Blood Coagulation: Hemostasis and Thrombin Regulation

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Perioperative bleeding is a major challenge particularly because of increasing clinical use of potent antithrombotic drugs. Understanding current concepts of coagulation is important in determining the preoperative bleeding risk of patients, and in managing hemostatic therapy perioperatively. The serine protease thrombin plays pivotal roles in the activation of additional serine protease zymogens (inactive enzymatic precursors), cofactors, and cell-surface receptors. Thrombin generation is closely regulated to locally achieve rapid hemostasis after injury without causing uncontrolled systemic thrombosis. During surgery, there are major disturbances in coagulation and inflammatory systems because of hemorrhage/hemodilution, blood transfusion, and surgical stresses. Postoperative bleeding often requires allogeneic blood transfusions, which support thrombin generation and hemostasis. However, procoagulant activity and inflammation are increased postoperatively; thus, antithrombotic therapy may be required to prevent perioperative thrombotic complications. There have been significant advances in the management of perioperative hemostasis and thrombosis because of the introduction of novel hemostatic and antithrombotic drugs. However, a limitation of current treatment is that conventional clotting tests do not reflect the entire physiological processes of coagulation making optimal pharmacologic therapy difficult. Understanding the *in vivo* regulatory mechanisms and pharmacologic modulation of thrombin generation may help control bleeding without potentially increasing prothrombotic risks. In this review, we focus on the regulatory mechanisms of hemostasis and thrombin generation using multiple, simplified models of coagulation.

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Blood has been considered sacred since ancient times. The Chinese character for blood originates from the hieroglyphical symbol of "a sacrifice placed in a vessel" (Fig. 1). The ability to transform blood components from liquid to solid form represents a network of enzymatic activation and inhibition. The modern concept of coagulation was presented in 1964 as the Waterfall/Cascade model, which might overwhelm many nonhematologists with its complexity (Fig. 2A).[1] The model has been further refined as a cell-based model describing a complex networking of various elements of coagulation (Fig. 2B).[2] Over the course of time, blood coagulation has become a highly sophisticated defense mechanism to detect injury to the body and prevent exsanguinations to enhance survival.[3–7] The importance of surveillance and rapid, localized hemostatic actions of the coagulation system are necessary given the multiple number of breaches to vascular integrity that occur over a lifetime.* This article presents a current perspective on the basic mechanisms of coagulation, hemostasis, and thrombin formation.

BASIC COAGULATION MECHANISM

To understand the evolution of hemostasis, it is useful to examine the primitive coagulation system of invertebrates. Horseshoe crabs (*Limulus*) have lived on Earth for more than 350 million years; thus, they are often called living fossils. In the blood (hemolymph) of *Limulus*, oxygen is transported by the copper-containing protein hemocyanin. The only circulating blood cells are amoebocytes (hemocytes) which contain bactericides and coagulation zymogen proteins that are released upon activation. The key components of coagulation in the *Limulus* are serine proteases,

*A typical person injures oneself 4000 times in lifetime—John Tayman, the author of "The Colony" on the Fresh Air, Fear and Loathing in Hawaii: "Colony", transcript available from www.npr.org.

Figure 1. The Chinese character of blood. A diagonal stroke over the symbol of the plate (皿) signifies "the sacrifice placed on the plate."

Figure 2. (A) Conventional cascade model of coagulation. The Waterfall/Cascade model consists of two separate initiations, intrinsic (contact) and extrinsic pathways, which ultimately merge at the level of Factor Xa (common pathway).[1] (B) Regulation of thrombin generation. Coagulation is triggered (initiation) by circulating trace amounts of fVIIa and locally exposed tissue factor (TF). Subsequent formations of fXa and thrombin are regulated by tissue factor pathway inhibitor (TFPI) and antithrombin (AT). When the threshold level of thrombin is generated, thrombin activates, platelets, fV, fVIII, and fXI to augment its own generation (propagation).

clottable proteins (coagulogen, Factor C), and transglutaminase (a Factor XIII-like enzyme). When amaebocytes are exposed to endotoxin (in seawater) after injury, serine proteases convert coagulogen to coagulin, which is polymerized by transglutaminase.[8] This basic coagulation system enables *Limulus* to locally isolate injuries and invading pathogens (Fig. 3A).

Limulus coagulation has several direct implications for humans. First, the *Limulus* (amoebocyte lysate) test is used clinically and commercially to detect bacterial contamination in blood, various materials (e.g., microchips), and the environment. This test is based on clotting of hemolymph exposed to endotoxin that may be present in human plasma or in the environment. Second, the final step of human coagulation is quite similar to that in *Limulus*, whereby thrombin (a serine protease) converts fibrinogen (a clottable protein) to fibrin monomer, which is polymerized by thrombin-activated Factor XIII (a transglutaminase) (Fig. 3B). One may ask that if the final step of hemostasis has been so simple and effective for hundreds of million years,[6] what is the advantage of having multiple coagulation factors and complex pathways to achieve

the same goal (clotting) as in invertebrates? Among nonmammalian and mammalian vertebrates, amino-acid sequences of prothrombin and fibrinogen are well conserved.[6] The important evolutionary difference between the vertebral coagulation system and that of invertebrate species is the need to provide localized thrombosis in high pressured closed networks of blood vessels in contrast to the low-pressure open circulation.

INITIATION OF COAGULATION

Similar to that by which amoebocytes detect a breach in the horseshoe crab's body armor, in humans, circulating blood reacts quickly to a disruption of the vascular endothelium to limit bleeding. The initial hemostatic response is triggered by tissue factor (TF; thromboplastin) expressed on subendothelial pericytes and fibroblasts. Activated Factor VII (fVIIa), a serine protease that normally circulates in blood in low concentration, binds to TF to activate Factor X to fXa. Subsequently, fXa (also a serine protease) generates trace amounts (0.1–1 nM) of thrombin. There are two inhibitors that regulate TF-triggered procoagulant responses, thus limiting serine protease actions to the site of vascular injury (Fig. 2B). Tissue factor pathway inhibitor (TFPI) neutralizes fXa when it is in a complex with TF-fVIIa.[9,10] The other regulator of TF-trigger procoagulant response is antithrombin (AT, formerly called antithrombin III; a serine protease inhibitor; SERPIN), which circulates at a high concentration (150 μg/mL) and neutralizes the initially formed fXa and thrombin. Thus, the procoagulant triggering reaction only proceeds when TF is exposed at a high enough level to overcome inhibition by TFPI and AT (Fig. 2B). In other words, fVIIa patrols the circulation in search of sites of vascular damage (i.e., where TF is exposed), and trace quantities of fXa and thrombin sound the "alarm" for any potential dangers. This activity is tightly monitored by naturally occurring inhibitors that prevent a "false alarm" or "too extensive of a response."

