
Challenges for Providing Effective Hemostasis in Surgery and Trauma

Jeffrey H. Lawson and Michael P. Murphy

Vascular injury, whether surgical or traumatic, triggers a complex series of regulatory events. The understanding of these events, their interdependence, and their effect on hemostasis and thrombosis, is slowly being unraveled. The current understanding of these processes is reviewed in this paper. The application of this knowledge to the operating theatre has been slow and is severely limited by the lack of effective tools to monitor the coagulopathic status of individual patients. Hence, the initial treatment of patients with severe hemorrhage relies on improving the patient's physiological status and on basic surgical techniques. Should these efforts fail, then a number of topical hemostatic agents, selective inhibitors of fibrinolysis, and procoagulant molecules, such as recombinant factor VIIa, may be utilized. However, many of these agents have not yet been tested in clinical trials and studies are urgently needed to determine efficacy, safety, optimal dosage and time of administration.

Semin Hematol 41(suppl 1):55-64. © 2004 Elsevier Inc. All rights reserved.

Blood Coagulation and the Regulation of Hemostasis and Thrombosis

BLOOD COAGULATION is a physiologic defense mechanism that maintains the integrity of the mammalian circulatory system in response to vascular damage. The hemostatic response to injury, whether traumatic or surgical, is a complex series of regulatory events that require the interaction of both cellular elements and blood plasma proteins. The regulation of these cellular and molecular events determines one of three potential outcomes: hemorrhage, controlled hemostasis, or thrombosis. The hemostatic response has been classically characterized as a cascading series of enzymatic reactions, which convert a group of plasma proenzymes to their active enzyme forms (Fig 1). This series of reactions leads to the formation of thrombin, a proteolytic enzyme, which is able to convert fibrinogen to fibrin by limited proteolysis, thus forming the basis of the blood clot.^{10,30} However, this model does not satisfactorily explain the dynamic regulation of blood coagulation reactions that occur in vivo, which requires localized reactions at the point of vascular damage, nor does it address the complex physiology that regulates the clinical outcomes following the major homeostatic challenge of surgery and trauma.

Functionally, hemostasis can be separated into four major regulatory steps, namely, initiation, propagation, termination, and resolution or fibrinolysis. Initiating events convert the normal vascular endothelium to a focal point of procoagulant enzymatic activity. Propagating events are the enzymatic steps that convert a small molecular signal to a multicomponent reaction, leading to the generation of thrombin. Terminating events inhibit or downregulate the procoagulant response, thus containing thrombus formation to a point of vascular damage. Resolution

of the hemostatic process occurs when a vessel filled with hemostatic thrombus becomes reorganized via fibrinolytic pathways allowing for the re-establishment of blood flow through the vascular channel.

Initiation

Endothelial cells line the vascular space which, under normal conditions, provides a barrier between the circulating blood and the extravascular tissue. Resting endothelial cells form a monolayer that is supported by a subendothelial matrix consisting of collagen, elastin, mucopolysaccharides, laminin, fibronectin, von Willebrand factor, and fibrin. The subendothelial matrix forms a second hemostatic barrier to prevent the extravasation of blood. Under normal conditions, the intraluminal surface of the endothelium maintains a nonthrombogenic environment by constitutive expression of heparan sulfate proteoglycans, thrombomodulin, and nonadherent phospholipids, and releases nitric oxide, prostacyclin (PGI₂), and tissue plasminogen activator (t-PA) into the vascular space to promote blood flow.

The initiation of blood coagulation is marked by a physical (traumatic or surgical) disruption or molecular signal, which converts the resting endothelium into a focal point of procoagulant activity. A number of biological agonists have been identified that promote endothelial cell activation by altering the interface of the endothelium with the circulating blood.

From the Departments of Surgery and Pathology, Duke University Medical Center, Durham, NC.

Address correspondence to Jeffrey H. Lawson, MD, PhD, Box 2622, MSRB, Department of Surgery, Duke University Medical Center, Durham, NC 27710.

© 2004 Elsevier Inc. All rights reserved.

0037-1963/04/4101-1011\$30.00/0

doi:10.1053/j.seminhematol.2003.11.012



Figure 1. Schematic representation of in vitro blood coagulation. The cascading reactions culminate in the formation of thrombin and the conversion of fibrinogen to fibrin. TF, tissue factor; PL, phospholipids; Fg, fibrinogen; Fn, fibrin. Adapted with permission from Mann et al.³²

One of the first events to occur following activation of endothelial cells is the proteolytic removal of heparan sulfate proteoglycans from the endothelial cell surface. These heparans function as anticoagu-

lant cofactors that bind to and increase the anti-protease activity of antithrombin III (AT-III). The removal of the heparan proteoglycans decreases the basal level of anti-protease cofactor activity on the cell surface, thus promoting a procoagulant environment.

A second procoagulant event that occurs during endothelial cell activation is the down-regulation, or loss, of thrombomodulin on the cell surface, which decreases the level of anticoagulant activity in the local environment.

The induction of tissue factor expression on the cell surface constitutes the third coagulant effect. Tissue factor functions as the major receptor/cofactor protein in the initiation of the hemostatic response. Once tissue factor appears on the cell surface it binds to the plasma zymogen factor VII or its activated protease factor VIIa, and initiates a series of procoagulant reactions commonly described as the "coagulation cascade."

The fourth event that occurs to initiate the hemostatic response is a change in the phospholipid composition of the endothelial cell membrane. This change results in an increase of acidic phospholipid headgroups in the outer leaflet of the endothelial cell membrane, resulting in the formation of an adherent cellular surface that allows the binding of clotting proteins and enzymatic complex assembly. This localization of clotting factors to the point of vascular damage initiates and propagates the clotting response by condensing the procoagulant enzymes and cofactors onto a single membrane, thus providing a catalytic surface for enzymatic activity (Fig 2).

