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Pharmacology of Hemostasis and Thrombosis

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Blood carries oxygen and nutrients to tissues and takes metabolic waste products away from tissues. Humans have developed a well-regulated system of **hemostasis** to keep the blood fluid and clot-free in normal vessels and to form a localized plug rapidly in injured vessels. **Thrombosis** describes a pathologic state in which normal hemostatic processes are activated inappropriately. For example, a blood clot (thrombus) may form as the result of a relatively minor vessel injury and occlude a section of the vascular tree. This chapter presents the normal physiology of hemostasis, the pathophysiology of thrombosis, and the pharmacology of drugs that can be used to prevent or reverse a thrombotic state. Drugs introduced in this chapter are used to treat a

variety of cardiovascular diseases, such as deep vein thrombosis and myocardial infarction.

**Case**

Mr. Soprano, a 55-year-old man with a history of hypertension and cigarette smoking, is awakened in the middle of the night with substernal chest pressure, sweating, and shortness of breath. He calls 911 and is taken to the emergency room. An EKG shows deep T-wave inversions in leads V2 to V5. A cardiac biomarker panel shows a creatine kinase level of 400 U/L (normal, <200 U/L) with 10% MB fraction (the heart-specific isoform), suggesting myocardial infarction. He is treated with IV **nitroglycerin, aspirin, unfractionated heparin**, and **eptifibatid**, but his chest

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pain persists. He is taken to the cardiac catheterization laboratory, where he is found to have a 90% mid-LAD (left anterior descending artery) thrombus with sluggish distal flow. He undergoes successful angioplasty and stent placement. At the time of stent placement, an intravenous loading dose of **clopidogrel** is administered. The heparin is stopped, the eptifibatide is continued for 18 more hours, and he is transferred to the telemetry ward. Six hours later, Mr. Soprano is noted to have an expanding hematoma (an area of localized hemorrhage) in his right thigh below the arterial access site. The eptifibatide is stopped and pressure is applied to the access site, and the hematoma ceases to expand. He is discharged 2 days later with prescriptions for clopidogrel and aspirin, which are administered to prevent subacute thrombosis of the stent.

QUESTIONS

- 1. How did a blood clot arise in Mr. Soprano's coronary artery?
- 2. If low molecular weight heparin had been used instead of unfractionated heparin, how would the monitoring of the patient's coagulation status during the procedure have been affected?
- 3. What accounts for the efficacy of eptifibatide (a platelet GPIIb–IIIa antagonist) in inhibiting platelet aggregation?
- 4. When the expanding hematoma was observed, could any measure other than stopping the eptifibatide have been used to reverse the effect of this agent?
- 5. How do aspirin, heparin, clopidogrel, and eptifibatide act in the attempt to treat Mr. Soprano's blood clot and to prevent recurrent thrombus formation?

PHYSIOLOGY OF HEMOSTASIS

An injured blood vessel must induce the formation of a blood clot to prevent blood loss and to allow healing. Clot formation must also remain localized to prevent widespread clotting within intact vessels. The formation of a localized clot at the site of vessel injury is accomplished in four temporally overlapping stages (Fig. 22-1). First, **localized vasoconstriction** occurs as a response to a reflex neurogenic mechanism and to the secretion of endothelium-derived vasoconstrictors such as endothelin. Immediately following vasoconstriction, **primary hemostasis** occurs. During this stage, platelets are activated and adhere to the exposed subendothelial matrix. **Platelet activation** involves both a change in shape of the platelet and the release of secretory granule contents from the platelet. The secreted granule substances recruit other platelets, causing more platelets to adhere to the subendothelial matrix and to aggregate with one another at the site of vascular injury. Primary hemostasis ultimately results in the formation of a **primary hemostatic plug**.

The goal of the final two stages of hemostasis is to form a stable, permanent plug. During **secondary hemostasis**, also known as the **coagulation cascade**, the activated endothelium and other nearby cells (see below) express a membrane-bound procoagulant factor called **tissue factor**, which complexes with coagulation factor VII to initiate the coagulation cascade. The end result of this cascade is the activation of thrombin, a critical enzyme. Thrombin serves two pivotal functions in hemostasis: (1) it converts soluble fibrinogen to an insoluble fibrin polymer that forms the matrix of the clot; and (2) it induces more platelet recruitment and activation. Recent evidence indicates that fibrin clot formation (secondary hemostasis) overlaps temporally with platelet plug formation (primary hemostasis), and that each process reinforces the other. During the final stage, platelet aggregation and fibrin polymerization lead to the formation of a stable, **permanent plug**. In addition, **antithrombotic mechanisms** restrict the permanent plug to the site of vessel injury, ensuring that the permanent plug does not inappropriately extend to occlude the vascular tree.

VASOCONSTRICTION

Transient arteriolar vasoconstriction occurs immediately after vascular injury. This vasoconstriction is mediated by a poorly understood reflex neurogenic mechanism. Local endothelial secretion of **endothelin**, a potent vasoconstrictor, potentiates the reflex vasoconstriction. Because the vasoconstriction is transient, bleeding would continue if primary hemostasis were not activated.

PRIMARY HEMOSTASIS

The goal of primary hemostasis is to form a platelet plug that rapidly stabilizes vascular injury. Platelets play a pivotal role in primary hemostasis. **Platelets** are cell fragments that arise by budding from megakaryocytes in the bone marrow; these small, membrane-bound discs contain cytoplasm but lack nuclei. Glycoprotein receptors in the platelet plasma membrane are the primary mediators by which platelets are activated. Primary hemostasis involves the transformation of platelets into a hemostatic plug through three reactions: (1) adhesion; (2) the granule release reaction; and (3) aggregation and consolidation.

Platelet Adhesion

In the first reaction, platelets adhere to subendothelial collagen that is exposed after vascular injury (Fig. 22-2). This adhesion is mediated by **von Willebrand factor (vWF)**, a large multimeric protein that is secreted by both activated platelets and the injured endothelium. vWF binds both to surface receptors (especially glycoprotein Ib [GPIb]) on the platelet membrane and to the exposed collagen; this “bridging” action mediates adhesion of platelets to the collagen. The GPIb:vWF:collagen interaction is critical for initiation of primary hemostasis, because it is the only known molecular mechanism by which platelets can adhere to the injured vessel wall.