PROPAGATION OF COAGULATION

Circulating platelets contribute to localized thrombus formation at the site of vascular injury first by adherence to subendothelial collagen-von Willebrand factor (vWF) via their glycoprotein (GP) Ib receptors. Thrombin generated by TF-fVIIa/fXa (the "extrinsic pathway") is capable of activating adherent platelets in its vicinity via protease-activated receptors 1 and 4 (PAR1 and PAR4).[11] Thrombin-activated platelets play a pivotal role in subsequent coagulation processes in several ways. First, platelet GPIb receptors bind to Factor XI, and they also localize Factor VIII to the site of endothelial disruption via its carrier protein vWF.[12] Furthermore, partially activated Factor V is released from platelet α-granules upon platelet activation.[13] Factors XI, VIII, and V are involved in sustaining procoagulant responses (the "intrinsic pathway")
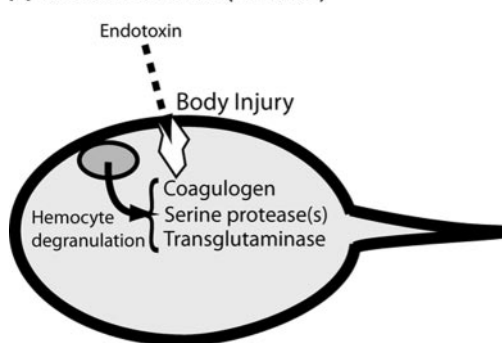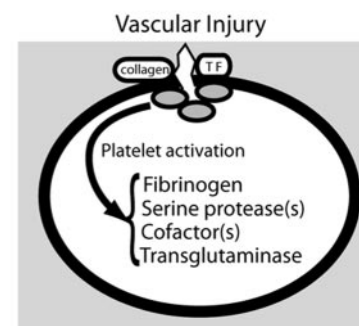
Figure 3. Schematic of hemostatic mechanisms in horseshoe crabs (*Limulus*) and humans. In the horseshoe crab, coagulation is regulated by hemocytes, which detects endotoxin, and localize serine proteases, coagulogen (clotting protein), and transglutaminase at the site of injury; in humans, serine proteases, cofactors, fibrinogen, and transglutaminase (Factor XIII) are localized on activated platelet surface after arterial injury.

Table 1. Coagulation Factor Level Required for Normal PT, aPTT, and *In Vivo* Hemostasis

Factor	PT	aPTT	*In vivo*[a]
Fibrinogen (mg/dL)	100	60	50–100
Prothrombin (%)	50	15	20–30
Factor V (%)	50	40	20
Factor VII (%)	50	NA	10
Factor X (%)	60	25%	20
Factor VIII (%)	NA	35%	40
Factor IX (%)	NA	20%	30
Factor XI (%)	NA	30%	50
Factor XII (%)	NA	20%	0
Factor XIII (%)	NA	NA	5
vWF (%)	NA	NA[b]	30

NA = not affected; vWF = von Willebrand factor; PT = prothrombin time; aPTT = activated partial thromboplastin time.

[a] Hemostatic data based on a single factor deficiency; these data cannot be simply inferred to surgical patients with multifactorial deficiency, e.g., hemodilution.

[b] Severe vWF deficiency may affect aPTT because the half-life of factor VIII is decreased.

after thrombin-mediated activation (Fig. 2B).[14,15] The serine protease Factor XIa mediates the activation of Factor IX to fIXa, and fVIIIa serves as a cofactor to fIXa. Factor IXa, a serine protease activates Factor X to fXa, and fVa serves as a cofactor to fXa. In the absence of fVIIIa or fIXa, as clinically observed in Hemophilia A or B, respectively, the initiation of coagulation is normal, but propagation steps are severely diminished (Fig. 2B). Patients with hemophilia develop recurrent bleeding in muscle and joints because of low-TF expression (thus, the initiation of coagulation is rapidly quenched by TFPI and AT). Using high-dose recombinant fVIIa (90–120 μg/kg), recurrent bleeding can be reduced by the increased fXa production to overcome TFPI and AT (thus improved thrombin generation) in hemophilia patients with inhibitory antibodies against Factor VIII or Factor IX.[16,17]

Three key components (substrate, enzyme, accelerator [cofactor]) concentrated on the activated platelet surface are needed to locally generate thrombin. A single thrombin-activated platelet exposes more than 12,000 copies of GPIIb/IIIa receptors that can concentrate fibrinogen for efficient fibrin formation.[18] Furthermore, plasma- and platelet-derived Factor XIII are activated by thrombin to fXIIIa, a transglutaminase that rapidly cross-links fibrin monomers.[19] Thus, localized fibrinogen and Factor XIII are final thrombin

substrates that play pivotal roles in stabilizing the primary hemostatic plug. In severe fibrinogen deficiency, platelets are localized by vWF-GPIb interactions but unable to recruit fibrinogen molecules to GPIIb/IIIa receptors. Lack of fibrin formation on the platelet surface results in a dislodgement of platelet plugs.[20] Clinically, this situation is observed as recurrent bleeds, and a paradoxical thrombosis in congenital afibrinogenemia.[21]

IN VITRO COAGULATION

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are by far the most common screening tests for coagulation abnormalities. These tests correspond respectively to extrinsic and intrinsic pathways of the Waterfall/Cascade model (Fig. 2A). PT was originally developed by Quick for measuring the prothrombin level by adding a large amount of TF (rabbit brain extract) to plasma.[22] It is now understood that PT is affected by reductions of Factors VII, X, V, and prothrombin such as occur with vitamin K antagonist therapy[23] or severe liver disease[24] (Table 1). In the PT assay, the amount of TF used to trigger *in vitro* clotting is in large excess compared with *in vivo* conditions, leading to rapid generation of thrombin, and its feedback activation of Factor V (Fig. 2B). However, it is evident that the *in vivo* coagulation mechanism is not fully reflected by PT because recurrent bleeding occurs in hemophilia (Factor VIII or Factor IX deficiency) despite normal PT values. The concentration of TF is presumably much lower *in vivo*, and thus, PT was modified for evaluating hemophilic plasma using "partial thromboplastin" (i.e., phospholipid with minimal TF isolated from crude thromboplastin by ultracentrifugation and dilution).[25] In the PTT (partial thromboplastin time) test, fVIIa-mediated fXa and thrombin productions are limited under the condition of low TF, and, as a result, the activities of fIXa and fVIIIa as an alternative source of fXa become critical for clotting (Fig. 2). The PTT was further improved for reproducibility by adding a contact activator (e.g., kaolin, celite, or ellagic acid) in an assay known as the activated PTT (aPTT).[25] In the presence of a contact system activator, a series of serine protease activations occur in the descending order of