Propagation

The propagation of blood coagulation has been characterized as proceeding by one of two enzymatic pathways commonly referred to as the intrinsic and extrinsic pathways. The intrinsic pathway of blood coagulation consists of a group of plasma proteins which, in vitro, are activated by "contact" with negatively charged surfaces, such as glass or kaolin. This pathway starts with the formation of factor XIIa. In the presence of high molecular weight kininogen, factor XIIa converts factor XI to XIa. In the presence of calcium ions, factor XIa converts factor IX to IXa. Factor IXa then binds with its cofactor protein factor VIIIa in the presence of calcium ions and the appropriate lipid surface and activates factor X to Xa. The formation of factor Xa then designates the "common pathway" where both intrinsic and extrinsic pathways converge to form the *prothrombinase* complex. Factor Xa binds to its cofactor protein factor Va, in the presence of calcium ions and the appropriate lipid surface, and activates factor II (prothrombin) to factor IIa (thrombin).

The intrinsic pathway has been well characterized

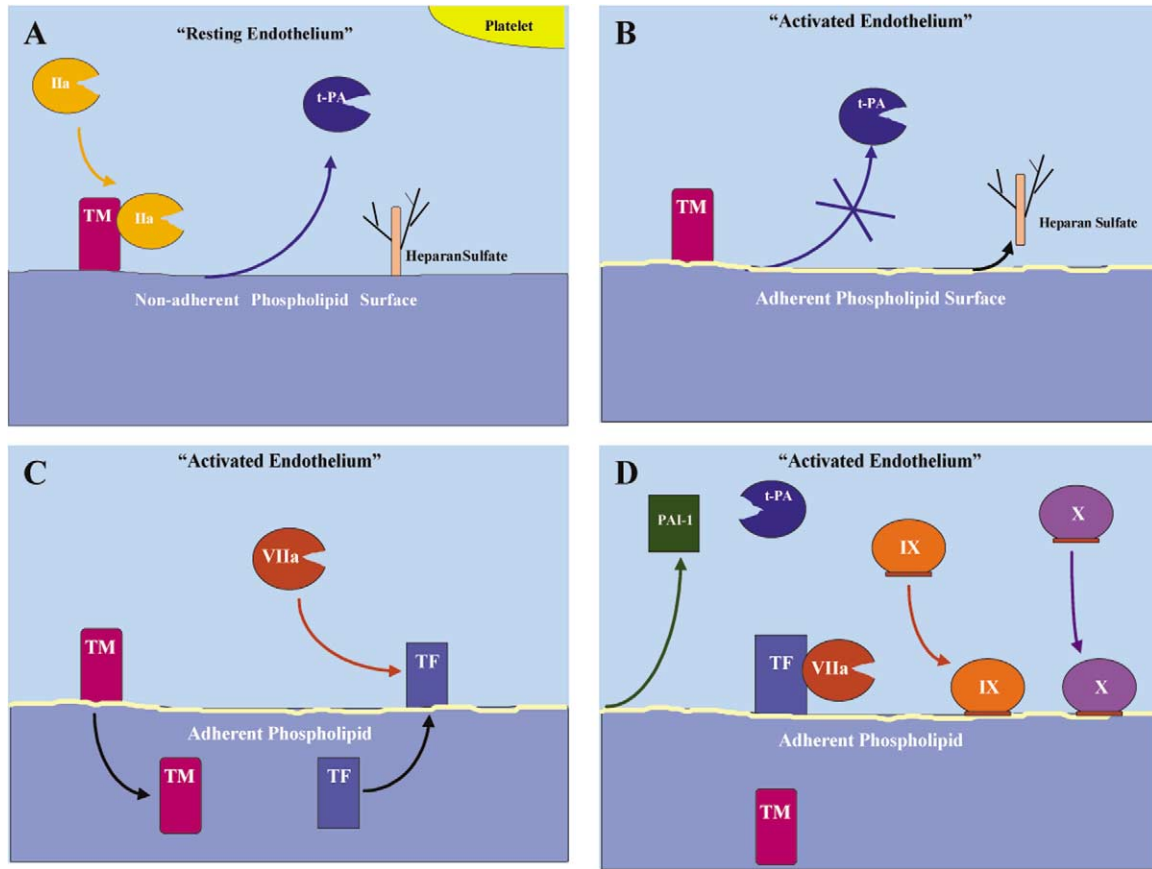


Figure 2. Schematic representation of the initiation of hemostatic pathways.

by in vitro studies. However, in vivo studies have failed to identify the physiologic activator of factor XII. Furthermore, although patients have been identified with deficiencies of prekallikrein,²² high-molecular-weight kinogen,⁷ and factor XII,²⁴ none has been reported to develop a significant bleeding diathesis, or to require prophylactic therapy prior to hemostatic challenge. Thus, the biological relevance of this pathway in the hemostatic response has become suspect.⁴⁶ However, patients with factor XI deficiencies, although rarely requiring therapy, do have significant episodes of bleeding as a complication of surgery.^{40,48} From these observations, it has been generally concluded that factor XI plays a role in the hemostatic response, but it is unclear from in vivo studies whether factor XIa is activated by the other proteins of the intrinsic pathway. An alternative role for the function of factor XI has recently been proposed whereby factor XI is activated to factor XIa by thrombin. In this setting thrombin activation of factor XIa forms a positive feedback loop in which the intrinsic pathway is activated only after a small amount of thrombin has been formed by the extrinsic pathway.¹⁸

The extrinsic pathway was classically defined as the interaction of blood elements with components "outside" the vasculature in response to vascular damage.^{14,33} This pathway is activated by the interaction of the plasma protein factor VIIa with the integral membrane protein tissue factor. This enzymatic complex activates factor X by limited proteolysis. Once factor Xa is formed, the enzyme binds with its cofactor protein, factor Va, to form the next level of enzyme complex. This pathway converges with the intrinsic pathway at the level of factor Xa formation and ultimately leads to the generation of thrombin via the prothrombinase complex.