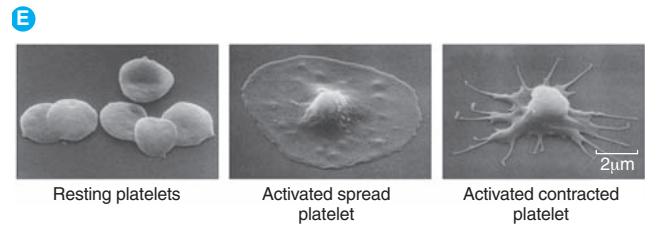
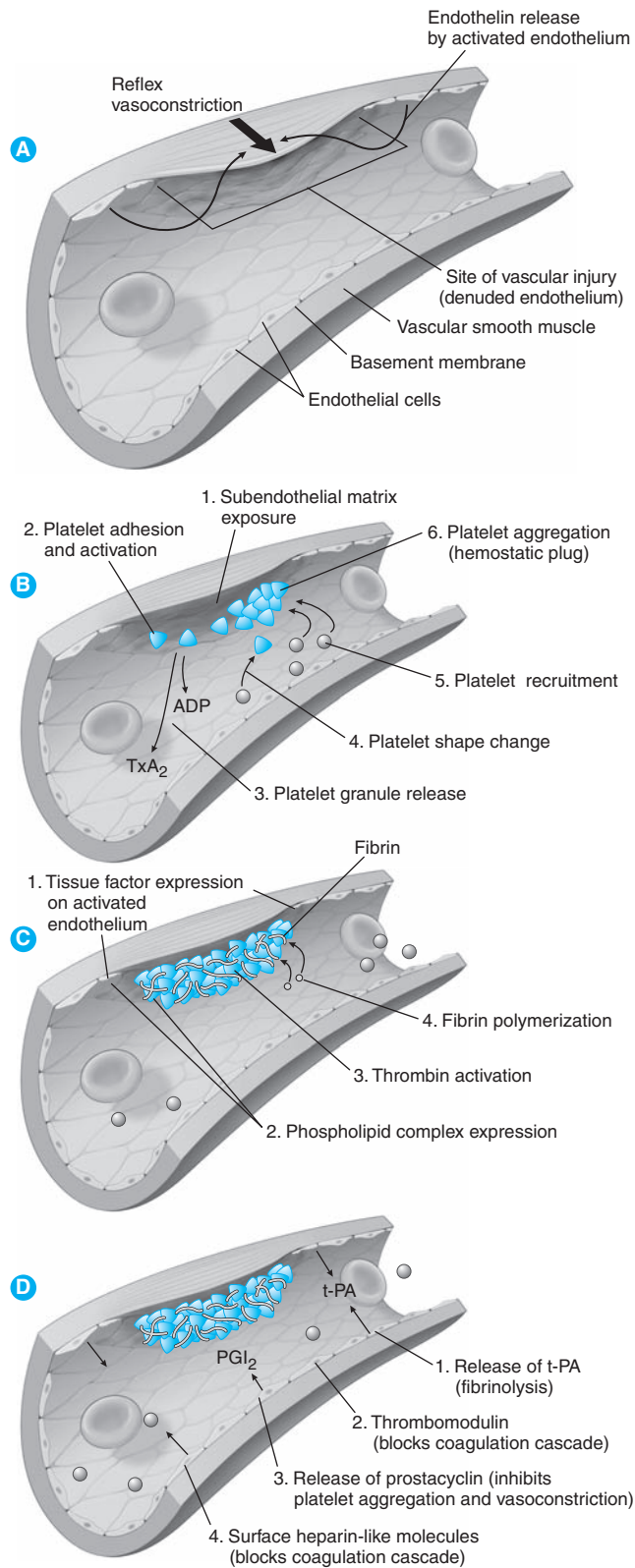


Figure 22-1. Sequence of events in hemostasis. The hemostatic process can be divided conceptually into four stages—vasoconstriction, primary hemostasis, secondary hemostasis, and resolution—although recent evidence suggests that these stages are temporally overlapping and may be nearly simultaneous. **A.** Vascular injury causes endothelial denudation. Endothelin, released by activated endothelium, and neurohumoral factor(s) induce transient vasoconstriction. **B.** Injury-induced exposure of the subendothelial matrix (1) provides a substrate for platelet adhesion and activation (2). In the granule release reaction, activated platelets secrete thromboxane A_2 (TxA_2) and ADP (3). TxA_2 and ADP released by activated platelets cause nearby platelets to become activated; these newly activated platelets undergo shape change (4) and are recruited to the site of injury (5). The aggregation of activated platelets at the site of injury forms a primary hemostatic plug (6). **C.** Tissue factor expressed on activated endothelial cells (1) and leukocyte microparticles (*not shown*), together with acidic phospholipids expressed on activated platelets and activated endothelial cells (2), initiate the steps of the coagulation cascade, culminating in the activation of thrombin (3). Thrombin proteolytically activates fibrinogen to form fibrin, which polymerizes around the site of injury, resulting in the formation of a definitive (secondary) hemostatic plug (4). **D.** Natural anticoagulant and thrombolytic factors limit the hemostatic process to the site of vascular injury. These factors include tissue plasminogen activator (t-PA), which activates the fibrinolytic system (1); thrombomodulin, which activates inhibitors of the coagulation cascade (2); prostacyclin, which inhibits both platelet activation and vasoconstriction (3); and surface heparin-like molecules, which catalyze the inactivation of coagulation factors (4). **E.** Scanning electron micrographs of resting platelets (1), a platelet undergoing cell spreading shortly after cell activation (2), and a fully activated platelet after actin filament bundling and crosslinking and myosin contraction (3).

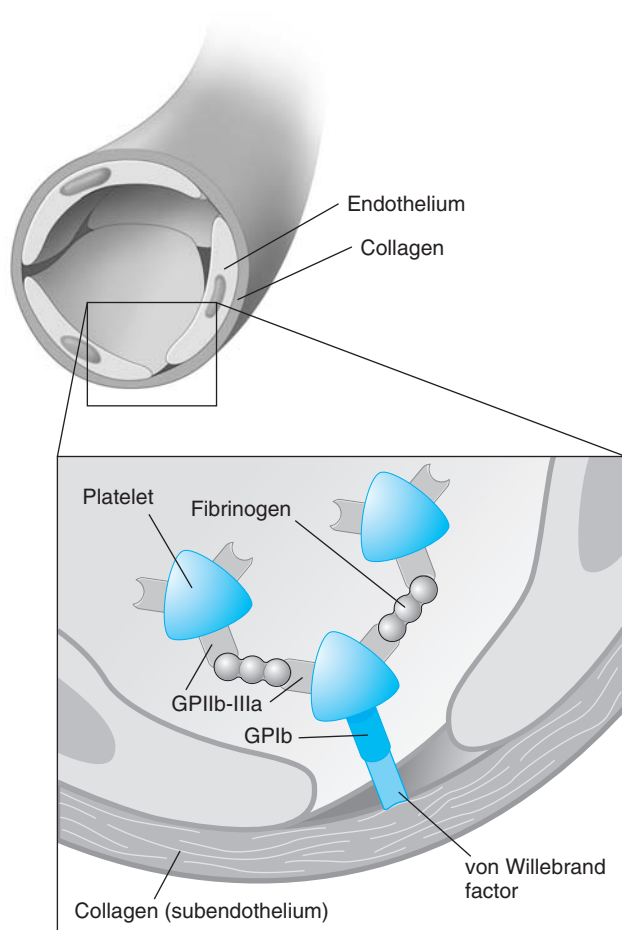


Figure 22-2. Platelet adhesion and aggregation. von Willebrand factor mediates platelet adhesion to the subendothelium by binding both to the platelet membrane glycoprotein GPIb and to exposed subendothelial collagen. During platelet aggregation, fibrinogen crosslinks platelets to one another by binding to GPIIb–IIIa receptors on platelet membranes.

Platelet Granule Release Reaction

Adherent platelets undergo a process of activation (Fig. 22-3) during which the cells' granule contents are released. The release reaction is initiated by agonist binding to cell surface receptors, which activates intracellular protein phosphorylation cascades and ultimately causes release of granule contents. Specifically, stimulation by ADP, epinephrine, and collagen leads to activation of platelet membrane phospholipase A₂ (PLA₂). PLA₂ cleaves membrane phospholipids and liberates **arachidonic acid**, which is converted into a **cyclic endoperoxide** by platelet cyclooxygenase. Thromboxane synthase subsequently converts the cyclic endoperoxide into **thromboxane A₂ (TxA₂)**. TxA₂, via a G protein-coupled receptor, causes vasoconstriction at the site of vascular injury by inducing a decrease in cAMP levels within vascular smooth muscle cells. TxA₂ also stimulates the granule **release reaction** within platelets, thereby propagating the cascade of platelet activation and vasoconstriction.