Factor XIIa → XIa → IXa → Xa, resulting in thrombin generation (Fig. 2A). Although Factor XII activation by a contact activator is not considered important for normal hemostasis (because Factor XII deficient patients do not bleed), aPTT is sensitive to gross reductions of Factors XII, XI, IX, VIII, V, and to a lesser extent, prothrombin (Table 1). The sequence of serine protease activations proceeds very slowly in aPTT because the cofactors, fVIIIa and fVa, are not available until thrombin is generated to activate them (Fig. 2B). Thus, aPTT is used clinically for monitoring of unfractionated heparin, argatroban, bivalirudin, and lepirudin anticoagulation (note: a specific calibration is required for each anticoagulant), because all these thrombin inhibitors reduce thrombin-mediated feedback activation of Factors VIII and V.[26,27]

Although PT/aPTT can be used to guide anticoagulation, several important limitations should be noted when they are being measured to evaluate bleeding. Perioperatively, bleeding is caused by multiple coagulation defects because of hemodilution, consumptive loss, fibrinolysis, anticoagulant use, hypothermia, and other mechanical and metabolic derangements.[28,29] Importantly, PT/aPTT do not provide any information on *in vivo* interaction of platelets with coagulation factors. Activated platelets are capable of locally accumulating coagulation factors, and thus, the extent of bleeding under prolonged PT/aPTT may vary according to the platelet count and/or function. Further, it is not possible to estimate the overall stability of a hemostatic thrombus using PT/aPTT because both tests are terminated before fibrin is polymerized by fXIIIa. Congenital Factor XIII deficiency is associated with umbilical cord bleeding and intracranial hemorrhage, but this deficiency is not detected by PT/aPTT screening.[30] PT/aPTT also remain normal when bleeding is caused by increased fibrin breakdown (i.e., hyperfibrinolytic state) such as occurs in congenital deficiency of α_2-antiplasmin.[31] In contrast to PT/aPTT, the use of thrombelastography/metry allow functional activities of fibrinogen, Factor XIII, and fibrinolytic proteins.[32–34]

ENDOTHELIAL REGULATION OF COAGULATION

Intact endothelium has multiple anticoagulant functions that maintain blood in a fluid state (Table 2). The endothelium attenuates platelet activity by releasing nitric oxide, prostacyclin, and ecto-ADPase (the latter degrades adenosine diphosphate). There are several coagulation inhibitors that are produced by endothelial cells. Endothelium-derived TFPI is localized on its surface,[42] and is rapidly released into circulation after heparin administration, reducing procoagulant activities of TF-fVIIa.[43] Endothelial cells also secrete heparan sulfate, a glycosaminoglycan which catalyzes anticoagulant activity of AT. Plasma AT binds to heparan sulfate located on the luminal surface, and in the basement membrane of the endothelium.[44] Thrombomodulin is another endothelium-bound protein with

Table 2. Coagulation Modulators Differentially Expressed in Endothelial Cells

Factor	Vascular distribution	References
ADAMTS-13	V/A	35
Endothelial nitric oxide synthase	A	
Endothelial protein C receptor	V/A	
Heparan sulfate	Variable EC expression	36
Plasminogen activator inhibitor-1	A	
Prostacyclin	A > V	37
Tissue factor	Undetected in quiescent EC	
Tissue factor pathway inhibitor	C	38
Tissue-type plasminogen activator	A	
Thrombomodulin	V/A/C except in brain	39
Von Willebrand factor	V > A	40

Modified from Aird WC.[41]

A = artery; V = vein; C = capillaries; EC = endothelial cells.

anticoagulant and antiinflammatory functions. In response to systemic procoagulant stimuli, tissue-type plasminogen activator (tPA) is transiently released from the Weibel-Palade bodies of endothelial cells to promote fibrinolysis.[45,46] Endothelium activated by inflammation modulates procoagulant responses by synthesizing TF, vWF, plasminogen activator inhibitor (PAI)-1, and PARs.[47]

The large surface area of endothelium requires constant repair, and thus, platelets and coagulation factors are consumed at a basal rate in the absence of clinically obvious vascular injury. Basal (homeostatic) thrombin generation is demonstrated by circulating thrombin-AT complexes and other coagulation markers (e.g., prothrombin fragment 1.2) even in the absence of overt bleeding.[48] Similarly, the basal consumption of platelets amounts to approximately 7×10^3 mm^{-3} per day (normal count 150–350 $\times$ 10^3 mm^{-3}), a level of platelets that corresponds to the threshold ($\leq 10 \times 10^3$ mm^{-3}) for spontaneous bleeding.[49]

The hemostatic response is generally limited to the site of vascular injury because key serine proteases are membrane-bound on activated platelet surfaces. Relatively few molecules of fXa and thrombin are carried out of the local milieu. Downstream to the vascular injury, the complex of TF-fVIIa/fXa is inhibited by TFPI. Plasma (free) fXa and thrombin are rapidly neutralized by heparan-bound AT. Thrombin is also taken up by endothelial surface-bound thrombomodulin.[12,50] The binding of thrombomodulin to Exosite I of thrombin optimizes the catalytic activity of thrombin toward generation of the natural anticoagulant protein C and TAFI (thrombin-activatable fibrinolysis inhibitor).[50] In the systemic circulation, activated

protein C (APC) has been shown to exert multiple antiinflammatory and cytoprotective functions by modulating endothelial protein C receptor and protease-activated receptor-1 (PAR-1, thrombin receptor) via mechanisms still under investigation.[51–53] TAFI also exerts antiinflammatory effects by cleaving bradykinin and C5a.[54,55]

The continuous release of fXa, thrombin, and soluble fibrin into the systemic circulation leads to the activation of the fibrinolytic system (tPA release),[45,46,56] which dissolves insoluble fibrin and prevents ischemia induced by thrombus deposition in end organs.[57] Large multimers of vWF are also increased during inflammation, and they are down-regulated by a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13 (ADAMTS–13), which is also synthesized by endothelial cells.[35] In patients with chronic recurrent thrombotic thrombocytopenic purpura, severely reduced ADAMTS–13 activity (<5%) may increase circulating ultralarge vWF multimers, which contribute to intravascular platelet aggregation and thrombosis.[58]