Over the past 15 years, it has become increasingly recognized that the majority of blood coagulation events are propagated via the tissue factor-dependent pathway.^{17,32,39} It is also clear that congenital deficiencies of factors VIII and IX lead to the classic bleeding tendencies described as hemophilia A and B, respectively.⁴³ Furthermore, patients who exhibit deficiencies in factors II,⁴¹ V,⁴⁷ VII, and X, although less common due to the autosomal recessive nature of the genes coding for these proteins, have bleeding episodes of equal magnitude to those more commonly

Table 1. Kinetic Properties of the Vitamin K–Dependent Enzymatic Complexes of Blood Coagulation

Enzyme	Substrate	K_m^* ($\mu\text{mol/L}$)	k_{cat}^\dagger (min^{-1})	k_{cat}/K_m ($\text{mol/L}^{-1} \cdot \text{min}^{-1}$)
Xa	II	131	0.6	4.58×10^3
Xa/Va/PCPS/ Ca^{2+}	II	1	1800.0	1.80×10^8
IXa	X	299	0.002	6.69
IXa/VIIIa/PCPS/ Ca^{2+}	X	0.063	500.0	7.94×10^9
VIIa	X	4.87	0.024	4.93×10^3
VIIa/TF/PCPS/ Ca^{2+}	X	0.45	69.0	1.53×10^8
VIIa	IX	NA	NA	NA
VIIa/TF/PCPS/ Ca^{2+}	IX	0.243	15.6	6.42×10^7
IIa	PC	60.0	1.2	2.00×10^4
IIa/TM/PCPS/ Ca^{2+}	PC	0.1	214.0	2.14×10^9

Abbreviation: NA, not available.

*Michaelis constant for substrate utilization.

†Turnover number.

Adapted from: Mann KG, et al: Surface-dependent reactions of the vitamin K-dependent enzyme complexes. *Blood* 1990;76:1-16. Copyright American Society of Hematology, used with permission.

described for the classic hemophilias. These observations illustrate that proteins from both the intrinsic and extrinsic pathway are essential for the propagation of normal hemostasis. This has led many investigators to combine the two-pathway system of hemostasis into a unified model where all of the proteins outlined above function in a common pathway of membrane-dependent enzyme complexes, leading ultimately to the generation of thrombin. A diagram of this model is presented in Fig 1. In this model the reaction is initiated after the appearance of tissue factor on the cell surface. Tissue factor then binds with factor VIIa and activates both factors IX and X to the activated proteases factors IXa and Xa, respectively. Factors IXa and Xa then bind with their respective cofactor proteins, factors VIIIa and Va, thus establishing the second level of enzyme complexes. A propagation loop of enzymatic reactions continues until sufficient quantities of factor Xa and thrombin are formed to overcome the local concentrations of protease inhibitors. Once a critical concentration of thrombin exists, it is able to convert sufficient fibrinogen to fibrin to generate a stable blood clot.

Complex Formation

One of the physiologic paradigms of the hemostatic response is the formation of membrane-bound enzymatic complexes. Coagulation enzymes appear only to be effective on a physiologic time scale when these proteins are assembled as a complex on an acidic phospholipid membrane in the presence of calcium ions.

The molecular details of complex assembly have been most rigorously described for the enzyme complex prothrombinase.^{27,31,34} The prothrombinase complex consists of factor Va, factor Xa, a charged

phospholipid surface, and divalent metal ions (Ca^{2+}). Kinetic studies of prothrombin activation by prothrombinase have demonstrated that the complete assembled complex consisting of factor Xa, factor Va, an acidic phospholipid surface, and calcium ions, activates prothrombin nearly 1×10^6 times more efficiently than does factor Xa alone.³⁵ The enhancement of prothrombin activation has been attributed to co-condensation of the enzyme and substrate on the same lipid surface and a 3,000-fold increase in the catalytic rate of prothrombin activation conferred by the interaction of factor Xa with factor Va and the phospholipid surface.³⁶

The other vitamin K–dependent blood clotting complexes have been studied using the prothrombinase complex as a model. Factors IXa and VIIIa form a 1:1 stoichiometric complex on both synthetic and natural cell membranes and in many ways appear to function in a manner similar to prothrombinase.⁴⁹ In contrast, studies of the tissue factor/factor VIIa complex have suggested a slightly different model, where assembly of the catalytic complex does not require acidic phospholipid but only tissue factor in the presence of calcium ions.^{2,29,42} The importance of complex formation for all of the procoagulant proteins outlined above is best illustrated by comparing the catalytic efficiency (k_{cat}/K_m) of each serine protease when bound in an enzyme complex or free in solution. The kinetic properties of complex formation of the various blood clotting enzyme complexes are shown in Table 1. In general, each of the assembled complexes is enzymatically 10,000-fold more efficient than the uncomplexed serine proteases.

Tissue Factor

Tissue factor is an integral membrane glycoprotein that propagates coagulation by its interaction with

plasma factor VII and/or two-chain factor VIIa on a phospholipid or cell surface. Tissue factor was initially characterized as "tissue thromboplastin," which was the functional component in tissue extracts taken from brain or lung tissue that initiated the extrinsic pathway in clotting reactions such as the prothrombin time (PT). Tissue factor has been characterized as a transmembrane glycoprotein with an apparent molecular weight of between 37,000 and 43,000. The mature protein contains three distinct structural domains: amino acids 1 to 219 form the extracellular domain of the protein; amino acids 220 to 242 form a small, hydrophobic transmembrane domain; and residues 243 to 263 form a short cytoplasmic tail.

Under normal conditions, tissue factor is not in contact with the blood. However, tissue factor is maintained in close proximity to the vascular space and has been shown by immunohistochemical techniques to envelop the endothelial cells in the subendothelial matrix and extravascular space.⁵¹ Under pathologic conditions, tissue factor has also been identified in foam cells of atherosclerotic plaques and in proliferating vascular smooth muscle cells.¹² It has also been demonstrated, both in vitro and in vivo, that tissue factor activity can be induced on vascular endothelial cells and monocytes by a number of growth factors and mitogens.^{3,4,9,21,26,45}

Tissue factor is unique among the protein cofactors involved in hemostasis because it requires no further proteolytic processing following its appearance on the cell membrane. Thus, as soon as tissue factor contacts blood elements it is ready to bind its plasma ligand factor VII and/or factor VIIa and initiate the hemostatic response. The exposure of tissue factor to the blood may be induced by a variety of agonists, which include physical, chemical, or cellular damage to the vascular lining. However, once tissue factor appears on the cell surface, the regulation of its activity is dependent on at least three potential events, namely: (1) availability of the ligand factor VIIa to bind the surface receptor; (2) presentation and orientation of substrates (factors IX and X) that will be activated by the tissue factor/factor VIIa complex; and (3) inhibition of the tissue factor/factor VIIa complex.