During the release reaction, large amounts of ADP, Ca²⁺, ATP, serotonin, vWF, and platelet factor 4 are *actively se-*

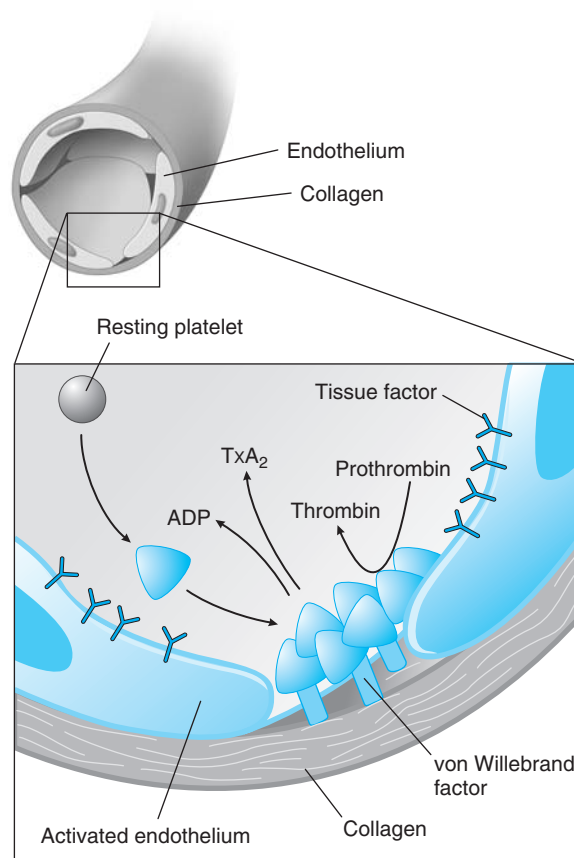


Figure 22-3. Platelet activation. Platelet activation is initiated at the site of vascular injury when circulating platelets adhere to exposed subendothelial collagen and are activated by locally generated mediators. Activated platelets undergo shape change and granule release, and platelet aggregates are formed as additional platelets are recruited and activated. Platelet recruitment is mediated by the release of soluble platelet factors, including ADP and thromboxane A₂ (TxA₂). Tissue factor, expressed on activated endothelium, is a critical initiating component in the coagulation cascade. The membranes of activated platelets provide a surface for a number of critical reactions in the coagulation cascade, including the conversion of prothrombin to thrombin.

creted from platelet granules. ADP is particularly important in mediating platelet aggregation, causing platelets to become “sticky” and adhere to one another (see below). Although strong agonists (such as thrombin and collagen) can trigger granule secretion even when aggregation is prevented, ADP can trigger granule secretion only in the presence of platelet aggregation. Presumably, this difference is caused by the set of intracellular effectors that are coupled to the various agonist receptors. Release of Ca²⁺ ions is also important for the coagulation cascade, as discussed below.

Platelet Aggregation and Consolidation

TxA₂, ADP, and fibrous collagen are all potent mediators of platelet aggregation. TxA₂ promotes platelet aggregation through stimulation of G protein-coupled TxA₂ receptors in the platelet membrane (Fig. 22-4). Binding of TxA₂ to platelet TxA₂ receptors leads to activation of phospholipase C

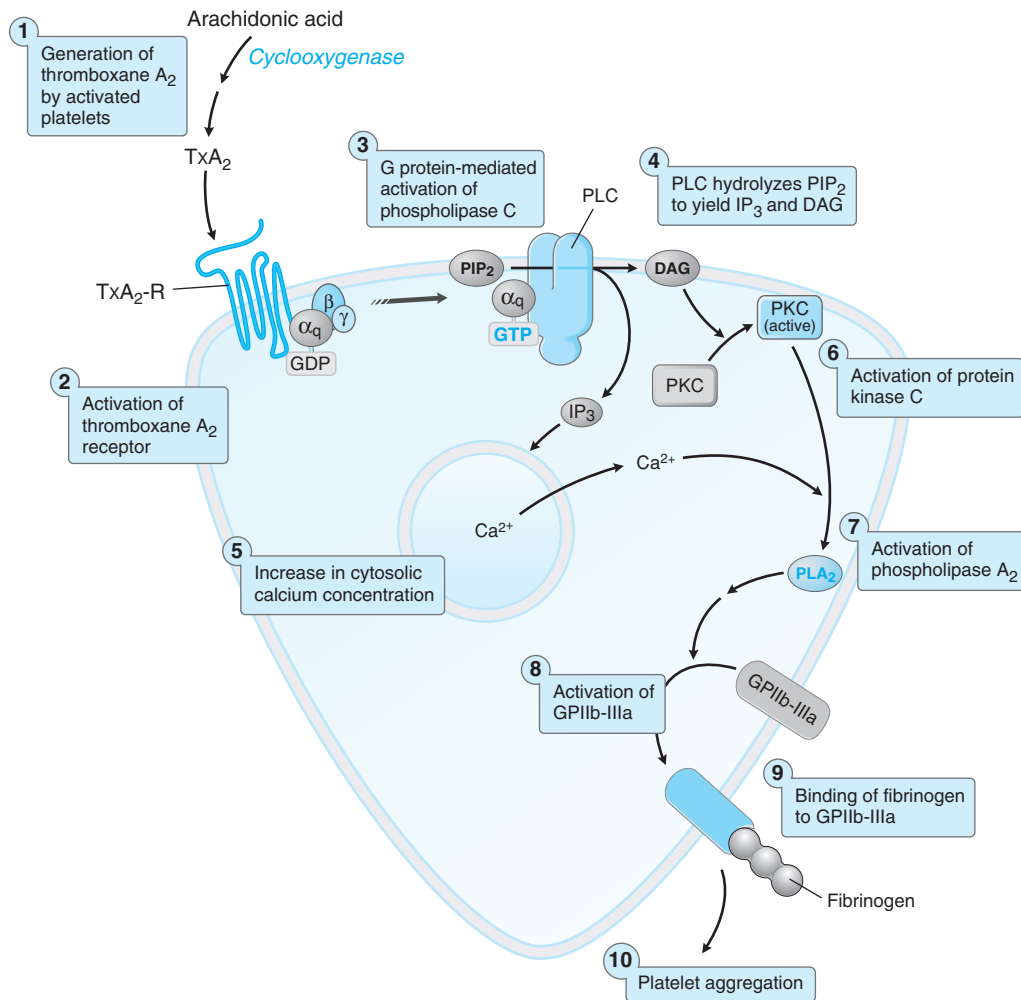


Figure 22-4. Platelet activation by thromboxane A₂. 1. Thromboxane A₂ (TxA₂) is generated from arachidonic acid in activated platelets; cyclooxygenase catalyzes the committed step in this process. 2. Secreted TxA₂ binds to the cell surface TxA₂ receptor (TxA₂-R), a G protein-coupled receptor. 3. The G_α isoform G_{αq} activates phospholipase C (PLC). 4. PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP₂) to yield inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG). 5. IP₃ raises the cytosolic Ca²⁺ concentration by promoting vesicular release of Ca²⁺ into the cytosol. 6. DAG activates protein kinase C (PKC). 7. PKC activates phospholipase A₂ (PLA₂). 8. Through a poorly understood mechanism, activation of PLA₂ leads to the activation of GPIIb-IIIa. 9. Activated GPIIb-IIIa binds to fibrinogen. 10. Fibrinogen crosslinks platelets by binding to GPIIb-IIIa receptors on other platelets. This crosslinking leads to platelet aggregation and formation of a primary hemostatic plug.

(PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PI[4,5]P₂) to yield inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG). IP₃ raises the cytosolic Ca²⁺ concentration and DAG activates protein kinase C (PKC), which in turn promotes the activation of PLA₂. Through a poorly understood mechanism, PLA₂ activation induces the expression of functional GPIIb-IIIa, the membrane integrin that mediates platelet aggregation. ADP triggers platelet activation by binding to G protein-coupled ADP receptors on the platelet surface (Fig. 22-5). The two subtypes of G protein-coupled platelet ADP receptors are termed **P2Y1 receptors** and **P2Y(ADP) receptors**. P2Y1, a G_q-coupled receptor, releases intracellular calcium stores through activation of phospholipase C. P2Y(ADP), a G_i-coupled receptor, inhibits adenylyl cyclase. The P2Y(ADP) receptor is the target of the antiplatelet agents **ticlopidine** and **clopidogrel** (see below).

Activation of ADP receptors mediates platelet shape change and expression of functional GPIIb-IIIa. Fibrous collagen activates platelets by binding directly to platelet glycoprotein VI (GPVI). Ligation of GPVI by collagen leads to phospholipase C activation and platelet activation, as described above.

Platelets aggregate with one another through a bridging molecule, **fibrinogen**, which has multiple binding sites for functional GPIIb-IIIa (Fig. 22-2). *Just as the vWF:GPIb interaction is important for platelet adhesion to exposed subendothelial collagen, the fibrinogen:GPIIb-IIIa interaction is critical for platelet aggregation.* Platelet aggregation ultimately leads to the formation of a reversible clot, or a **primary hemostatic plug**.

Activation of the **coagulation cascade** proceeds nearly simultaneously with the formation of the primary hemostatic plug, as described below. Activation of the coagulation cascade leads to the generation of fibrin, initially at the periph-

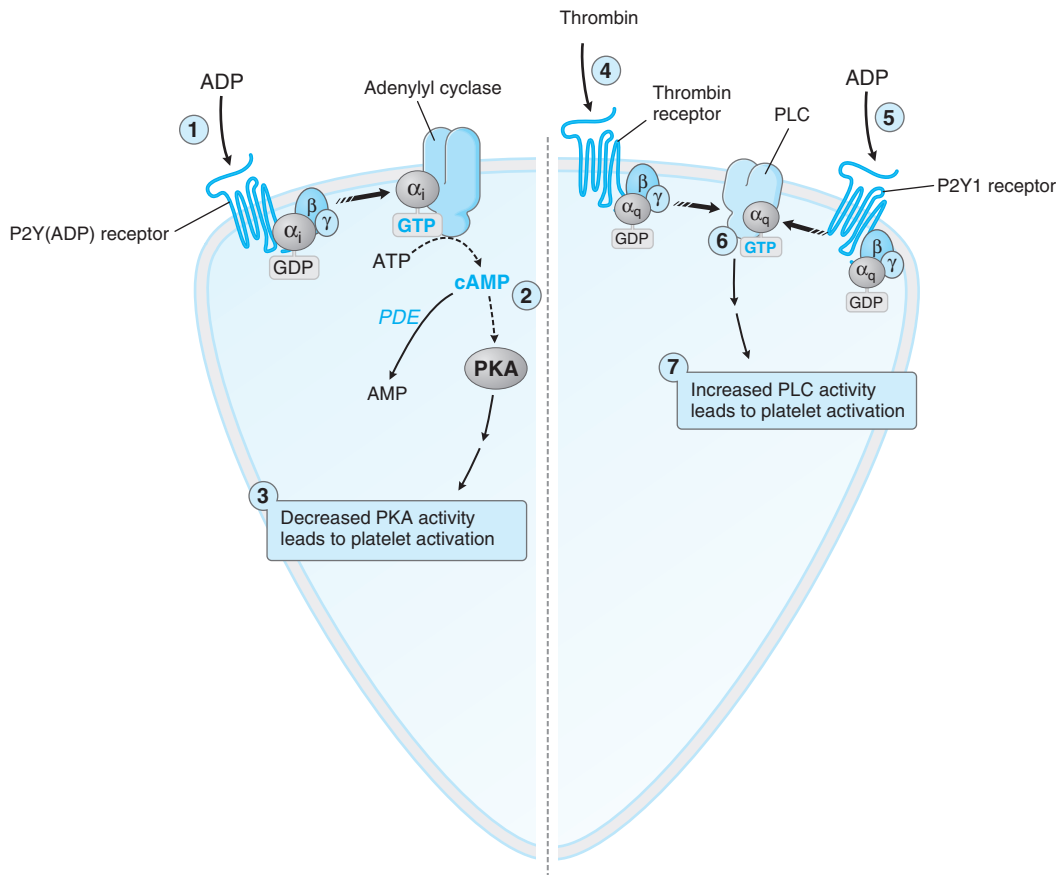


Figure 22-5. Platelet activation by ADP and thrombin. **Left panel:** 1. Binding of ADP to the P2Y(ADP) receptor activates a G_i protein, which inhibits adenylyl cyclase. 2. Inhibition of adenylyl cyclase decreases the synthesis of cAMP, and hence decreases protein kinase A (PKA) activation (*dashed arrow*). cAMP is metabolized to AMP by phosphodiesterase (PDE). 3. PKA inhibits platelet activation through a series of poorly understood steps. Therefore, the decreased PKA activation that results from ADP binding to the P2Y(ADP) receptor causes platelet activation. **Right panel:** 4. Thrombin proteolytically cleaves the extracellular domain of its receptor. This cleavage creates a new N-terminus, which binds to an activation site on the thrombin receptor to activate a G_q protein. 5. ADP also activates G_q by binding to the P2Y1 receptor. 6. G_q activation (by either thrombin or ADP) activates phospholipase C (PLC). 7. PLC activity leads to platelet activation, as shown in Figure 22-4. Note that ADP can activate platelets by binding to either the P2Y(ADP) receptor or the P2Y1 receptor, although recent evidence suggests that full platelet activation requires the participation of both receptors.

ery of the primary hemostatic plug. Platelet pseudopods attach to the fibrin strands at the periphery of the plug and *contract*. Platelet contraction yields a compact, solid, irreversible clot, or a **secondary hemostatic plug**.

SECONDARY HEMOSTASIS: THE COAGULATION CASCADE

Secondary hemostasis is also termed the **coagulation cascade**. The goal of this cascade is to form a stable fibrin clot at the site of vascular injury. Details of the coagulation cascade are presented schematically in [Figure 22-6](#). Several general principles should be noted.

First, the coagulation cascade is a sequence of enzymatic events. Most plasma coagulation factors circulate as inactive *proenzymes*, which are synthesized by the liver. These proenzymes are proteolytically cleaved, and thereby activated, by the activated factors that precede them in the cascade.

The activation reaction is catalytic and not stoichiometric. For example, one “unit” of activated factor X can potentially generate 40 “units” of thrombin. This robust amplification process rapidly generates large amounts of fibrin at a site of vascular injury.

