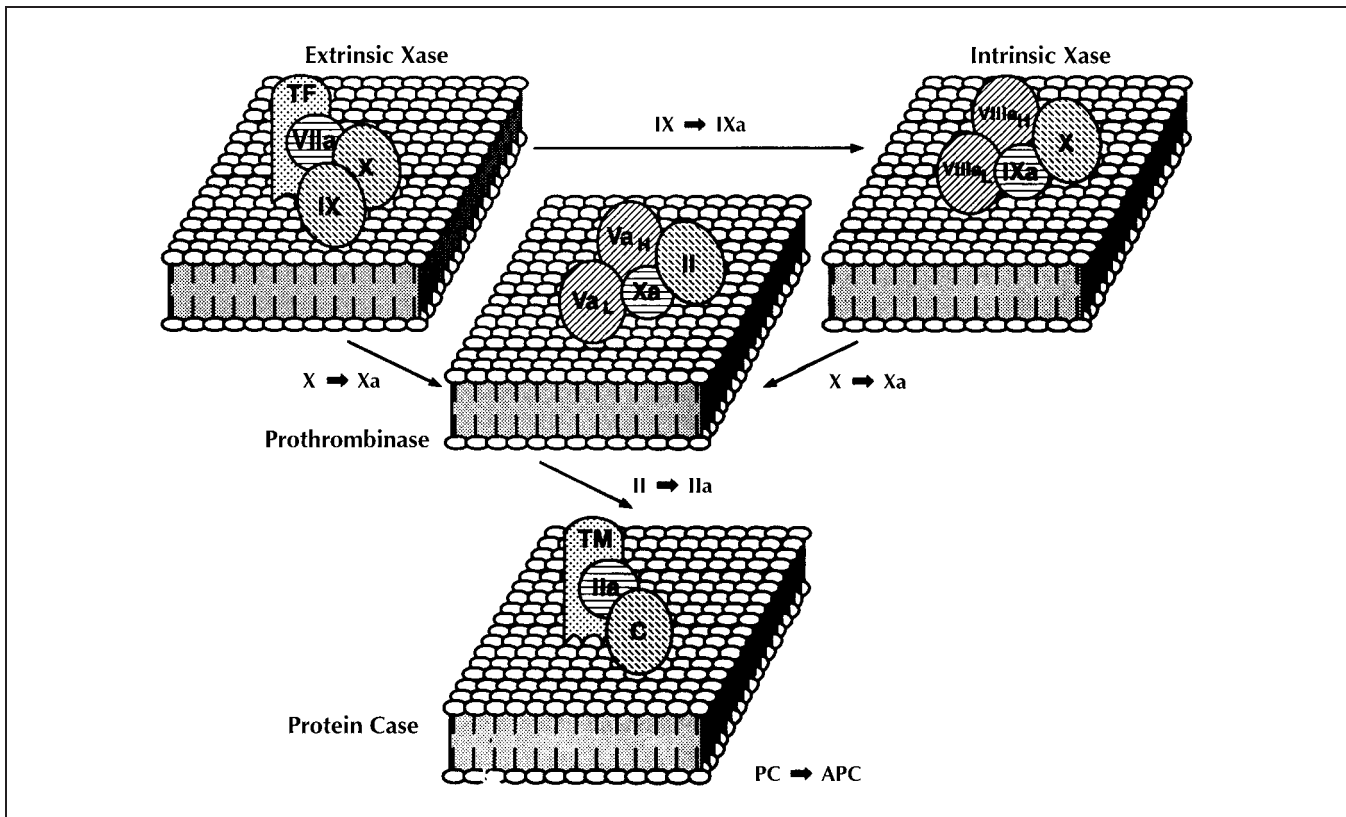
A schematic representation of the vitamin K–dependent blood coagulation enzyme complexes. Each complex is represented assembled on a phospholipid surface. The cofactors—tissue factor (TF), thrombomodulin (TM), factor VIIIa, and factor Va—are represented associated with their respective complementary serine proteases—factor VIIA, factor Xa, factor Ixa, and thrombin (IIa). The substrates of the complexes—factor IX, factor X, prothrombin (II), and protein C (C)—are also represented; APC corresponds to the activated form of protein C. The reactants and products associated with each reaction complex are also represented. Reprinted with permission from Mann KG, Lorand L. Introduction: blood coagulation. In: Methods in Enzymology. Vol 222. San Diego, Calif: Academic Press Inc; 1993:6.