The binding of factor VIIa to tissue factor proceeds independently of the phospholipid component of the cell surface. The formation of the binary complex is only dependent on two variables: the available number of tissue factor binding sites, and the concentration of factor VII/VIIa in the blood plasma. Tissue factor binds both the zymogen factor VII and the protease factor VIIa with the dissociation constant for either ligand generally reported to be in the range of 10^{-10} to 10^{-9} mol/L.^{13,16} Interestingly, factor VII is found in blood at the lowest concentration of all the

plasma zymogens (10^{-8} mol/L), which is only slightly above the dissociation constant for the tissue factor/factor VIIa interaction. Teleologically, this low concentration of factor VII may function as a protective mechanism in the setting of tissue factor-induced disseminated intravascular coagulation (DIC). It has been demonstrated that animals treated with *Escherichia coli*, endotoxin, or tumor necrosis factor, develop a DIC syndrome that is induced by a tissue factor-based mechanism.⁵⁰ This DIC response can be blocked in vivo by the addition of anti-tissue factor antibodies or high-dose tissue factor pathway inhibitor (TFPI).¹¹ In this setting, once a significant concentration of factor VII has been depleted from the plasma by complex formation and subsequent inhibition, no further procoagulant complexes can be formed, blunting any further hemostatic response. Thus, the very low levels of factor VII in plasma may function as a protective ceiling in the setting of overwhelming tissue factor stimulus, so that the extent of pathologic thrombosis is constrained. This may also explain the hemostatic effect of high-dose factor VIIa observed in coagulopathic patients with massive trauma.

The interaction of the physiological substrates, factors IX and X, which propagate the tissue factor response is a second level of regulation of the catalytic complex. It is well established that proteolytic activation of both factors IX and X is needed for a functional procoagulant response and that these substrates function in concert to propagate the hemostatic reaction. Furthermore, detailed biochemical analysis supports a model whereby these substrates are delivered into the reaction center by an activated cellular membrane surface.²⁸ Although the importance of this may not seem obvious, the mechanism of substrate delivery to the catalytic complex provides a second key regulatory step in the activity of the tissue factor/factor VIIa complex where cellular activation of the membrane surface is required for maximal activity of the tissue factor complex. In this case, the cell would not only require the expression of tissue factor, but also alterations in the phospholipid components of the outer leaflet of the plasma membrane that allow for binding of factors IX or X to the cell surface prior to their activation.

A third major area of regulation of the tissue factor-induced procoagulant response involves the inhibition of the catalytic complex once it is formed. Of the known plasma inhibitors of blood coagulation, only TFPI and AT-III have been characterized as physiological inhibitors of the tissue factor/factor VIIa complex.³⁸ The role of these inhibitors is discussed in detail below.

Termination

Protease inhibitors and anticoagulants. Regulation of the hemostatic response requires the dynamic balance between the procoagulant enzymes and the negative feedback of inhibitor proteins and anticoagulant proteases. In general terms, the blood coagulation response must be tightly localized to the point of vascular damage to prevent widespread thrombosis of the normal blood vessels. This control is maintained by three major classes of regulatory molecules, which include the serine protease inhibitors (serpins), heparins, and the anticoagulant proteases.

Protease inhibitors. The major protease inhibitors that inhibit or regulate the blood coagulation response include the serpins, namely, AT-III, heparin cofactor II (HC-II), plasminogen activator inhibitor 1 (PAI-1), and α_2 -antiplasmin (α_2 AP). Once formed, complexes of proteases and serpins are rapidly cleared from the circulation.

AT-III is the major inhibitor of thrombin and has also been shown to inhibit factors VIIa, IXa, Xa, and XIa. Kinetic studies of AT-III inhibition of these proteases have demonstrated that the inhibitory rate is slow in the absence of heparin, but when heparin is added to the reaction, the rate of inhibition is, in general, increased by 1,000-fold. Only small amounts of free heparin circulate in the plasma and the major source of biologically active heparin is thought to be heparan proteoglycans found on the endothelial cell surface. Heparins function as a cofactor and bind to both the protease and AT-III, thus potentiating the molecular interaction of the protease and inhibitor. Congenital and acquired deficiencies of AT-III lead to hypercoagulable states, which commonly present as spontaneous venous thrombosis or pulmonary embolus.

HC-II is similar to AT-III in that it inhibits serine proteases activated during the procoagulant process and its rate of inhibition is also accelerated by heparin. However, HC-II is much less selective than AT-III in its cofactor requirements and is able to function well with many polyanions and glycosaminoglycans. HC-II circulates at a lower concentration than AT-III and has a slower K_{ass} , suggesting that it is a less effective inhibitor of free thrombin; however, its role as an inhibitor on the cell surface in association with cellular glycosaminoglycans remains to be established.

α_2 -macroglobulin (α_2 M) is an ancient class of protein that circulates in plasma at a concentration of 2 to 5 $\mu\text{mol/L}$. The protein contains two to four identical 180-kd subunits. There are no known deficiency states of α_2 M, a fact that leads some authors to postulate that the inhibitor is biologically so important that a congenital deficiency is incompatible with life. The specific mechanism of α_2 M inhibition has been

studied in detail and a model has been developed in which the large α_2 M molecule physically traps the protease in a molecular cage following the cleavage by the protease of a specific portion of α_2 M termed the "bait region." Following cleavage of the bait region, the α_2 M subunit undergoes a major conformational change which traps the protease and renders it incapable of macromolecular substrate hydrolysis. Once α_2 M-protease complexes are formed, they are rapidly cleared from the circulation by specific high-affinity α_2 M receptors, which are found on a wide range of cell types, including hepatocytes, reticuloendothelial cells, fibroblasts, and adipocytes. α_2 M appears to function as a second line of defense against widespread systemic activation of proteases found in pathologic conditions such as DIC.