Second, the major activation reactions in the cascade occur at sites where a **phospholipid-based protein–protein complex** has formed ([Fig. 22-7](#)). This complex is composed of a membrane surface (provided by activated platelets, activated endothelial cells, and possibly activated leukocyte microparticles [see below]), an enzyme (an activated coagulation factor), a substrate (the proenzyme form of the downstream coagulation factor), and a cofactor. The presence of negatively charged phospholipids, especially phosphatidylserine, is critical for assembly of the complex. Phosphatidylserine, which is normally sequestered in the inner leaflet of the plasma membrane, translocates to the outer leaflet of the membrane in response to agonist stimulation of platelets, endothelial cells, or leukocytes. Calcium is required for the enzyme, substrate, and cofactor to adopt the

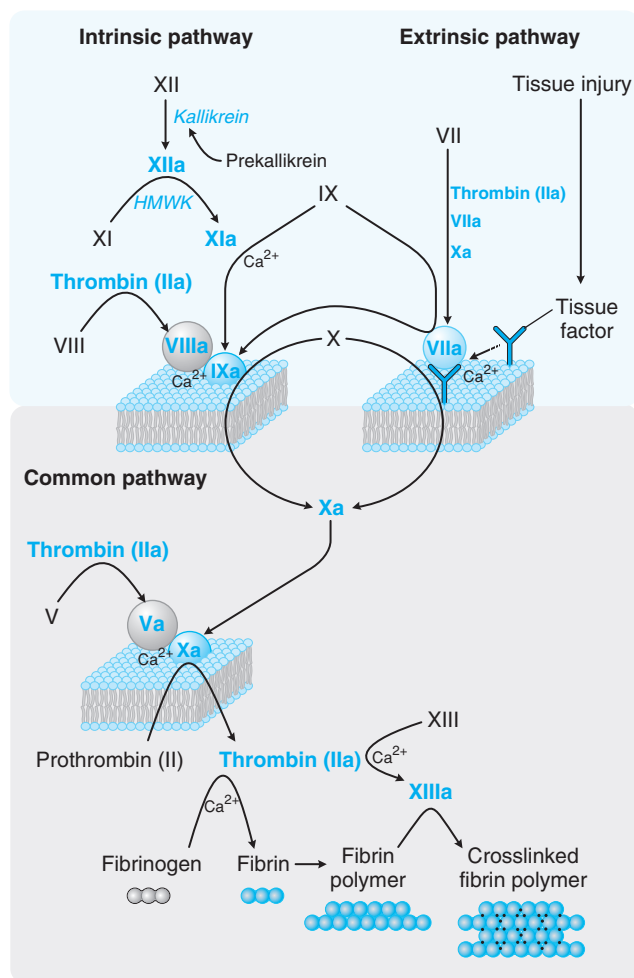


Figure 22-6. Coagulation cascade. The coagulation cascade is arbitrarily divided into the intrinsic pathway, the extrinsic pathway, and the common pathway. The intrinsic and extrinsic pathways converge at the level of factor X activation. The intrinsic pathway is largely an *in vitro* pathway, while the extrinsic pathway accounts for the majority of *in vivo* coagulation. The extrinsic pathway is initiated at sites of vascular injury by the expression of tissue factor on several different cell types, including activated endothelial cells, activated leukocytes (and leukocyte microparticles), subendothelial vascular smooth muscle cells, and subendothelial fibroblasts. Note that Ca^{2+} is a cofactor in many of the steps, and that a number of the steps occur on phospholipid surfaces provided by activated platelets, activated endothelial cells, and activated leukocytes (and leukocyte microparticles). Activated coagulation factors are shown in blue and indicated with a lower case "a." HMWK, high-molecular-weight kininogen.

proper conformation for the proteolytic cleavage of a coagulation factor proenzyme to its activated form.

Third, the coagulation cascade has been divided traditionally into the **intrinsic** and **extrinsic pathways** (Fig. 22-6). This division is a result of *in vitro* testing and is essentially arbitrary. The intrinsic pathway is activated *in vitro* by factor XII (Hageman factor), while the extrinsic pathway is initiated *in vivo* by **tissue factor**, a lipoprotein expressed by activated leukocytes (and microparticles derived from activated leukocytes; see below), activated endothelial cells, subendothelial smooth muscle cells, and subendothelial fibro-

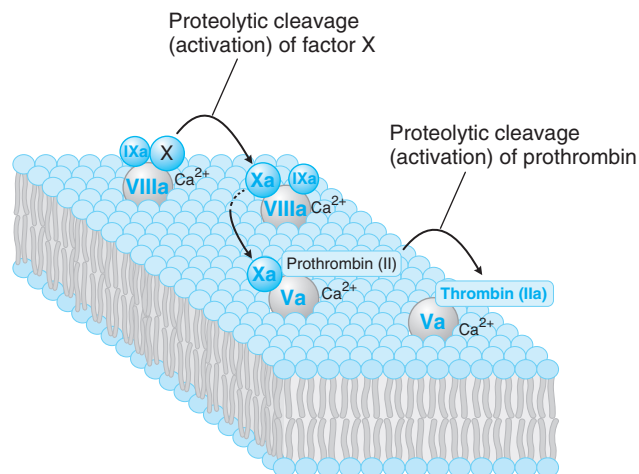


Figure 22-7. Coagulation factor activation on phospholipid surfaces. Surface catalysis is critical for a number of the activation reactions in the coagulation cascade. Each activation reaction consists of an enzyme (e.g., factor IXa), a substrate (e.g., factor X), and a cofactor or reaction accelerator (e.g., factor VIIIa), all of which are assembled on the phospholipid surface of activated platelets, endothelial cells, and leukocytes. Ca^{2+} allows the enzyme and substrate to adopt the proper conformation in each activation reaction. In the example shown, factor VIIIa and Ca^{2+} act as cofactors in the factor IXa-mediated cleavage of factor X to factor Xa. Factor Va and Ca^{2+} then act as cofactors in the factor Xa-mediated cleavage of prothrombin to thrombin.

blasts at the site of vascular injury. Although these two pathways converge at the activation of factor X, there also exists much interconnection between the two pathways. Because factor VII (activated by the extrinsic pathway) can proteolytically activate factor IX (a key factor in the intrinsic pathway), the extrinsic pathway is regarded as the primary pathway for the initiation of coagulation *in vivo*.

Fourth, both the intrinsic and extrinsic coagulation pathways lead to the activation of factor X. In an important reaction that requires factor V, activated factor X proteolytically cleaves prothrombin (factor II) to **thrombin** (factor IIa) (Fig. 22-8). Thrombin is a multifunctional enzyme that acts in the coagulation cascade in four important ways: (1) it converts the soluble plasma protein fibrinogen into fibrin, which then forms long insoluble polymer fibers; (2) it activates factor XIII, which crosslinks the fibrin polymers into a highly stable meshwork or clot; (3) it amplifies the clotting cascade by catalyzing the feedback activation of factors VIII and V; and (4) it strongly activates platelets, causing granule release, platelet aggregation, and platelet-derived microparticle generation. In addition to its procoagulant properties, thrombin acts to modulate the coagulation response. Thrombin binds to thrombin receptors on the *intact* vascular endothelial cells adjacent to the area of vascular injury, and stimulates these cells to release the platelet inhibitors prostacyclin (PGI_2) and nitric oxide (NO), the profibrinolytic protein tissue-type plasminogen activator (t-PA), and the endogenous t-PA modulator plasminogen activator inhibitor 1 (PAI-1) (see below).

The thrombin receptor, a protease-activated G protein-coupled receptor, is expressed in the plasma membrane of

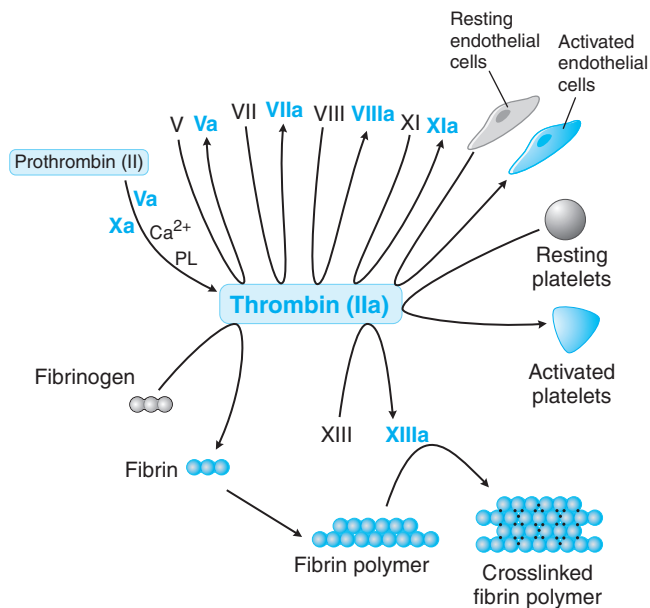


Figure 22-8. Central role of thrombin in the coagulation cascade. In the coagulation cascade, prothrombin is cleaved to thrombin by factor Xa; factor Va and Ca^{2+} act as cofactors in this reaction, and the reaction takes place on an activated (phosphatidylserine-expressing) phospholipid surface (PL). Thrombin converts the soluble plasma protein fibrinogen to fibrin, which spontaneously polymerizes. Thrombin also activates factor XIII, a transglutaminase that crosslinks the fibrin polymers into a highly stable meshwork or clot. Thrombin also activates co-factors V and VIII, as well as coagulation factors VII and XI. In addition, thrombin activates both platelets and endothelial cells. Finally, thrombin stimulates the release of several antithrombotic factors—including PGI_2 , NO, and t-PA—from resting (intact) endothelial cells near the site of vascular injury; these factors limit primary and secondary hemostasis to the injured site (not shown).