date biological and biochemical knowledge of how these agents interfere with the coagulation system. In addition, the monitoring systems for evaluating the efficacy of anticoagulation have not been effectively improved since their introductions. The activated partial thromboplastin time depends on a totally artifactual initiation of the coagulation system. The prothrombin time as a tool for monitoring anticoagulants has been improved (''patched'') by standardization techniques for the tissue components used to initiate the reaction. However, the prolongation of the prothrombin time used to identify ''appropriate'' anticoagulation is based on clinical experience and tends to err on the side of hemorrhage rather than risk thrombosis. The anticoagulant is influenced by individual variations, including diet, adsorption, genetics, etc, and hemorrhagic risk may also be influenced by other therapies. Evaluation of heparin therapy using plasma clot–based assays has a limited predictive quality, and many laboratories do not monitor heparin at all, a process in vogue with the newer heparin products on the market.

It is somewhat ironic that while we have sophisticated knowledge of the protein and membrane structures, and functions and interactions in the coagulation-fibrinolysis systems, including in many instances the 3-dimensional structure of the proteins, the intellectual capital of coagulation research has not found ready application to the diagnosis of risk and evaluation of prophylaxis for thrombosis. This is especially troublesome when one considers the social and economic costs of thrombotic disease. In the ideal world, we would be available to diagnose those at risk for thrombosis prior to the morbid expression of a thrombotic condition. If such diagnosis is indeed possible, one could provide prophylactic treatment using pharmaceutical and/or lifestyle interventions to reduce the risk of a thrombotic episode. It is my opinion that modern technology has at its disposal the potential to efficiently diagnose thrombotic risk and provide adequate supervision of prophylaxis using both existing anticoagulants and those being developed to identify and treat those at risk. What is required involves identification of each individual's mosaic pattern of coagulation, inhibition, and fibrinolysis parameters using modern diagnostic methodology and to scale this mosaic with population-based epidemiology studies. Scaling will provide a ''thrombosis altimeter,'' which will allow us to survey the immediate risks of a thrombotic event. Through the use of the thrombosis altimeter, we can adjust to fly at a safe height.

Future Directions for Anticoagulant Therapy—Bovill et al

GLYCOPROTEIN IIb-IIIa IN PLATELET AGGREGATION: AN EMERGING TARGET FOR THE PREVENTION OF ACUTE CORONARY THROMBOTIC OCCLUSIONS

David R. Phillips, PhD; Debbie Law, PhD; Robert M. Scarborough, PhD

Platelet aggregates provide the structural foundation for the thrombotic occlusions in coronary arteries. Such occlusions cause the ischemic events associated with acute coronary thrombotic syndromes, such as unstable angina, acute myocardial infarction, and the acute complications associated with coronary angioplasty or stent deployment. Much effort has been directed toward understanding the molecular mechanisms regulating platelet aggregation and in developing pharmacologic agents that prevent aggregation and thrombosis within coronary arteries[1] (Figure).