PERIOPERATIVE CHANGES IN COAGULATION

Trauma and surgical patients have varied degrees of vascular injury and exsanguinations. Massive hemorrhage results in progressive dilution of coagulation factors to 30% of normal after a loss of 1 blood volume and down to 15% after a loss of 2 blood volumes.[59,60] In the presence of severe hemodilution, the initiation of thrombin generation is delayed by reduced Factor VII, and the propagation is reduced by the gross reduction of procoagulatant serine protease zymogens and accelerators. The efficiency of thrombin generation is further reduced by decreased platelet count, which decreases to 50×10^3 mm^{-3} over the loss of 2 blood volumes.[59] PT and aPTT also indicate a "hypocoagulable state" as they approach more than 1.5 times the normal. Two important thrombin substrates, fibrinogen and Factor XIII also decrease rapidly during hemodilution; fibrinogen decreases to 100 mg/dL after a loss of 142% of blood volume. Reduced thrombin generation, low fibrinogen, and low Factor XIIIa activity render fibrin clot susceptible to tPA induced fibrinolysis. Unstable fibrin clot formation because of low fibrinogen and/or Factor XIII result in a profuse bleeding but may also represent a problem in localizing procoagulant activity. Polymerized fibrin seems to have a role in containing excess thrombin and fXa molecules inside the thrombus.[61] In fact, AT I which was originally described as an anticoagulant protein turned out to be fibrin.[62] In severe hemodilution, anticoagulant proteins, including TFPI, AT, protein C, protein S, and endothelium-bound thrombomodulin, are progressively decreased.[63,64] Thus, thrombin's procoagulant and proinflammatory activities may not be quickly suppressed after thrombin is released (from

weak clots at the injury site) into circulation. The deficiency in either fibrinogen or Factor XIII seems to be associated with elevated plasma markers of thrombin generation.[62,65]

Current hemostatic therapies for postoperative bleeding consist of allogeneic fresh frozen plasma, cryoprecipitate, and platelet concentrates.[66] More recently, plasma-derived factor concentrates, such as fibrinogen, Factor XIII, and recombinant-activated fVIIa, have been increasingly used in postoperative patients in a empirical manner.[67–69] These components may allow rapid restoration of specific elements of coagulation without the need for a cross-match, while avoiding intravascular volume overload. In addition, a risk of infectious transmission is decreased because they are pasteurized against currently known viruses. However, additional studies are required to prove their safety in the perioperative setting because surgical patients demonstrate multiple deficiencies in procoagulant and anticoagulant proteins.[70,71] For example, severe perioperative bleeding after massive transfusion is often improved by the use of recombinant fVIIa (20–90 μg/kg); however, there are a few reported cases of systemic thrombosis.[72,73]

Decreased coagulation factors and inhibitors recover after surgery over the course of several days. Acute inflammatory responses associated with vascular injury and wound healing often result in elevated cytokines, platelet count, fibrinogen, vWf-Factor VIII, and PAI-1 levels over the normal limit.[74,75] The syntheses of TFPI and AT are not increased, and endothelial thrombomodulin expression is decreased by inflammatory cytokines (e.g., tumor necrosis factor, interleukin-1β). The imbalance of procoagulant and anticoagulant elements may increase the risk for prothrombotic complications in the postoperative period. Prophylactic use of antithrombotic therapy should be considered based on the type of surgery, hematological history, and other patient characteristics (e.g., age, obesity).[76]

CONTROL OF HYPERCOAGULABILITY

A disrupted balance between procoagulant and anticoagulant elements of coagulation can lead to prothrombotic events and associated morbidity and mortality. Thromboses can be induced by the excess function of procoagulant factors or by the failure of anticoagulant proteins to suppress coagulation response. These conditions may result from physiological changes (e.g., aging), hereditary causes, acquired disease state (e.g., atheromatous disease, cancer, antiphospholipid syndrome), drugs (e.g., oral contraceptive), and iatrogenic causes (e.g., drug-eluting stent) (Table 3).[77,78]

The incidence of venous and arterial thrombosis increases exponentially with age, and this may be explained, in part, by advanced atheromatous disease,

Table 3. Risks for Venous and Arterial Thrombosis

Venous thrombosis	Arterial thrombosis
Age	Age
Major surgery or Trauma	Atrial fibrillation
Abdominal surgery	Hypercholesterolemia
Pelvic surgery	Hypertension
Hip/knee replacement	Diabetes
Cancer	Smoking
Antiphospholipid syndrome	Thrombotic thrombocytopenic purpura
Heparin-induced thrombocytopenia	Heparin-induced thrombocytopenia (less frequent)
Medication (e.g., lenalidomide)	Medication (e.g., COX-2 inhibitor)
Immobility (e.g., air travel)	Devices (e.g., mechanical valve, drug-eluting stent)
Obesity	Hereditary factors
Pregnancy	Hyperhomocystinemia, MTHFR 677C→T variant
Contraceptive use or hormone replacement	Fibrinogen β-chain polymorphism (inconsistent)
Devices (e.g., central venous catheter)	PAI-1 polymorphism (inconsistent)
Hereditary factors	TAFI polymorphism (inconsistent)
Factor V Leiden	
Prothrombin G20210A	
Antithrombin deficiency	
Protein C deficiency	
Protein S deficiency	

Based on the literature.[77,78]

COX = cyclooxygenase; MTHFR = methylenetetrahydrofolate reductase; PAI = plasminogen activator inhibitor; TAFI = thrombin activatable fibrinolsysis inhibitor.

cancer, various surgical procedures, and immobility.[79,80] Thrombotic occlusions of coronary and cerebral arteries are associated with platelet activation and coagulation triggered by the rupture of atherosclerotic plaque, resulting in myocardial infarction and ischemic stroke.[81,82] In cancer patients, mediators of tumor growth, including TF and other cytokines (e.g., interleukin-1β), may trigger coagulation and downregulate the anticoagulant system, increasing the incidence of venous thromboembolism.[83] A number of chemotherapeutic drugs are also associated with increased thrombosis (L-asparaginase, lenalidomide, tamoxifen, etc.).[83] Osteoarthritis and osteoporosis predispose elderly patients to bone fractures, and subsequent reparative surgery and immobility increase the risk of venous thrombosis.