platelets, vascular endothelial cells, smooth muscle cells, and fibroblasts. Activation of the thrombin receptor involves proteolytic cleavage of an extracellular domain of the receptor by thrombin. The new NH_2 -terminal-tethered ligand binds intramolecularly to a discrete site within the receptor and initiates intracellular signaling. Activation of the thrombin receptor results in G protein-mediated activation of PLC (Fig. 22-5) and inhibition of adenylyl cyclase.

Finally, recent evidence from intravital (in vivo) microscopy experiments suggests that leukocyte-derived microparticles have an important role in coupling platelet plug formation (primary hemostasis) to fibrin clot formation (secondary hemostasis). A subpopulation of these microparticles, released from monocytes that are activated in the context of tissue injury and inflammation, appears to express both tissue factor and PSGL-1, a protein that binds to the P-selectin adhesion receptor expressed on activated platelets. By recruiting tissue factor-bearing microparticles throughout the developing platelet plug (primary hemostasis), thrombin generation and fibrin clot formation (secondary hemostasis) could be greatly accelerated within the plug itself. Indeed, it appears that vessel wall tissue factor (expressed by activated endothelial cells and subendothelial fibroblasts and smooth muscle cells) and microparticle tissue factor are both important for formation of a stable clot.

REGULATION OF HEMOSTASIS

Hemostasis is exquisitely regulated for two major reasons. First, hemostasis must be restricted to the local site of vascular injury. That is, activation of platelets and coagulation factors in the plasma should occur only at the site of endothelial damage, tissue factor expression, and procoagulant phospholipid exposure. Second, the size of the primary and secondary hemostatic plugs must be restricted so that the vascular lumen remains patent. After vascular injury, intact endothelium in the immediate vicinity of the injury becomes “activated.” This activated endothelium presents a set of procoagulant factors that promote hemostasis at the site of injury, and anticoagulant factors that restrict propagation of the clot beyond the site of injury. The procoagulant factors, such as tissue factor and phosphatidylserine, tend to be *membrane-bound* and *localized* to the site of injury — these factors provide a surface on which the coagulation cascade can proceed. In contrast, the anticoagulant factors are generally *secreted* by the endothelium and are *soluble* in the blood. Thus, *the activated endothelium maintains a balance of procoagulant and anticoagulant factors to limit hemostasis to the site of vascular injury.*

After vascular injury, the endothelium surrounding the injured area participates in five separate mechanisms that limit the initiation and propagation of the hemostatic process to the immediate vicinity of the injury. These mechanisms involve prostacyclin (PGI_2), antithrombin III, proteins C and S, tissue factor pathway inhibitor (TFPI), and tissue-type plasminogen activator (t-PA).

Prostacyclin (PGI_2) is an eicosanoid (i.e., a metabolite of arachidonic acid) that is synthesized and secreted by the endothelium. By acting through G_s protein-coupled platelet surface PGI_2 receptors, this metabolite increases cAMP levels within platelets and thereby inhibits platelet aggregation and platelet granule release. PGI_2 also has potent vasodilatory effects; this mediator induces vascular smooth muscle relaxation by increasing cAMP levels within the vascular smooth muscle cells. (Note that these mechanisms are physiologically antagonistic to those of TxA_2 , which induces platelet activation and vasoconstriction by decreasing intracellular cAMP levels.) Therefore, PGI_2 both prevents platelets from adhering to the intact endothelium that surrounds the site of vascular injury and maintains vascular patency around the site of injury.

Antithrombin III inactivates thrombin and other coagulation factors (IXa, Xa, XIa, and XIIIa, where “a” denotes an “activated” factor) by forming a stoichiometric complex with the coagulation factor (Fig. 22-9). These interactions are enhanced by a heparin-like molecule that is expressed at the surface of intact endothelial cells, ensuring that this mechanism is operative at all locations in the vascular tree *except* where endothelium is denuded at the site of vascular injury. (These endothelial cell surface proteoglycans are referred to as “heparin-like” because they are the physiologic equivalent of the pharmacologic agent heparin, discussed below.) Heparin-like molecules on the endothelial cells bind to and activate antithrombin III, which is then primed to complex with (and thereby inactivate) the activated coagulation factors.