Platelet aggregation is mediated by the binding of adhesive proteins, such as fibrinogen and von Willebrand factor, to glycoprotein (GP) IIb-IIIa, a member of the integrin family of adhesion receptors. Integrins are widely distributed in most cell types and play critical roles in both cell-cell and cell-matrix adhesion events.[2] Glycoprotein IIb-IIIa expressed on the surface of circulating discoid platelets does not bind soluble adhesive proteins. When platelets are stimulated by agents such as adenosine 5′-diphosphate and thrombin, which are generated within coronary arteries that display active vascular lesions or that are injured by interventive procedures, GP IIb-IIIa becomes a receptor for soluble adhesive proteins. The GP IIb-IIIa recognition motifs on these adhesive proteins are known; for example, fibrinogen contains 2 Arg-Gly-Asp (RGD) sequences found on the α chain, as well as an RGD-like sequence, Lys-Gln-Ala-Gly-Asp-Val, found on the γ chain. These proteins are capable of binding GP IIb-IIIa. The multivalent nature of these proteins allows them to bridge the surfaces of activated platelets, causing platelet aggregation.

Unlike most integrins, GP IIb-IIIa has a narrow tissue distribution, being found only on platelets and cells of the megakaryocytic lineage, increasing its accessibility as a therapeutic target. The members of the first class of therapeutic agents that target GP IIb-IIIa are designed to have an intravenous mode of administration, are effective inhibitors of the adhesive protein-binding function of this receptor, and have proven useful in reducing the ischemic complications of acute coronary thrombotic syndromes. One example of this class of agents is eptifibatide (Integrilin), a synthetic heptapeptide containing a modified Lys-Gly-Arg sequence, which is a high-affinity, specific antagonist of fibrinogen binding to GP IIb-IIIa and is therefore a potent inhibitor of platelet aggregation.[3] The phase III IMPACT II trial showed that eptifibatide inhibits acute thrombotic events (death and myocardial infarction) following angioplasty; the phase III PURSUIT trial showed that eptifibatide also reduces death and myocardial in-
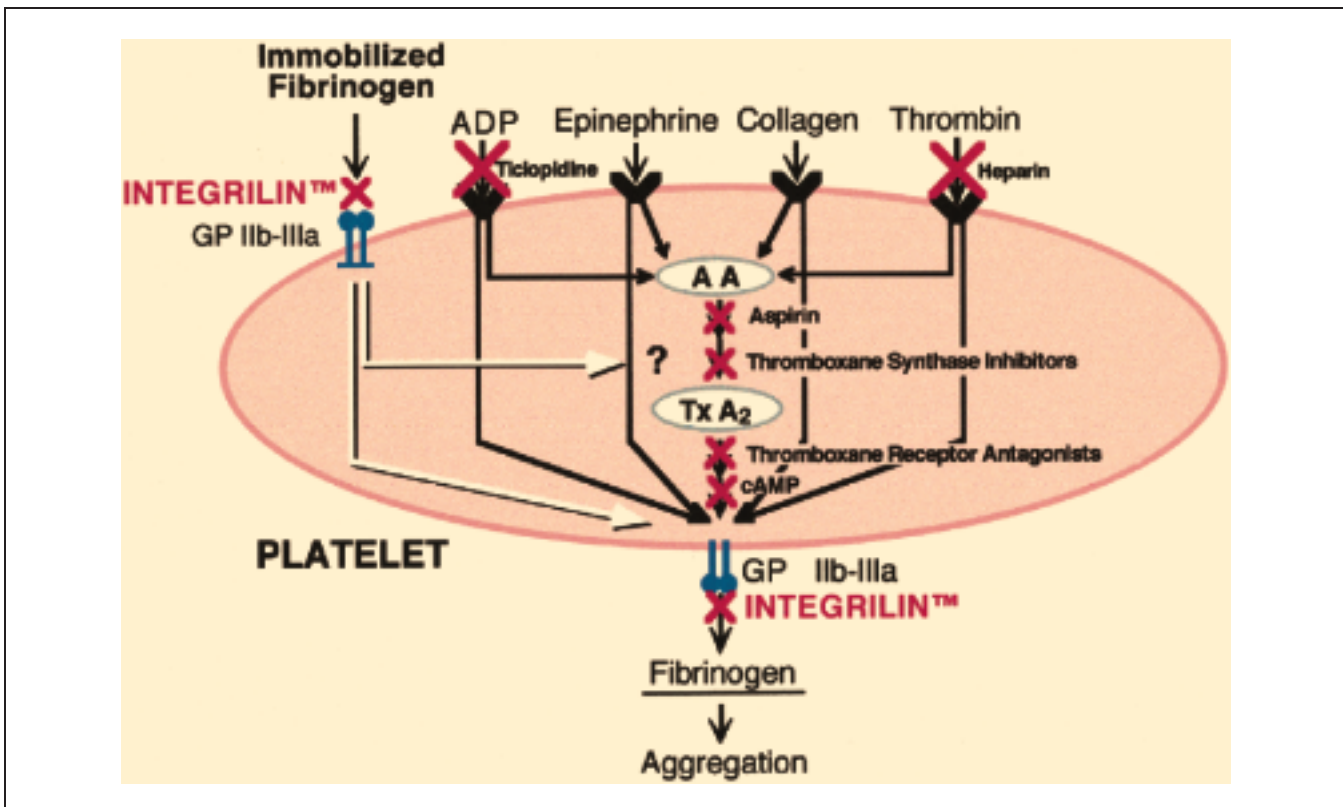
Schematic representation of biochemical targets to antiplatelet agents.