Kunins are a superfamily of proteins that are structurally homologous to aprotinin. The unique structural element common to all kunins has been localized to a small 58-amino acid kunin domain. Of the various kunin-type proteins found in blood, the most important inhibitor of the hemostatic process is thought to be TFPI. TFPI has been described as a protease inhibitor consisting of three tandem Kunitz-type domains.²⁰ A unique mechanism of action has been invoked to describe the inhibition of tissue factor/factor VIIa by TFPI. This requires the binding of factor Xa to TFPI before the inhibitor can interact with the tissue factor/factor VIIa complex.⁵ In this setting, inhibition of the complex can only occur after factor Xa has been generated by the reaction. However, the work of Callander et al has challenged this notion.⁶ These investigators have reported that TFPI binds to and inhibits the tissue factor/factor VIIa complex directly with a dissociation constant of 11.9 nmol/L in the absence of factor Xa, and a dissociation constant of 4.5 nmol/L in the presence of factor Xa. From these studies the authors concluded that factor Xa is not an obligate requirement for the inhibition of the tissue factor/factor VIIa complex by TFPI, but only enhances the overall binding affinity. In spite of TFPI being a high-affinity inhibitor of the tissue factor pathway, its plasma concentration is below its reported K_{ass} . These data have caused some investigators to question the role of TFPI as a true inhibitor of the tissue factor pathway and have led to the suggestion that it may function more as a regulator of tissue factor-stimulated reactions rather than as a true inhibitor. In light of these data, the true role of TFPI in the regulation of tissue factor-dependent reactions awaits further study.

Heparins. Heparins are naturally occurring glycosaminoglycans, which function in the anticoagulant arm of the hemostatic process by potentiating the inhibitory action of AT-III. The binding of heparin to a specific domain of AT-III greatly accelerates the rate at which AT-III inactivates procoagulant enzymes.

This property forms the basis for the widespread clinical use of heparin as anticoagulant therapy.

Thrombomodulin. Thrombomodulin is an integral membrane protein, which does not require proteolytic processing to become an active cofactor. Thrombomodulin can be classified as cellular “receptor,” which binds thrombin with a dissociation constant of 1×10^{-9} mol/L.¹⁹ The binding of thrombin to thrombomodulin alters the enzymatic specificity of thrombin, switching it from a procoagulant enzyme that converts fibrinogen to fibrin, to an anticoagulant enzyme that activates protein C to activated protein C (APC). Once formed, APC inactivates factors Va and VIIIa via limited proteolysis. Thrombomodulin is constitutively expressed on the surface of vascular endothelial cells and platelets¹⁵ thus promoting an anticoagulant posture on the surface of normal cells in contact with blood elements.

Fibrinolysis

Under normal hemostatic functioning, t-PA is secreted by endothelial cells and, on binding to a fibrin clot, locally activates plasminogen to plasmin. It is the direct binding of t-PA to fibrin that gives this molecule the reputation for “clot-specific” fibrinolysis. Once plasmin is activated by t-PA, it begins to degrade fibrin into soluble products, including D-dimers, which are often used as a marker of fibrinolytic processes. The regional control of plasmin activation is essential to the specific lysis of a local thrombus. However, in the setting of trauma and surgery, systemic plasmin activation may result in systemic fibrinolysis and degradation of both hemostatic fibrin polymers and circulating fibrinogen. This is a likely explanation for the use of systemic antifibrinolytics, such as ϵ -aminocaproic acid and aprotinin, in the setting of massive surgery in which systemic fibrinolysis has been induced.

Like all other proteolytic pathways that are activated during the hemostatic response, fibrinolysis involves a complex set of inhibitory pathways that, under normal conditions, are downregulated to prevent overactivation. PAI-1 has been characterized as a potent inhibitor of t-PA, two-chain t-PA, and urokinase. PAI-1 downregulates the activity of these plasminogen activators, thus preventing widespread plasmin formation and systemic fibrinolysis. PAI-1 is normally synthesized and secreted from endothelial cells. However, during endothelial cell stimulation this process is diminished leading initially to a loss of local anti-fibrinolytic activity.

α_2 AP is the primary inhibitor of plasmin, which is formed during fibrinolysis. α_2 AP inhibits free plasmin 100 times more effectively than plasmin which is bound to fibrin during fibrinolysis. These data have led to the hypothesis that α_2 AP functions to inhibit

free plasmin as it diffuses from fibrin, thus inhibiting systemic fibrinolysis, while leaving plasmin which is bound to fibrin free to function as a protease. Unlike AT-III and HC-II, α_2 AP does not appear to be potentiated by heparin or polyanionic surfaces.

Physiology of Hemostasis

Although this brief review outlines various individual aspects of complex hemostatic pathways, it fails to emphasize the interdependence of these various physiologic systems. In the setting of human physiology and stress, competing biochemical pathways often appear to push and pull against each other. However, when surgical stress, trauma, or illness pushes one of these competing pathways out of balance, pathologic events can occur, leading to either hemorrhage or thrombosis. Unfortunately, few studies have evaluated the interdependence of these pathways in vivo and even fewer have attempted to characterize how these events may proceed amid the complex physiologic events observed in surgical and trauma patients.

Hemostasis in Surgery and Trauma

In the best-case scenario, surgery can be thought of as a controlled form of trauma in which patients are exposed to hemostatic stress. In the worst-case scenario, major surgery or trauma tests the limits of hemostatic function by stimulating an array of physiologic systems that “push” to the limit bleeding from the operative or traumatic wound while maintaining essential blood flow to vital organs. This problem is highlighted in a review by Sauaia and associates, which places exsanguination second to central nervous system injuries as a cause of death in trauma patients.⁴⁴ Coagulopathy is the most frequent complication encountered in the management of traumatic injuries. A clinical model developed using multiple logistic regression analysis revealed four significant risk factors that contribute to bleeding complications in trauma patients, namely, pH less than 7.10, temperature less than 34°C, injury severity score greater than 25, and systolic blood pressure less than 70 mm Hg. Of these risk factors, hypothermia is the most important as it impedes the temperature-dependent enzymatic reactions of both the intrinsic and extrinsic pathways, and depresses platelet function. Furthermore, the fibrinolytic system is immediately activated following trauma, returning to normal after the first 24 hours in patients with mild to moderate injuries, but remaining elevated in those with major injuries.²³ Platelet counts decrease after major injury, and in survivors platelet counts normalize in the first few days. However, in fatal cases platelet counts are lower and remain depressed throughout

resuscitation.^{1,23} Blood tests that are most frequently abnormal are PT (97%), platelet count (72%), and activated partial thromboplastin time (aPTT) (70%).¹