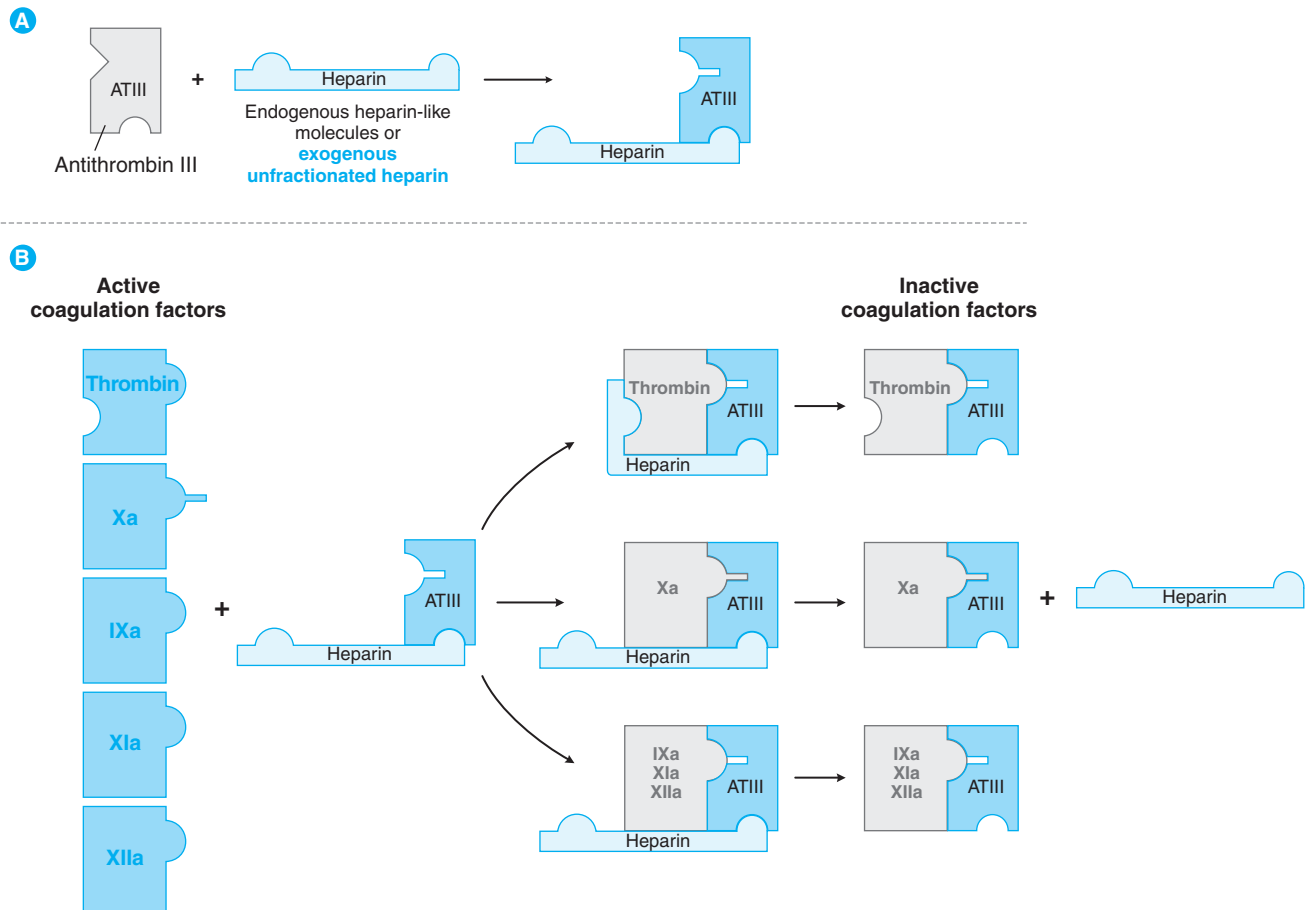


Figure 22-9. Antithrombin III action. Antithrombin III (ATIII) inactivates thrombin and factors IXa, Xa, XIa, and XIIa by forming a stoichiometric complex with these coagulation factors. These reactions are catalyzed physiologically by heparin-like molecules expressed on healthy endothelial cells; sites of vascular injury do not express heparin-like molecules because the endothelium is denuded or damaged. Pharmacologically, these reactions are catalyzed by exogenously administered heparin. In more detail, the binding of heparin to ATIII induces a conformational change in ATIII (**A**) that allows the ATIII to bind thrombin or coagulation factors IXa, Xa, XIa or XIIa. The stoichiometric complex between ATIII and the coagulation factor is highly stable, allowing heparin to dissociate without breaking up the complex (**B**).

Protein C and **protein S** are vitamin K-dependent proteins that slow the coagulation cascade by inactivating coagulation factors Va and VIIIa. Protein C and protein S are part of a feedback control mechanism, in which excess thrombin generation leads to activation of protein C which, in turn, helps to prevent the enlarging fibrin clot from occluding the vascular lumen. Specifically, the endothelial cell surface protein **thrombomodulin** is a receptor for both thrombin and protein C in the plasma. Thrombomodulin binds these proteins in such a way that thrombomodulin-bound thrombin cleaves protein C to activated protein C (also known as *protein Ca*). In a reaction that requires the cofactor protein S, activated protein C then inhibits clotting by cleaving (and thereby inactivating) factors Va and VIIIa.

Tissue factor pathway inhibitor (TFPI), as its name indicates, limits the action of tissue factor (TF). The coagulation cascade is initiated when factor VIIa complexes with TF at the site of vascular injury (Fig. 22-6). The resulting VIIa:TF complex catalyzes the activation of factors IX and X. After limited quantities of factors IXa and Xa are generated, the VIIa:TF complex becomes feedback inhibited by

TFPI in a two-step reaction. First, TFPI binds to factor Xa and neutralizes its activity in a Ca^{2+} -independent reaction. Subsequently, the TFPI:Xa complex interacts with the VIIa:TF complex via a second domain on TFPI, so that a quaternary Xa:TFPI:VIIa:TF complex is formed. The molecular “knots” of the TFPI molecule hold the quaternary complex tightly together and thereby inactivate the VIIa:TF complex. In this manner, TFPI prevents excessive TF-mediated activation of factors IX and X.

Plasmin exerts its anticoagulant effect by proteolytically cleaving fibrin into fibrin degradation products. Because plasmin has powerful antithrombotic effects, the *formation* of plasmin has intrigued researchers for many years, and a number of pharmacologic agents have been developed to target the plasmin formation pathway (Fig. 22-10). Plasmin is generated by the proteolytic cleavage of plasminogen, a plasma protein that is synthesized in the liver. The proteolytic cleavage is catalyzed by **tissue-type plasminogen activator (t-PA)**, which is synthesized and secreted by the endothelium. Plasmin activity is carefully modulated by three regulatory mechanisms in order to restrict plasmin action to

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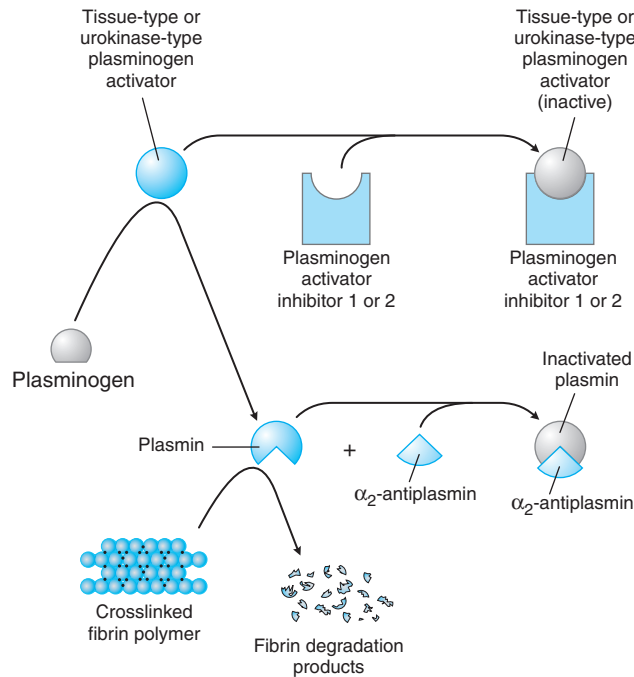


Figure 22-10. The Fibrinolytic System. Plasmin is formed by the proteolytic cleavage of plasminogen by tissue-type or urokinase-type plasminogen activator. Plasmin formation can be inhibited by plasminogen activator inhibitor 1 or 2, which binds to and inactivates plasminogen activators. In the fibrinolytic reaction, plasmin cleaves crosslinked fibrin polymers into fibrin degradation products. α_2 -Antiplasmin, which circulates in the bloodstream, neutralizes free plasmin in the circulation.

the site of clot formation. First, t-PA is most effective when it is bound to a fibrin meshwork. Second, t-PA activity can be inhibited by **plasminogen activator inhibitor (PAI)**. When local concentrations of thrombin and inflammatory cytokines (such as IL-1 and TNF- α) are *high*, endothelial cells *increase* the release of PAI, preventing t-PA from activating plasmin. This ensures that a stable fibrin clot forms at the site of vascular injury. Third, **α_2 -antiplasmin** is a plasma protein that neutralizes free plasmin in the circulation and thereby prevents random degradation of plasma fibrinogen. Plasma fibrinogen is important for platelet aggregation in primary hemostasis (see above), and it is also the precursor for the fibrin polymer that is required to form a stable clot.

PATHOGENESIS OF THROMBOSIS

Thrombosis is the pathologic extension of hemostasis. In thrombosis, coagulation reactions are inappropriately regulated so that a clot uncontrollably enlarges and occludes the lumen of a blood vessel. The pathologic clot is now termed a **thrombus**. Three major factors predispose to thrombus formation—endothelial injury, abnormal blood flow, and hypercoagulability. These three factors influence one another, and are collectively known as **Virchow's triad** (Fig. 22-11).