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farction in patients with unstable angina. Other agents in this class include tirofiban and lamifiban, both additional small molecular weight inhibitors, and abciximab, a human/mouse chimeric Fab fragment of a GP IIb-IIIa monoclonal antibody. These agents have also been shown in phase III trials to reduce the thrombotic complications in selected acute coronary thrombotic syndromes.[4]

The members of the second class of GP IIb-IIIa antagonists are also designed to inhibit the adhesive protein-binding function of GP IIb-IIIa, but these antagonists are designed to be orally available and will be tested to determine whether they are effective in reducing thrombotic complications in patients who are chronically at risk for arterial thrombosis (eg, individuals who have had previous myocardial infarction or thrombotic stroke). Recent studies have investigated a potential novel mechanism of platelet inhibition through outside-in GP IIb-IIIa signaling, which occurs when platelets bind to fibrinogen immobilized on the vessel wall or to fibrinogen immobilized on a platelet aggregate. Signaling through this integrin causes platelet stimulation (platelet secretion, GP IIb-IIIa activation) and attachment of cytoskeletal proteins to the plasma membrane (allowing, eg, for clot retraction), thus facilitating the recruitment of unstimulated platelets and the stabilization of a growing thrombus.

To determine whether it is possible to therapeutically inhibit platelet aggregation by regulating the signal transduction reactions of GP IIb-IIIa, we have initiated studies to determine mechanisms by which GP IIb-IIIa signals. These studies have shown that GP IIIa becomes tyrosine-phosphorylated during GP IIb-IIIa outside-in signaling. Biochemical studies have established that GP IIb-IIIa tyrosine phosphorylation provides a mechanism for the induction of outside-in GP IIb-IIIa signal transduction reactions within platelets and for the interaction of the platelet plasma membrane with the cytoskeleton.[5] These studies may provide the frame work to determine whether disruption of outside-in integrin signaling will provide yet an additional strategy to regulate GP IIb-IIIa function and thrombosis.

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# THE NEW ANTICOAGULANTS: NEW OPPORTUNITIES, NEW ISSUES

George P. Vlasuk, PhD

The use of anticoagulants in the treatment and prevention of both acute and chronic thrombosis-related disorders is growing at a rapid pace, in part owing to an increasing geriatric population and the recognition of intravascular clot formation as a causative factor in a number of important cardiovascular diseases, such as myocardial infarction, unstable angina, deep vein thrombosis, pulmonary embolism, and ischemic stroke. The mechanism of all current anticoagulant strategies is the inhibition of 1 or more of the trypsinlike serine proteases that catalyze blood coagulation through the sequential amplified activation of the corresponding zymogens for these enzymes.[1] To date there are only 2 anticoagulant strategies that have been used clinically for more than 50 years in the treatment of acute and chronic thrombosis: (1) heparin and related derivatives (low-molecular-weight heparins) and (2) vitamin K antagonists (warfarin).