Coagulation abnormalities occur with greater severity in trauma patients with head injuries, followed by those with gunshot wounds, blunt trauma, and stab wounds.⁸ Brain injury causes the release of tissue thromboplastin into the circulation, rapidly activating the extrinsic coagulation pathway and leading to a consumptive coagulopathy. Massive transfusion is associated with a significant increase in mortality in trauma. Dilution of platelets and coagulation factors occurs with red blood cell replacement alone, and prolongation of the PT and aPTT are due to low levels of factors V and VIII. Other abnormalities associated with transfusion are thrombocytopenia and hypothermia-induced platelet dysfunction.^{8,37} In addition, surgical and trauma patients tend to evolve from a fibrolytic state in the first 24 to 48 hours after the insult to a hypercoagulable condition notable for an increased risk of thrombotic complications, such as deep venous thrombosis, myocardial infarction, thrombotic occlusion of bypass grafts, and stroke.^{23,37}

Thus, the challenge of providing effective hemostasis in surgery is to be able to recognize the unique situation of each patient undergoing hematologic stress and to maintain their physiology between the delicate balance of bleeding or clotting to death. In spite of the observation that surgery and trauma cause some of the most extreme hemostatic challenges to patients, much is still unknown about the cellular or biochemical function of patients during the critical period of surgical stress. Such knowledge might enable the operative team to make critical decisions regarding risks for hemorrhage, thrombosis, or both.

The clinical magnitude of this problem is highlighted by a recent 2-year review of surgical deaths and complications from our own institution. From this review, nearly 50% of the surgical complications could be attributed to either hemorrhage or thrombosis in the operative or postoperative period. An even more unsettling observation was that the majority of these complications were “nontechnical,” meaning that perioperative bleeding or thrombosis was out of balance causing the adverse outcome. Obvious reasons for these complications include highly complex surgical procedures, severe trauma, patients presenting to the operating room on an array of systemic antiplatelet medication or anticoagulants, and unknown underlying hypercoagulable states.

The problem of perioperative hemorrhage or thrombosis is exacerbated by the fact that a given patient may swing from one extreme to the other during the course of the operative and postoperative period. This hemostatic swing is illustrated in Fig 3.

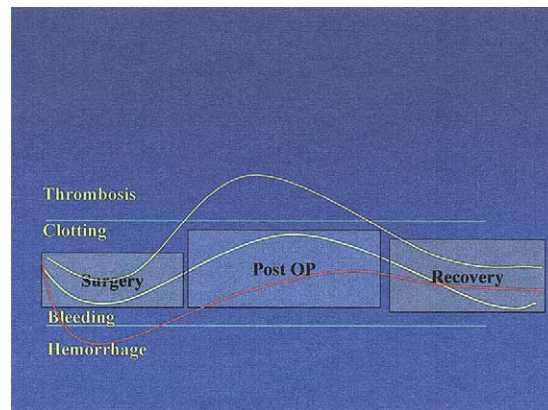


Figure 3. Schematic representation of physiologic swings between hemorrhage and thrombosis during surgery.

In this setting, patients may be initially hypothermic and/or hemodiluted, and have systemic activation of fibrinolytic pathways leading to a hemorrhagic phenotype.²³ However, if oversupported in the inflammatory postoperative period, this situation may rapidly swing back to that of a thrombotic state with the associated risk of myocardial infarction, pulmonary embolus, or deep vein thrombosis. This scenario is well-illustrated by a recent report from our institution where 14 cases of children undergoing cardiopulmonary bypass procedures were prospectively followed during the operative period in an attempt to describe changes in hemostatic function. These children quickly slipped into a hemorrhagic state with the loss of factor V activity (30%) and a systemic increase in circulating t-PA levels. However, this state quickly reversed in the postoperative period with the recovery of PAI-1 levels and an increase in inflammatory cytokines that could be shown to induce tissue factor on cultures of endothelial cells.²⁵

Effective Hemostasis: What Can Be Done in Surgery and Trauma?

In the past 20 years much has improved in the medical management of hemostasis and thrombosis. Unfortunately, this improved understanding of hemostatic biology and the use of innovative therapies has been slow to translate to the operating room. One major limitation for many clinicians is the lack of accurate point-of-care testing that would allow operative teams to make real-time decisions regarding the complex interplay of the various hemostatic pathways outlined above. Activated clotting times serve only as a rough measure of heparinization, while the usefulness of thrombelastograms is limited by variation in interpretation. Thus, many clinicians have to make intraoperative therapeutic decisions based on

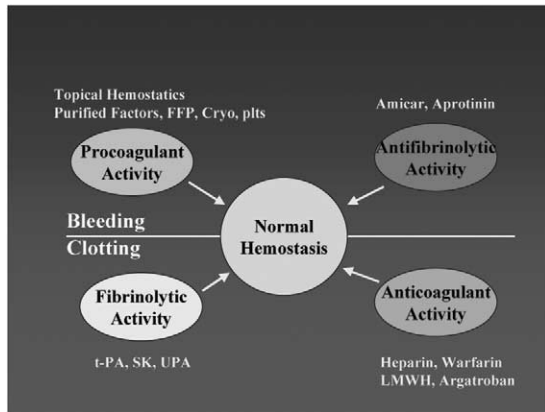


Figure 4. Various therapeutics for use in control of hemostatic pathways during surgery. FFP, fresh-frozen plasma; Cryo, cryoprecipitate; plts, platelets; SK, streptokinase; UPA, urokinase-type plasminogen activator; LMWH, low-molecular-weight heparin.