Congenital deficiencies of AT, protein C, and protein S result in a reduced ability to regulate thrombin generation, and thus, predispose affected individuals to deep venous thrombosis and pulmonary embolism.[84] A single nucleotide polymorphism in the Factor V gene (commonly Arg506→Gln; Factor V Leiden)

is another example of dysfunctional thrombin regulation because APC-mediated inactivation of Factor Va Leiden is slower than normal. Leiden V mutation is common in northern Europeans (heterozygous 5%–10%) increasing the risk for venous thrombosis by three to eightfold in heterozygotes, and up to 80-fold in homozygotes. A polymorphisms of prothrombin (G20210A variant) is associated with high-plasma prothrombin levels (>115% of normal) and the increased risk of deep venous thrombosis and pulmonary embolism.[84,85] Antiphospholipid syndrome is an example of acquired thrombophilic state associated with increased risk of venous embolism and pregnancy loss. It is characterized by the presence of phospholipids binding antibodies (so-called lupus anticoagulant). Despite prolonged PT and aPTT, lupus antibodies do not exert anticoagulation. Rather, the complex formed between the autoantibodies and β_2-GP I (apolipoprotein H) is presumed to interfere with endogenous anticoagulation (e.g., TFPI, APC)[86,87] and upregulate coagulation and inflammation systems. Although the incidence of thromboembolism is low (3–30 per 10,000 per year), use of oral contraceptives (a combination of estrogen and progestogen) can potentially induce a procoagulant state. This is due to estrogen-induced increases in Factors VII, IX, X, and XIII, and decreases in AT and protein S.[88]

After the clinical diagnosis of thrombophilia manifests as arterial and venous thrombosis, prophylactic and therapeutic antithrombotic therapies are usually necessary to prevent the reoccurrence of thrombosis, and to reduce morbidity and mortality from a vascular occlusion in major organs. Since the beginning of the 20th century, coumarin derivatives, and unfractionated heparin (hereinafter referred to as heparin) have been used for prophylaxis and treatment for various thrombotic conditions.[89] Over the last several years, new drugs with more predictable pharmacokinetics than these drugs have been introduced, including low molecular weight heparin (LMWH) and a synthetic pentasaccharide (Fondaparinux®).[90] In the arterial circulation, platelet activation is a triggering event for thrombosis, a process which cannot be adequately suppressed with heparin and coumarins. Thus, antiplatelet therapy is the primary strategy for the prevention or treatment of arterial thrombosis.[91] The main drugs for this purpose are aspirin and clopidogrel (Plavix®). Other parenteral antiplatelet drugs, including GP IIb/IIIa inhibitors, may be administered during percutaneous coronary artery interventions. Novel oral and IV antiplatelet drugs are currently being investigated as reviewed elsewhere.[92] As summarized in Table 4, anesthesiologists today face increasingly complex anticoagulation regimens. It is thus important to understand the mechanism of action and pharmacokinetic/pharmacodynamic profiles of these antithrombotic drugs.

VITAMIN K ANTAGONISTS

Coumarin derivatives are currently the sole drugs available for oral anticoagulation therapy (warfarin in North America and phenprocoumon in Europe).[93,94] Coumarin derivatives are vitamin K antagonists that inhibit γ-carboxylation of serine protease zymogens, including prothrombin, Factors VII, IX, X, protein C, and protein S.[95] The γ-carboxylated domain (called the Gla-domain) is critical for enzymatic functions of vitamin K-dependent proteins because this protein domain binds to calcium ions on negatively charged phospholipid surfaces. Coumarins thus reduce the enzymatic activation of serine proteases, but they do not directly antagonize thrombin activity in contrast to heparin-AT complex. Both procoagulant (prothrombin, Factors VII, IX, X) and anticoagulant (protein C and S) proteins are affected, but the net clinical effect of vitamin K antagonists is anticoagulation because thrombin generation is suppressed by nonfunctional prothrombin and Factor X.[95] However, during the induction of oral anticoagulation, functions of Factor VII and protein C are acutely lost because of their short half-life (6–9 h), but prothrombin and Factor X remain functional for 2–3 days (half-life 42–72 h, and 27–48 h, respectively).[96] Although PT and its international normalized ratio (INR) may be prolonged by the decreased Factor VII, thrombin generation *in vivo* from residual Factor X and prothrombin may continue during the induction of coumarins.[97] Normally thrombin self-regulates its formation via activation of APC (i.e., inactivation of fVa and fVIIIa) after binding to endothelial thrombomodulin (Fig. 4), but severely reduced protein C may render thrombin generation unsuppressed. Limb gangrene and skin necrosis have been reported as rare but serious thrombotic complications of coumarins. The use of coumarins is not recommended in the acute phase of thrombosis (e.g., heparin-induced thrombocytopenia [HIT]), and the induction of coumarins should be overlapped with heparin (if not contraindicated) or direct thrombin inhibitors (DTIs) to suppress residual thrombin activity.[97]

Coumarins are effective for anticoagulation for patients with prosthetic heart valves,[98] atrial fibrillation,[99] and ischemic strokes.[100] It is often difficult to maintain the therapeutic range of coumarins (INR 2.0–4.0), and thus, hemostatic balance,[93,98] because the metabolism of coumarins, is affected by genetic factors, various medications, and diet. Bleeding in the gastrointestinal and urinary tracts occurs in up to 6.5% of coumarin-treated patients per year, whereas intracranial hemorrhage, the most serious complication, affects up to 1% of patients annually.[101] The management of coumarin therapy in patients undergoing surgery is complicated because the recovery of hemostatic function requires a few days, and either hemorrhage or thrombosis can potentially occur.[102] Warfarin is generally discontinued 4 days before surgery to allow the INR to be normalized on the day of surgery.[102] The use of vitamin K alone is not adequate for an acute reversal of coumarin due in part to its slow onset of action (4–6 h after IV administration). Fresh frozen plasma is most frequently used for this purpose in the United States, but as much as 30 mL/kg is required to achieve hemostatic levels of vitamin K-dependent factors.[103] The concentrated vitamin K-dependent factors are available in most European countries (known as prothrombin complex concentrate, which is not currently approved for this indication in the United States).[23] These factor concentrates circumvent a large volume-load and time required for preparation (thawing and cross-matching) in high-risk surgical patients.[104,105]

HEPARIN, LMWH, AND FONDAPARINUX

Heparin is a mixture of glycosaminoglycans with a range of molecular weights (3000–30,000 Da). Heparin exerts anticoagulation by binding to AT, which circulates in plasma at a high concentration (150 μg/mL, 2.6 μM) relative to prothrombin (90 μg/mL, 1.4 μM) or Factor X (10 μg/mL, 0.17 μM).[106] High molecular weight fractions of heparin catalyze 1:1 interaction between AT and thrombin, whereas low molecular weight fractions catalyze the interaction between AT and Factor Xa (fXa).[107] Despite its potent anticoagulant effects, there are limitations associated with heparin, including a relatively short half-life (30–90 min) after IV injection, unpredictable pharmacokinetics by subcutaneous administration, hypersensitivity, and thrombocytopenia.[108,109] LMWH is predominantly composed of heparin fractions with shorter chain length (4000–6000 Da). Compared with unfractionated heparin, LMWH and fondaparinux have advantages in excellent bioavailability, long half-lives (3–6 h), less frequent HIT, and fewer major bleeds.[108,109] Thus, LMWH and fondaparinux are increasingly part of standard prophylaxis for deep venous thrombosis in perioperative patients (Table 4).[76]