Both the heparins and vitamin K antagonists have been used effectively as parenteral and oral anticoagulants, respectively, despite significant limitations in maintaining a therapeutic dose and efficacy in certain clinical indications, as well as unwanted side effects, the most prominent of which is the risk for prolonged bleeding. The side effect profiles of both the heparins[2] and vitamin K antagonists[3,4] are thought to be principally due to their lack of specificity toward numerous serine proteases involved in thrombus development. In the case of the vitamin K antagonists, this includes a significant impairment of the natural anticoagulant pathway mediated through the serine protease activated protein C. The limitations of both the heparins and warfarin have stimulated the search for new therapeutic alternatives based on the selective inhibition of the principal serine proteases catalyzing thrombus formation, namely, thrombin, factor Xa, and the factor VIIa/tissue factor enzymatic complex (Figure).

As the terminal enzyme in the coagulation cascade, thrombin primarily acts to form insoluble fibrin from circulating fibrinogen and activate circulating platelets leading to aggregation.[5] The action of thrombin coupled with the particular rheologic environment found in diseased or damaged vascular beds result in thrombi with compositions that range from platelet-rich clots, which are found primarily in the arterial vasculature, to fibrin-rich, platelet-poor clots, which are found in the venous vasculature. As opposed to the heparins, thrombin inhibitors, which act directly to neutralize the procoagulant effects of this en-
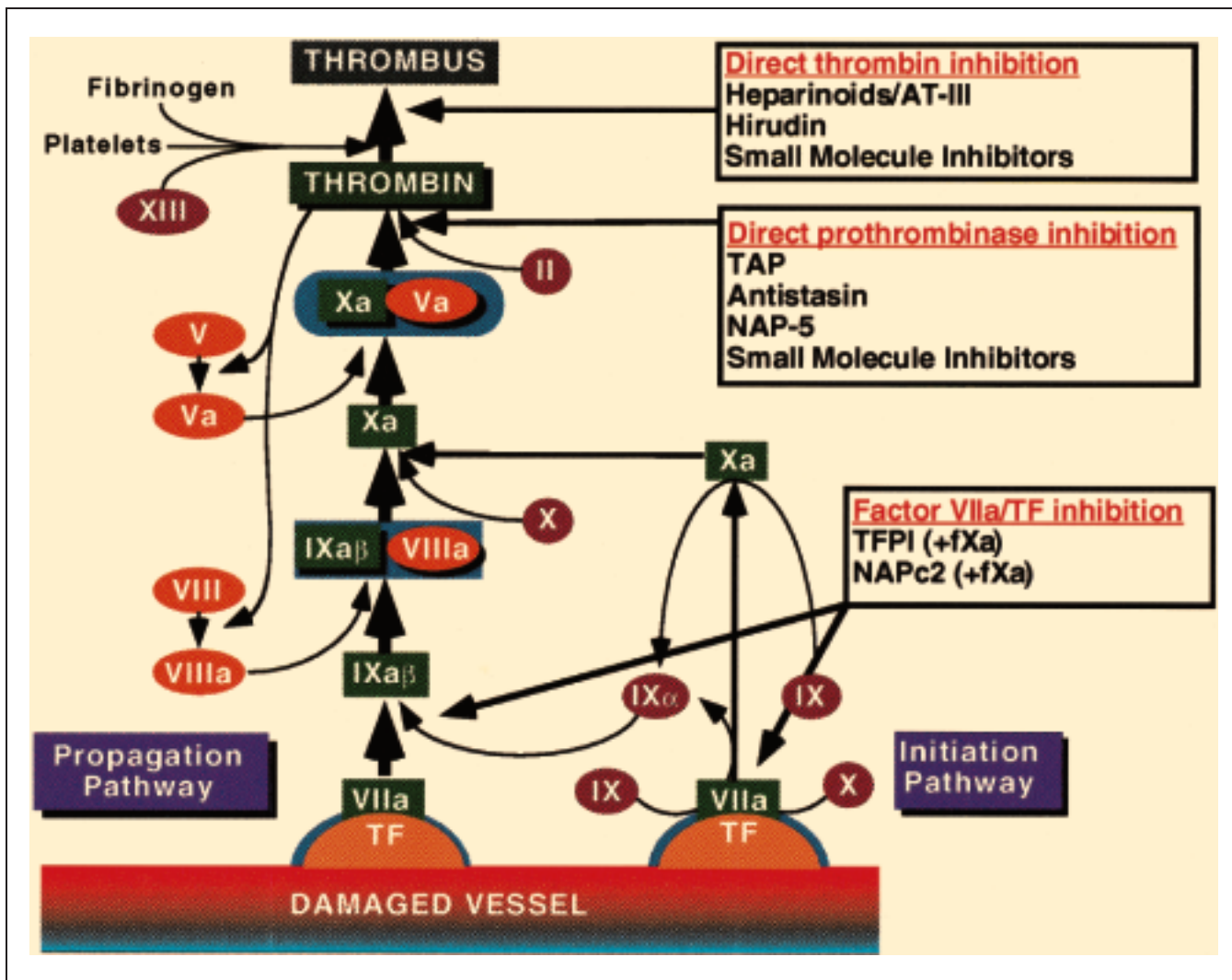
Schematic representation of the serine protease zymogen activation cascade resulting in thrombus formation and examples of various pharma-cologic intervention strategies. Relevant references for the various anticoagulant agents described are as follows: direct thrombin inhibitors and heparanoids[2]; hirudin[21]; small molecule inhibitors[27]; direct prothrombinase inhibitors, tick anticoagulant protein, antistasin, and nematode anti-coagulant protein-5[13]; small molecule inhibitors[27]; factor VIIa/tissue factor inhibitors and tissue factor pathway inhibitor[16]; nematode anticoagulant protein-c2[19,20]; and inactivated factor VIIa (factor VIIai).[17,18]