physiologic parameters, simple observation such as the ability of a patient to make a clot, and the complexity of the surgical procedure. With clinical experience of the operative team as the leading guide to hemostasis management, many tools are now available to manage both hemorrhage and thrombosis in the operative environment. Figure 4 illustrates a general list of agents that may be used to direct the physiology of any given patient as they drift away from the “safe center” of hemostatic function. This array of topical hemostatic products includes matrix proteins, fibrin polymers, and/or thrombin, which can be applied by the surgeon directly onto a bleeding wound. In addition, standard blood products are still considered to be extremely useful to replace lost factors or platelets and aid in volume resuscitation. In exsanguinating patients, the use of high-dose recombinant factor VIIa has been shown to be beneficial in selected cases of severe trauma. Finally, ϵ -aminocaproic acid (Amicar, Lederle Laboratories, Pearl River, NY) and aprotinin (Trasylol Bayer Corp, Leverkusen, Germany) are widely used in cardiac surgery when systemic fibrinolysis is identified; these agents are slowly gaining acceptance in other major surgical and traumatic procedures.

Many vascular surgeons use intraoperative lytic therapy to augment the fibrinolytic system; t-PA, urokinase, or streptokinase may be applied directly to a vascular bed compromised by intraluminal thrombus.

To enhance the anticoagulant system, heparin is routinely the drug of choice. However, there is now an array of newer anticoagulants that await formal testing in the operative environment.

When severe hemorrhage is encountered in a patient, we recommend compliance with the following basic principles. The best results start with good patient physiology. The operative team must make

every effort to keep the patient warm, well resuscitated, and balanced with respect to pH, divalent metal ions (Ca^{2+} , Mg^{2+}), and electrolytes. If basic surgical techniques are exhausted, the selective use of topical hemostatic agents to areas of bleeding appears to have merit. However, many of these reagents still lack formal documentation of efficacy, and they should never be used as a substitute for surgical skill. Finally, the use of selective, systemic inhibitors of fibrinolysis, and procoagulant molecules such as recombinant factor VIIa, is increasing. However, the benefit of these reagents will only truly be demonstrated after careful studies have addressed the following issues: (1) the optimal dose of the reagent; (2) the best time in the surgical procedure to use these powerful molecular tools; and (3) how to avoid “overshooting the mark” in patients at risk for a myriad of thrombotic complications.

References

1. Aasen A, Kierulf P, Vaage J, et al: Determination of the plasma proteolytic enzyme systems gives information of prognostic value in patients with multiple trauma. *Adv Exp Med Biol* 156:1037-1047, 1983
2. Bach R, Gentry R, Nemerson Y: Factor VII binding to tissue factor in reconstituted phospholipid vesicles: induction of cooperativity by phosphatidylserine. *Biochemistry* 25:4007-4020, 1986
3. Bevilacqua MP, Pober JS, Majeau GR, et al: Interleukin 1 (IL-1) induces biosynthesis and cell surface expression of procoagulant activity in human vascular endothelial cells. *J Exp Med* 160:618-623, 1984
4. Brox JH, Osterud B, Bjorklid E, et al: Production and availability of thromboplastin in endothelial cells: The effects of thrombin, endotoxin and platelets. *Br J Haematol* 57:239-246, 1984
5. Broze GJ, Warren LA, Novotny WF, et al: The lipoprotein-associated coagulation inhibitor that inhibits the factor VII-tissue factor complex also inhibits factor Xa: Insight into its possible mechanism of action. *Blood* 71:335-343, 1988
6. Callander NS, Rao LVM, Nordfang O, et al: Mechanisms of binding of recombinant extrinsic pathway inhibitor (rEPI) to cultured cell surfaces. Evidence that rEPI can bind to and inhibit factor VIIa-tissue factor complexes in the absence of factor Xa. *J Biol Chem* 267:876-882, 1992
7. Colman RW, Bagdasarian A, Talama RC: Human kininogen deficiency with diminished levels of plasminogen proactivator and prekallikrein associated with abnormalities of the Hageman factor-dependent pathways. *J Clin Invest* 56:1650-1656, 1975
8. Counts RB, Haisch C, Simon T, et al: Homeostasis in massively transfused trauma patients. *Ann Surg* 190:91-99, 1979
9. Crossman DC, Carr DP, Tuddenham EGD, et al: The regulation of tissue factor mRNA in human endothelial cells in response to endotoxin or phorbol ester. *J Biol Chem* 265:9782-9787, 1990
10. Davie EW, Ratnoff OD: Waterfall sequence for intrinsic blood clotting. *Science* 145:1310-1312, 1964
11. Day KC, Hoffman LC, Palmier MO, et al: Recombinant lipoprotein-associated coagulation inhibitor inhibits tissue thromboplastin-induced intravascular coagulation in the rabbit. *Blood* 76:1538-1545, 1990