LMWH is more selective toward fXa compared with heparin because longer saccharide units are needed to bridge thrombin (Exosite II) and AT.[110] Fondaparinux is a synthetic LMWH composed of five saccharide chains (pentasaccharides). AT bound to fondaparinux exclusively inhibits fXa. These agents are very effective in inhibiting procoagulant fXa, and thus, *in vivo* coagulation triggered by TF-fVIIa is effectively quenched. However, PT and aPTT are not sensitive to therapeutic LMWH and fondaparinux because of supraphysiological Factor Xa production in PT/aPTT. A specific anti-Xa activity assay is needed to measure LMWH and fondaparinux anticoagulation effects. Although major bleeding complications with LMWH and fondaparinux are less likely compared with heparin, anti-Xa activity of AT resulting from these compounds is not readily reversed with protamine sulfate.[111] The bleeding

Table 4. Summary of Antithrombotic Drugs

Drug	Heparin	Warfarin	LMWH	Fondaparinux	Rivaroxaban[a]
Mechanism of Action	Inhibit thrombin/fXa	Reduce vitamin K factors	Inhibit fXa > thrombin	Inhibit fXa	Inhibit fXa
Indication	PCI, CPB, thrombosis	Stroke/DVT prophylaxis	DVT/PE prophylaxis	DVT/PE prophylaxis	DVT/PE prophylaxis
Route	IV, SQ	Oral	SQ	SQ	Oral
Half life	1–2.5 h	36–42 h	3–5 h	17 h	5–10 h
Elimination	Hepatic	Hepatic	Renal	Renal	Renal
Monitoring	aPTT/ACT	PT/INR	Anti-Xa	Anti-Xa	Anti-Xa
Comments	May cause HIT/HITT	↓ Protein C/protein S	↓ Dose in reduced kidney fx	↓ Dose in reduced kidney fx	

IV = intravenous; SQ = subcutaneous; LMWH = low molecular weight heparin; APC = activated protein C (drotrecogin alpha); sTM = recombinant human soluble thrombomodulin; PCI = percutaneous coronary intervention; DVT = deep venous thrombosis; PE = pulmonary embolism; DIC = disseminated intravascular coagulation; PT/INR = prothrombin time/international normalized ratio; aPTT = activated partial thromboplastin time; ACT = activated clotting time; kidney fx = kidney function; HITT = heparin-induced thrombocytopenia with thrombosis; HIT = heparin-induced thrombocytopenia; EPCR = endothelial protein C receptor; CPB = cardiopulmonary bypass.

[a] Under clinical development.

[b] Argatroban blocks catalytic site of thrombin.

[c] Lepirudin and bivalirudin block catalytic site and Exosite I of thrombin.

[d] Available in Japan for DIC/United States Phase II trial for DVT/PE.

[e] Antibody may increase anticoagulant effect, but allergic reaction is rare.
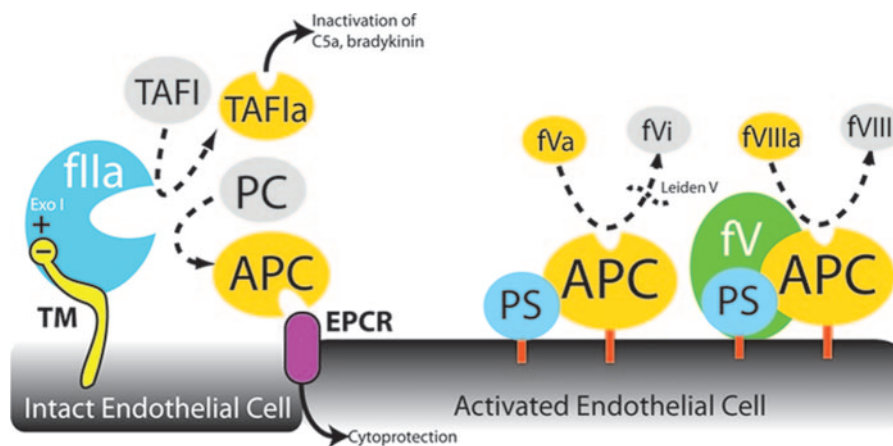

Figure 4. Endothelium and protein C activation. Thrombomodulin (TM) expressed on intact endothelium rapidly binds to thrombin (fIIa), which then preferentially cleaves protein C and thrombin activatable fibrinolysis inhibitor (TAFI) to activated protein C (APC) and TAFIa, respectively. APC exerts antiinflammatory functions via the endothelial protein C receptor, triggering intracellular cytoprotective signals. TAFIa also exerts antiinflammatory function by degrading complements (C5a) and bradykinin. APC binds to protein S (PS) and inactivates activated Factor V (fV) and Factor VIII on the activated endothelium. Inactivation of fVa is delayed in case of Factor V Leiden. Inactivation of fVIIIa is enhanced by fV.

risk may also be increased if these drugs are not discontinued for 12–24 h before invasive procedures.[112,113] Unlike heparin, LMWH is mainly excreted in kidneys, and the dose reduction for LMWH should be considered to avoid bleeding complications in patients with creatinine clearance <30 mL/min.[114]

Heparin and its derivatives are very useful in both acute and chronic prevention of thrombosis, but their dependence on AT, an endogenous SERPIN may pose several problems. The efficacy of heparin may be reduced when AT activity is low (<60%).[115–117] Although congenital AT deficiency is rare, acquired AT deficiency is observed in 10%–20% of patients presenting for cardiac surgery, which is particularly common in individuals receiving preoperative heparin therapy because heparin increases the rate of AT turnover.[115,116,118] Other clinical conditions associated with low AT levels include pregnancy, severe burn, hepatic dysfunction, nephrotic syndrome, sepsis, and the use of estrogen, or L-asparaginase.[118] Heparin binds to a number of plasma proteins (LMWH to a lesser degree), including platelet factor 4 (PF4, a chemokine released from platelets).[119] In about 50% of patients who received heparin during cardiac surgery, an IgG antibody against heparin-PF4 complex may be detected using immunological antibody assay.[120] In only a fraction of cardiac surgery patients (1%–3%), thrombocytopenia and a prothrombotic state known as HIT develops. A higher incidence (5%) of HIT has been demonstrated in orthopedic surgical patients who received heparin for thromboprophylaxis for more than 2 wk. *In vivo*, the PF4-heparin complex on the platelet surface activates platelets by cross-linking FcγRIIa receptors and a resultant calcium mobilization (Fig. 5).[121] This