zyme without the requirement of antithrombin III, have been shown in some cases to be more effective antithrombotic agents in the prevention of arterial and venous thrombosis.[5,6] However, the issue of whether direct thrombin inhibitors will be more beneficial from both an efficacy and safety viewpoint compared to the heparin anticoagulants has not been resolved especially in the clinical setting of acute arterial thrombosis.[7–9]

The pathway leading from the initiation of the coagulation response to thrombin proceeds through the serine protease factor Xa. This enzyme directly activates the circulating zymogen of thrombin, prothrombin. This process occurs following the formation of a macromolecular catalytic complex (prothrombinase), which is composed of factor Xa and the nonenzymatic cofactor factor Va.[10] The assembly of prothrombinase and binding of the substrate prothrombin require an appropriate phospholipid surface, which is typically provided by activated platelets or inflammatory cells adhered to the site of vascular damage. Attempts to target the formation of thrombin rather than

directly inhibit its catalytic activity have relied on direct inhibitors of the prothrombinase complex via the inhibition of factor Xa. Both standard heparin and, more specifically, the family of low-molecular-weight heparins have been shown to effectively, and in some cases selectively, inhibit uncomplexed factor Xa via antithrombin III. However, the contribution of this inhibition to the overall antithrombotic effect of these agents in vivo remains speculative, since it has been shown that factor Xa is restricted from inhibition by antithrombin III when assembled in the prothrombinase complex.[11,12] In contrast, several studies that have compared selective direct and potent inhibitors of factor Xa in the prothrombinase complex with other anticoagulant strategies, such as direct and indirect thrombin inhibition, have suggested a potential advantage of intervening at this point in the cascade in indications where thrombin generation is prominent due to an accumulation of a significant prothrombotic platelet surface, such as the arterial vasculature.[13]

Initiation of the blood coagulation response to vascular

injury follows the formation of a catalytic complex composed of serine protease factor VIIa and the nonenzymatic cofactor, tissue factor.[1]