12. Drake TA, Morrissey JH, Edgington TS: Immunohistochemical detection of tissue factor in human atherosclerotic plaques. *Circulation* 80:II-182, 1989 (suppl 2, abstr)
13. Drake TA, Ruf W, Morrissey JH, et al: Functional tissue factor is entirely cell surface expressed on lipopolysaccharide-stimulated human blood monocytes and a constitutively tissue factor-producing neoplastic cell line. *J Cell Biol* 109:389-395, 1989
14. Edgington TS, Mackman N, Brand K, et al: The structural biology of expression and function of tissue factor. *Thromb Haemost* 66:67-79, 1991
15. Esmon CT: The roles of protein C and thrombomodulin in the regulation of blood coagulation. *J Biol Chem* 264:4743-4746, 1989
16. Fair DS, MacDonald MJ, Fair DS: Cooperative interaction between factor VII and cell surface-expressed tissue factor. *J Biol Chem* 262:11692-11698, 1987
17. Furie B, Furie BC: The molecular basis of blood coagulation. *Cell* 53:505-518, 1988
18. Gailani D, Broze GJ: Factor XI activation in a revised model of blood coagulation. *Science* 253:909-912, 1991
19. Galvin JB, Kurosawa S, Moore K, et al: Reconstitution of rabbit thrombomodulin into phospholipid vesicles. *J Biol Chem* 262:2199-2206, 1987
20. Girard TJ, Warren LA, Novotny WF, et al: Functional significance of the Kunitz-type inhibitory domains of lipoprotein-associated coagulation inhibitor. *Nature* 338:518-520, 1989
21. Hartzell S, Ryder K, Lanahan A, et al: A growth factor-responsive gene of murine BALB/c 3T3 cells encodes a protein homologous to human tissue factor. *Mol Cell Biol* 9:2567-2573, 1989
22. Hathaway WE, Belhanson LP, Hathaway HS: Evidence for a new plasma thromboplastin factor I. Case report, coagulation studies and physicochemical properties. *Blood* 26:521-525, 1965
23. Hewson JR: Homeostatic alterations with major trauma: Massive transfusion. *Can Anaesth Soc J* 32:239-240, 1985
24. Hoak JC, Swenson LW, Warnar ED: Myocardial infarction associated with severe factor-XII deficiency. *Lancet* 2:884-886, 1966
25. Jagers JJ, Neal MC, Smith PK, et al: Infant cardiopulmonary bypass: A procoagulant state. *Ann Thorac Surg* 68:513-520, 1999
26. Janco RL, Morris PJ: Regulation of monocyte procoagulant by chemoattractants. *Blood* 65:545-552, 1985
27. Krishnaswamy S, Jones KC, Mann KG: Prothrombinase complex assembly. Kinetic mechanism of enzyme assembly on phospholipid vesicles. *J Biol Chem* 263:3823-3834, 1988
28. Krishnaswamy S, Field KA, Edgington TS, et al: Role of the membrane surface in the activation of factor X. *Circulation* 82:III-133, 1990 (abstr)
29. Lawson JH, Butenas S, Mann KG: The evaluation of complex-dependent alterations in human factor VIIa. *J Biol Chem* 267:4834-4843, 1992
30. Macfarlane RG: An enzyme cascade in the blood clotting mechanism, and its function as a biochemical amplifier. *Nature* 202:4998-4999, 1964
31. Mann KG, Jenny RJ, Krishnaswamy S: Cofactor proteins in the assembly and expression of blood clotting enzyme complexes. *Annu Rev Biochem* 57:915-956, 1988
32. Mann KG, Nesheim ME, Church WR, et al: Surface-dependent reactions of the vitamin K-dependent enzyme complexes. *Blood* 76:1-16, 1990
33. Nemerson Y: Tissue factor and hemostasis. *Blood* 71:1-8, 1988 (erratum 71:1178, 1988)
34. Nesheim ME, Kettner C, Shaw E, et al: Cofactor dependence of factor Xa incorporation into the prothrombinase complex. *J Biol Chem* 256:6537-6540, 1981
35. Nesheim ME, Taswell JB, Mann KG: The contribution of bovine factor V and factor Va to the activity of prothrombinase. *J Biol Chem* 254:10952-10962, 1979
36. Nesheim ME, Tracy RP, Mann KG: "Clotspeed," a mathematical simulation of the functional properties of prothrombinase. *J Biol Chem* 259:1447-1453, 1984
37. Ordog GT, Wasserberger J, Balasubramaniam S, et al: Coagulation abnormalities in traumatic shock. *Ann Emerg Med* 14:650-655, 1985
38. Rapaport SI: Inhibition of factor VIIa/tissue factor-induced blood coagulation: with particular emphasis upon a factor Xa-dependent inhibitory mechanism. *Blood* 73:359-365, 1989
39. Rapaport SI: The extrinsic pathway inhibitor: A regulator of tissue factor-dependent blood coagulation. *Thromb Haemost* 66:6-15, 1991
40. Rapaport SI, Proctor RR, Patch MJ, et al: The mode of inheritance of PTA deficiency: Evidence for the existence of major PTA deficiency and minor PTA deficiency. *Blood* 18:149-165, 1961
41. Roberts HR, Foster PA: Inherited disorders of prothrombin conversion, in Colman RW, Hirsh J, Marder VJ, et al (eds): *Hemostasis and Thrombosis*. Philadelphia, PA, Lippincott, 1987, pp 162-181
42. Ruf W, Rehemtulla A, Edgington TS: Phospholipid-independent and -dependent interactions required for tissue factor receptor and cofactor function. *J Biol Chem* 266:2158-2166, 1991
43. Sadler JE, Davie EW: Hemophilia A, hemophilia B, and von Willebrand's disease, in Stamatoyannopoulos G, Nienhuis AW, Leder P, et al (eds): *The Molecular Basis of Blood Diseases*. Philadelphia, PA, Saunders, 1987, pp 575-630
44. Sauaia A, Moore FA, Moore FE, et al: Epidemiology of trauma deaths: A reassessment. *J Trauma* 38:185-193, 1995
45. Scarpatti EM, Sadler JE: Regulation of endothelial cell coagulant properties. Modulation of tissue factor, plasminogen activator inhibitors, and thrombomodulin by phorbol 12-myristate 13-acetate and tumor necrosis factor. *J Biol Chem* 264:20705-20713, 1989
46. Schmaier AH, Silverberg M, Kaplan AP, et al: Contact activation and its abnormalities, in Colman RW, Hirsh J, Marder VJ, et al (eds): *Hemostasis and Thrombosis. Basic Principles and Clinical Practice*. Philadelphia, PA, Lippincott, 1987, pp 18-33
47. Seeler RA: Parahemophilia. factor V deficiency. *Med Clin North Am* 56:119-124, 1972
48. Sidi A, Seligsohn U, Jonas P, et al: Factor XI deficiency: Detection and management during urological surgery. *J Urol* 119:528-537, 1978
49. van Dieijen G, Tans G, Rosing J, et al: The role of phospholipid and factor VIIIa in the activation of bovine factor X. *J Biol Chem* 256:10952-10959, 1981
50. Warr TA, Rao VM, Rapaport SI: Disseminated intravascular coagulation in rabbits induced by administration of endotoxin or tissue factor: Effect of anti-tissue factor antibodies and measurement of plasma extrinsic pathway inhibitor activity. *Blood* 75:1481-1489, 1990
51. Wilcox JN, Smith KM, Schwartz SM, et al: Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. *Proc Natl Acad Sci USA* 86:2839-2843, 1989