Table 4. (Continued)

Apixaban[a]	Argatroban	Lepirudin	Bivalirudin	APC	sTM[d]
Inhibit fXa	Inhibit thrombin[b]	Inhibit thrombin[c]	Inhibit thrombin[c]	EPCR modulation ↓ fVa/fVIIIa	Protein C activation
DVT/PE prophylaxis	HIT/HITT, PCI	HIT/HITT	PCI	Severe sepsis	DIC, DVT prophylaxis[d]
Oral	IV	IV	IV	IV	IV
5–18 h	40–50 min	1.3 h	25 min	13 min	3–4 h
Renal	Hepatic	Renal	Plasma/Renal	Plasma	Renal (>50%)
Anti-Xa	aPTT/ACT Prolong PT/INR	aPTT/ACT ↓ Dose in reduced kidney fx; antibody formation[e]	aPTT/ACT ↓ Dose in reduced kidney fx	— May prolong aPTT	aPTT
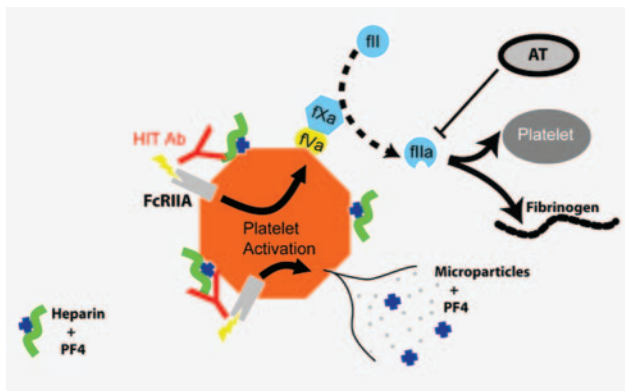
Figure 5. Procoagulant mechanism of heparin-induced thrombocytopenia (HIT). IgG antibodies (Ab) binds to heparin-platelet factor 4 (PF4) complexes on the platelet surface. These antibodies induce platelet activation by cross-linking FcγRIIA receptors, and a resultant intraplatelet calcium mobilization. Platelet activation supports systemic thrombin generation by catalyzing prothrombinase (fXa–fVa complex), and releasing procoagulant microparticles. The release of PF4 from platelet α-granules also increases antigenic PF4-heparin complex.

results in the release of platelet granule contents and procoagulant microparticles, which support thrombin generation.[122] Thus, HIT is generally a procoagulant state despite moderate thrombocytopenia (nadir count, 50–$60 \times 10^3 \ mm^{-3}$). If HIT is suspected and HIT antibody titers are present, heparin and LMWH should be stopped and the use of DTIs should be considered. When the history of HIT is remote (>3 mo) and the antibody titer is low, it is considered safe to administer heparin, particularly for cardiopulmonary bypass procedures.[123] This is because the immune response of HIT is not associated with an anamnestic response.

DIRECT THROMBIN INHIBITORS

After proteolytic activation by fXa, thrombin rapidly binds to platelets and fibrinogen via its Exosite I (anion-binding site, Fig. 6).[124] Thrombin activates platelets by cleaving PAR1 and PAR4 receptors on the platelet surface and generates fibrin by releasing Fibrinopeptides A and B. DTIs compete with platelets and fibrinogen for thrombin Exosite I and/or catalytic site (Table 4; Fig. 6). Argatroban is a catalytic site inhibitor of thrombin, whereas bivalirudin and lepirudin are bivalent inhibitors which occupy both the Exosite I and active site (Fig. 6).[125] DTIs exert anticoagulant activity independent of endogenous AT, and these agents have much smaller molecular size compared with heparin (Table 4). Thus, DTIs inhibit thrombin bound to fibrin, whereas a heparin-AT complex does not.[126,127] The indication of DTIs include anticoagulation for HIT, percutaneous coronary interventions, and other heparin contraindications (e.g., allergy).

DTIs exert potent antithrombotic activities in the arterial and venous circulations by preventing thrombin from activating platelets, Factors V, and VIII.[128,129] In patients with HIT, DTIs are effective in reducing thrombotic complications, including a limb loss.[130] Hemorrhagic complications, particularly from overdosing are a concern in DTI-treated patients because there is currently no available antidote. The dose adjustment according to aPTT is particularly important to reduce hemorrhagic complications when bivalent inhibitors are used in patients with renal impairment.[130] Argatroban requires dose reduction in patients with hepatic insufficiency.[131]

The difference in molecular weights among DTIs has clinical implications. Anticoagulation with DTIs is monitored using the aPTT to maintain a two to threefold increase in aPTT over baseline. Although both lepirudin and argatroban are used at similar concentrations in isolated HIT, the molar concentration of argatroban is much higher than lepirudin. Prolonged PT results from more extensive inhibition of thrombin by a higher number of argatroban molecules compared with lepirudin
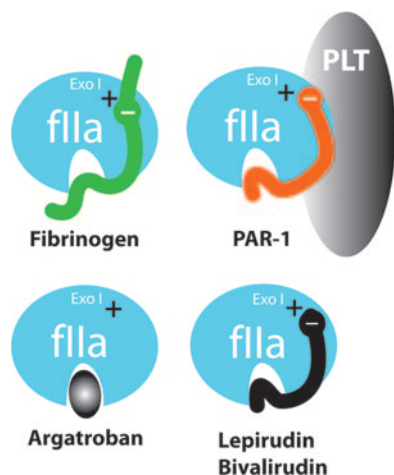
Figure 6. Interaction of thrombin (fIIa) with fibrinogen, platelets, and direct thrombin inhibitors. Negatively charged domains of fibrinogen and platelet PAR1 (protease-activated receptor 1) bind to thrombin via positively charged Exosites I. Thrombin cleaves fibrinogen and PAR1 receptor at the catalytic domain, resulting in fibrin formation and platelet activation. Argatroban occupies the catalytic domain of thrombin, and inhibits thrombin's enzymatic activity. Lepirudin and bivalirudin occupy both Exosite I and catalytic domain of thrombin.

within therapeutic levels.[132,133] Plasma fibrinogen assay using the Clauss method is also more susceptible to argatroban than lepirudin because added bovine thrombin reagents are quenched by a higher number of argatroban molecules within therapeutic levels.[122]

Although the first oral thrombin inhibitor, ximelagatran, was recalled because of drug-associated hepatic dysfunction, novel oral and IV DTIs are currently undergoing clinical trials. A new class of drugs that are oral (direct) Xa inhibitors (e.g., rivaroxaban and apixaban, Table 4) represent the drugs furthest along in development. The armamentarium of different classes of anti-Xa and DTI drugs should allow a better selection of antithrombotic therapy depending on pharmacokinetic profiles, type of intervention, and the underlying condition.