This response appears to be exclusively regulated by the exposure of subendothelial tissue factor to trace circulating levels of factor VIIa and its zymogen factor VII following a focal breakdown in vascular integrity or the recruitment of activated inflammatory cells, such as monocytes that have surface-exposed tissue factor. It is believed that the small amount of factor Xa that is initially formed catalyzes further activation of factor VII and primes the coagulation response through the proteolytic modification of factor IX to factor IXα, which in turn is converted to the active serine protease factor IXaβ by the factor VIIa/tissue factor complex.[14] This leads to the propagation of the coagulation response through increased factor Xa (prothrombinase) generation following the assembly of factor IXaβ and the nonenzymatic cofactor, factor VIIIa, on an appropriate phospholipid surface.[15] Inhibitors of the factor VIIa–tissue factor complex, such as tissue factor pathway inhibitor,[16] factor VIIai,[17,18] and nematode anticoagulant protein c2,[19,20] have been demonstrated to be effective and potent antithrombotic agents.

How do these different, more direct strategies aimed at intervening in the coagulation cascade compare with the relatively nonspecific agents represented by the heparins and warfarin? The answer to this question will principally depend on how well separated the antithrombotic versus the antihemostatic effects of these agents are, which in essence will define the side-effect profile of which bleeding is of primary concern. This therapeutic window will be the principal determinate of how widely accepted these agents will be in the clinical community once they have completed the necessary clinical trials. One of the most crucial issues that has been and will continue to be considered in the clinical evaluation of these new agents is related to the choice of an appropriate monitoring assay that can accurately reflect the anticoagulant and antihemostatic effects in patients. The conventional clotting time–based assays, such as the activated partial thromboplastin time, prothrombin time, and activated clotting time, have proven useful in monitoring most of the new agents. However, care needs to be taken in developing paradigms that correlate changes in these clotting time assays with associated efficacy and/or bleeding for one agent versus another. This caution is principally due to the differences in mechanism by which these agents prolong clotting time. For example, the associated efficacy and risk of bleeding expected in a patient with twofold to threefold prolongation of the activated partial thromboplastin time or activated clotting time observed with standard heparin may be significantly underestimated with a direct thrombin inhibitor such as hirudin.[21,22] This requires a rigorous determination, during controlled clinical trials, of the correlation between efficacy and safety for any particular agent, avoiding cross-compound comparisons that can lead to a significant underestimate of benefit or risk. The introduction of newer, more specific clinical monitoring tests[23] offers the possibility of more accurate assessments of anticoagulant activity. These tests, which include measures of thrombin activity (fibrinopeptide A[24]), thrombin generation (prothrombin activation peptide F_{1+2},[25]

and thrombin-antithrombin complexes), as well as more experimental approaches, such as factor IX and factor X activation peptide assays,[26] may be used in the future to give more guidance to the clinician as to what exactly is happening in a patient treated with any anticoagulant. However, as with other clinical diagnostic assays, widespread use will depend on a rigorous evaluation of the cost benefit ratio.

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A CLINICIAN'S VIEW OF THROMBOTIC DISORDERS OF THE CARDIOVASCULAR SYSTEM

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The availability and clinical use of anticoagulants in the prophylaxis and treatment of thrombotic disorders involving the venous and arterial circulatory systems date back more than 50 years. Despite intermittent periods of wide-scale use and endorsements from varying societies, associations, and governing bodies, the early clinical experience was limited by poorly standardized drug preparations, monitoring techniques, and the virtual absence of clinical trials to support ''proof of concept.'' As a result, the risks, benefits, and required level of anticoagulation for a variety of clinical indications remained a mystery for many years. More recent attention to carefully designed and coordinated large-scale clinical trials, coupled with an understanding of vascular biology, atherosclerosis, and thrombosis, has paved the way for science and technology to develop new and innovative approaches designed to change the natural history of a variety of common disorders, particularly those characterized by thrombosis occurring at sites of atheromatous plaque erosion or rupture.

Perhaps an appropriate question that should accompany the field's evolution is as follows: What are the perceived priorities for clinicians directly involved with the care of individuals at risk for thrombotic events or for those with ongoing and overt expression of atherothrombotic disease? From a public health perspective, the major goal is to prevent ''hemostasis in the wrong place,'' which is responsible for substantial morbidity, mortality, and health care expenditures. Although the prevention of atherosclerotic vascular disease plays a dominant role in this regard, events may still occur as a result of genetically or environmentally determined prothrombotic potential. Thus, widescale preventive measures may be indicated; however, a preferred approach would focus more on those at greatest risk. Despite the availability of biochemical markers of thrombin activity, thrombin generation, fibrin degradation, and acute-phase reactions that are associated with clinical events in large populations of patients, the overall predictive value of these markers in individual patients and common clinical scenarios is limited. This reality is highlighted by the infrequent use of biochemical markers of thrombus formation or degradation in clinical practice. In essence, the tools used to assess risk and to guide patient management, although somewhat refined, are no different today for clinicians than they were 20 to 30 years ago.

The direct thrombin antagonist hirudin provides an interesting perspective of anticoagulant therapy development.[1–5] The hirudins, hirudin variant 1 (HV-1) and PA-hirudin, are 65- and 66-amino acid proteins, respectively, with 3 sulfide bridges. Hirudin has an unusual asymmetry of structural elements, consisting of (1) a compact hydrophobic core region in the amino-terminal half of the molecule, containing alternating polar and nonpolar segments and all 3 disulfide bonds, and (2) a more extended and extremely hydrophilic carboxy-terminal region. Qualitatively, hirudin has structural properties similar to those of epidermal growth factor, which is a common structural motif found in many proteins. The molecular weight of hirudin is approximately 6964 d. Recent studies suggest that both natural and recombinant hirudin are multimeric under physiologic conditions, but are monomeric when interacting with thrombin under equilibrium-binding conditions. In vitro studies, which were soon followed by studies in animal models, of coronary thrombosis were encouraging and stimulated great interest in a new class of anticoagulants that were potent, specific, and capable of inhibiting clot-bound thrombin. The pharmaceutical industry seized an opportunity and supported phase I and phase II clinical trials in patients with acute myocardial infarction. Stimulated by encouraging early results,[6,7] phase III trials were conducted in the United States and abroad. With increasing experience it became clear, however, that hirudin had a narrow safety window and was not particularly effective at preventing thrombin generation.[8–11] It was an unfortunate, costly, and disappointing turn of events that delivered a strong message to scientists and clinicians alike. Could monitoring (other than the activated partial thromboplastin time) have predicted the narrow safety margin and modest clinical benefit offered by hirudin? Although other direct thrombin antagonists are still being investigated, serious questions remain concerning their application in clinical practice, at least in the treatment of disorders characterized by coronary arterial thrombosis.

Beyond prevention and the identification of individuals at risk for clinical events, clinicians desire safe and cost-effective therapies that are widely available, easy to administer, and easy to monitor (if monitoring is necessary at all). The latter point is one worth emphasizing given the current challenges. A prime example is unfractionated heparin, which is used commonly in the treatment of acute coronary syndromes. Over the years, clinicians have struggled with heparin's complex pharmacokinetics, which necessitates frequent monitoring and complicated titration nomograms. The challenge becomes greater with delays in laboratory test results, making most adjustments in dosing a retrospective exercise. Improvements in the activated partial thromboplastin time assay have helped define the safe level of anticoagulation, which in reality is quite narrow; however, the fact remains that monitoring techniques, as they currently exist, poorly predict efficacy and do not reflect either thrombotic capacity or disease activity.

The mission at hand for future clinicians is not only to develop more safe and effective anticoagulant or antithrombotic agents with predictable pharmacokinetics, pharmacodynamics, and therapeutic response, but also to change our thinking about what actually needs to be monitored, that is, the disease or the drug. Clearly, prior and present day clinicians' time and effort have focused predominantly on the latter. It is time for a change.

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