FIBRINOLYTIC DRUGS

Acute interventions with fibrinolytic drugs can be lifesaving in patients with pulmonary emboli,[134] ischemic stroke (e.g., middle cerebral arterial occlusion),[135] and in patients suffering acute myocardial infraction without immediate access to percutaneous coronary interventions.[136] Bleeding complications (5%–30%) may occur whether fibrinolytics are injected systemically or directly into the affected artery.[137] Currently available fibrinolytics include streptokinase, urokinase, and tPA. These drugs activate plasminogen to plasmin, a serine protease that degrades fibrin (ogen) and Factors V and VIII. In clinical practice, tPA is most commonly used because of its localized catalytic effect on plasminogen activation in the presence of fibrin.[138] Blood flow to the thrombus is vital for the delivery of tPA, and thus, localized
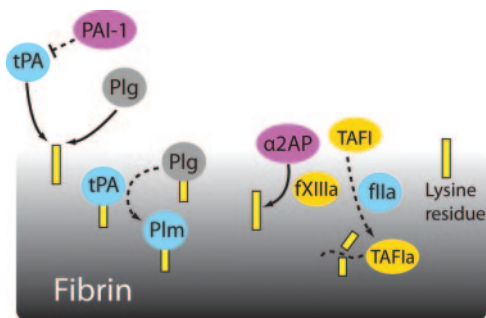

Figure 7. Regulation of fibrinolysis. Fibrin express positively charged lysine residues (shown as bars) to which tissue plasminogen activator (tPA) and plasminogen (Plg) bind resulting in plasmin (Plm) formation on fibrin. Plasminogen activator inhibitor-1 (PAI-1) inhibits + PA. Thrombin (fIIa) exerts antifibrinolytic effects by activating Factor XIII and thrombin-activatable fibrinolysis inhibitor (TAFI). The fXIIIa cross-links α_2-antiplasmin (inhibitor of plasmin) and TAFI to lysine residues. Activated TAFI (TAFIa) cleaves off lysine residues (plasminogen binding sites) from the fibrin, and reduces plasmin activation.

activation of fibrinolysis via catheter-directed drug delivery is theoretically more favorable than systemic administration.

tPA-induced fibrinolysis is normally regulated by protease inhibitors. PAI-1 is a serine protease inhibitor synthesized in the liver and endothelium, and its expression is increased in inflammatory states.[139] PAI-1 rapidly binds to and neutralizes tPA in plasma; PAI-1 also inhibits urokinase but not streptokinase. Both plasminogen and tPA bind to positively charged lysine residues expressed on the fibrin surface. Analogous to procoagulant serine protease activation on negatively charged platelet surfaces, fibrin-bound tPA efficiently catalyzes plasmin activation. In the systemic circulation, plasmin is rapidly inhibited by α_2-antiplasmin, but plasmin bound to fibrin is more resistant to α_2-antiplasmin (Fig. 7).[140] Thrombin modulates fibrinolytic activation by activating FXIIIa and TAFI. Plasma Factor XIII circulates in a heterotetramer of two A subunits and two B subunits (platelets also contain Factor XIII A subunits in their cytoplasm). Fibrin monomers are cross-linked by active A subunits of Factor XIIIa, which renders fibrin more resistant by cross-linking α_2-antiplasmin and TAFI to fibrin.[141,142] A high local thrombin concentration (approximately 150 nM) that is achieved inside a thrombus results in TAFI activation,[143–145] and activated TAFI cleaves lysine residues from the fibrin surface, thereby preventing the binding of tPA and plasminogen.[146,147]

APC AND THROMBOMODULIN

Thrombin generation for hemostasis occurs in a localized manner in healthy individuals because anticoagulant activities of endothelial cells inhibit systemic release of thrombin and other procoagulant proteases. APC is a unique serine protease that exerts anticoagulant and antiinflammatory activities. Its zymogen protein C circulates in plasma at 4–5 μg/mL

(0.08 μM) and is proteolytically activated by thrombin. Within the thrombus, the degree of protein C activation is limited because plasma fibrinogen level is high (2500 μg/mL, 7.6 μM), and the latter is preferably cleaved by thrombin. However, when plasma-free thrombin binds to endothelial thrombomodulin, protein C can be preferably activated to APC as a result of thrombomodulin blocking fibrinogen binding to thrombin Exosite I (Figs. 4 and 6). APC inactivates Factor Va and Factor VIIIa, two critical cofactors in propagating thrombin generation (Fig. 2B). Protein S functions as a cofactor for the enzymatic activity of APC. Severely low plasma protein C levels may be associated with thromboses, such as in purpura fulminans[148] and warfarin-induced skin necrosis.[97] Prospective randomized trials of APC in severely ill septic patients have shown APC reduces the rate of multiple organ failure and mortality.[149,150] The improved survival in sepsis is attributed to the antiinflammatory (rather than anticoagulant) activity of APC. Antiinflammatory roles of APC are mediated by activation of endothelial protein C receptor, triggering intracellular cytoprotective signals.[53]

CONCLUSION

Blood coagulation plays an important role in containing blood loss and in repairing the vascular injury (wound). With increasing longevity, vascular injuries are incurred through various disease processes (e.g., atherosclerosis, diabetes), which may result in inadvertent activation of procoagulant and proinflammatory responses, resulting in a thrombotic vascular occlusion.[80] An array of antithrombotic and antiplatelet drugs has been developed to prevent vascular complications in vulnerable patients using chemical synthesis and biomedical engineering techniques. Increasingly better control over thrombosis has been achieved by the targeted inhibition of serine protease(s) activity or platelet activation.[90,91] However, perioperative hemostatic management is increasingly complicated because of the need for reducing hemorrhage without thrombotic complications.[76,151] Nevertheless, our understanding of the coagulation system has evolved over the last century, and the technological improvement also provide specialized coagulation assays, which monitor different phases of blood coagulation. These include platelet function (aggregation) assays, thrombelastography/metry, and calibrated thrombin generation assay (readers should refer to a detailed discussion elsewhere[32–34,152]). The specific, point-of-care monitoring of coagulation will become more important in the future because hemostatic interventions are increasingly available as plasma-derived or recombinant factor(s) concentrates in addition to conventional fresh frozen plasma and cryoprecipitate.[23,70,153] Further validation and use of suitable coagulation monitors will likely help us improve our understanding and management of balancing hemostatic and antithrombotic functions in blood coagulation.

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