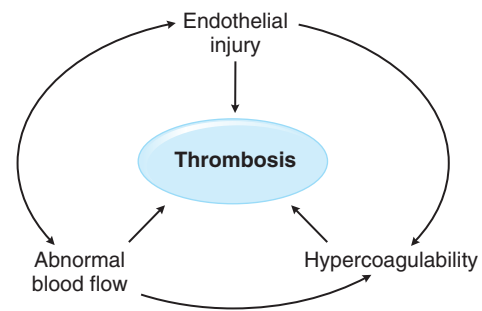


Figure 22-11. Virchow's triad. Endothelial injury, abnormal blood flow, and hypercoagulability are three factors that predispose to thrombus formation. These three factors are interrelated; endothelial injury predisposes to abnormal blood flow and hypercoagulability, while abnormal blood flow can cause both endothelial injury and hypercoagulability.

ENDOTHELIAL INJURY

Endothelial injury is the most dominant influence on thrombus formation in the *heart* and the *arterial circulation*. There are many possible causes of endothelial injury, including changes in shear stress associated with hypertension or turbulent flow, hyperlipidemia, elevated blood glucose in diabetes mellitus, traumatic vascular injury, and some infections. (Recall that Mr. Soprano developed coronary artery thrombosis, which was probably attributable to endothelial injury secondary to hypertension and cigarette smoking.)

Endothelial injury predisposes the vascular lumen to thrombus formation through three mechanisms. First, platelet activators, such as exposed subendothelial collagen, promote platelet adhesion to the injured site. Second, exposure of tissue factor on injured endothelium initiates the coagulation cascade. Third, natural antithrombotics, such as t-PA and PGI₂, become depleted at the site of vascular injury because these mechanisms rely on the functioning of an intact endothelial cell layer.

ABNORMAL BLOOD FLOW

Abnormal blood flow refers to a state of **turbulence** or **stasis** rather than laminar flow. Atherosclerotic plaques commonly predispose to turbulent blood flow in the vicinity of the plaque. Bifurcations of blood vessels can also create areas of turbulent flow. Turbulent blood flow causes endothelial injury, forms countercurrents, and creates local pockets of stasis. Local stasis can also result from formation of an aneurysm (a focal out-pouching of a vessel or a cardiac chamber) and from myocardial infarction. In the latter condition, a region of noncontractile (infarcted) myocardium serves as a favored site for stasis. Cardiac arrhythmias, such as atrial fibrillation, can also generate areas of local stasis. Stasis is the major cause for the formation of *venous* thrombi.

Disruption of normal blood flow by turbulence or stasis promotes thrombosis by three major mechanisms. First, the absence of laminar blood flow allows platelets to come into close proximity to the vessel wall. Second, stasis inhibits the flow of fresh blood into the vascular bed, so that activated coagulation factors in the region are not removed or diluted.

Third, abnormal blood flow promotes endothelial cell activation, which leads to a prothrombotic state.

HYPERCOAGULABILITY

Hypercoagulability is generally less important than endothelial injury and abnormal blood flow in predisposing to thrombosis, but this condition can be an important factor in some patients. Hypercoagulability refers to an abnormally heightened coagulation response to vascular injury, resulting from either: (1) **primary (genetic) disorders**; or (2) **secondary (acquired) disorders** (see [Table 22-1](#)). (Hypocoagulable states, or **hemorrhagic disorders**, can also result from primary or secondary causes; see [Box 22-1](#) for an example.)

Among the genetic causes of hypercoagulability, the most prevalent known mutation resides in the gene for coagulation factor V. It is estimated that 6% of the Caucasian population in the U.S. carries mutations in the factor V gene. The most common mutation is the Leiden mutation, in which glutamine is substituted for arginine at position 506. This position is important because it is part of a site in factor Va that is marked for proteolytic cleavage by activated protein C. The mutant **factor V Leiden** protein is resistant to proteolytic cleavage by activated protein C. As a result of the Leiden mutation, factor Va is allowed to accumulate and thereby to promote coagulation.

A second common mutation (2% incidence) is the **prothrombin G20210A mutation**, in which adenine (A) is substituted for guanine (G) in the 3'-untranslated region of the

prothrombin gene. This mutation leads to a 30% increase in plasma prothrombin levels. Both the factor V Leiden mutation and the prothrombin G20210A mutation are associated with a significantly increased risk of venous thrombosis and a modestly increased risk of arterial thrombosis. Other genetic disorders that predispose some individuals to thrombosis include mutations in the fibrinogen, protein C, protein S, and antithrombin III genes. Although the latter disorders are relatively uncommon (less than 1% incidence), patients with a genetic deficiency of protein C, protein S, or antithrombin III often present with spontaneous venous thrombosis.

Hypercoagulability can sometimes be acquired (secondary) rather than genetic. An example of acquired hypercoagulability is the **heparin-induced thrombocytopenia** syndrome. In some patients, administration of the anticoagulant heparin stimulates the immune system to generate circulating antibodies directed against a complex consisting of heparin and platelet factor 4. Because platelet factor 4 is present on platelet and endothelial cell surfaces, antibody binding to the heparin:platelet factor 4 complex results in antibody-mediated removal of platelets from the circulation; that is, in thrombocytopenia. In some patients, however, antibody binding also causes platelet activation, endothelial injury, and a prothrombotic state. Although both unfractionated and low molecular weight heparin (see below) can cause thrombocytopenia, it appears that low molecular weight heparin has a lower incidence of thrombocytopenia than unfractionated heparin.

TABLE 22-1 Major Causes of Hypercoagulability

CONDITION	MECHANISM OF HYPERCOAGULABILITY
Primary (Genetic)	
Factor V Leiden mutation (factor V R506Q) (common)	Resistance to activated protein C → excess factor Va
Hyperhomocysteinemia (common)	Endothelial damage due to accumulation of homocysteine
Prothrombin G20210A mutation (common)	Increased prothrombin level and activity
Antithrombin III deficiency (less common)	Decreased inactivation of factors IIa, IXa, and Xa
Protein C or S deficiency (less common)	Decreased proteolytic inactivation of factors VIIIa and Va
Secondary (Acquired: Disease or Drug-Induced)	
Antiphospholipid syndrome	Autoantibodies to negatively charged phospholipids → ↑ platelet adhesion
Heparin-induced thrombocytopenia	Antibodies to platelet factor 4 → platelet activation
Malignancy	Tumor cell induction of tissue factor expression
Myeloproliferative syndromes	Elevated blood viscosity, altered platelets
Nephrotic syndrome	Loss of antithrombin III in urine, ↑ fibrinogen, ↑ platelet activation
Oral contraceptive use, estrogen replacement therapy	↑ Hepatic synthesis of coagulation factors and/or effects of estrogen on endothelium (effect may be more prominent in patients with underlying primary hypercoagulability)
Paroxysmal nocturnal hemoglobinuria	Unknown, possibly “leaky” platelets
Postpartum period	Venous stasis, increased coagulation factors, tissue trauma
Surgery/trauma	Venous stasis, immobilization, tissue injury

BOX 22-1. Hemorrhagic Disorders: The Case of Hemophilia A

When the vascular endothelium is injured, the hemostatic process ensures localized, stable clot formation without obstruction of the vascular lumen. Just as thrombosis constitutes a pathologic variation on this otherwise orchestrated physiologic process, disorders involving insufficient levels of functional platelets or coagulation factors can lead to a hypocoagulable state characterized clinically by episodes of uncontrolled hemorrhage. Hemorrhagic disorders result from a multitude of causes, including disorders of the vasculature, vitamin K deficiency, and disorders or deficiencies of platelets, coagulation factors, and von Willebrand factor. Hemophilia A serves as an example of a hemorrhagic disorder in which hypocoagulability is the underlying pathology.

Hemophilia A is the most common genetic disorder of serious bleeding. The hallmark of the disorder is a reduction in the amount or activity of coagulation factor VIII. The syndrome has an X-linked mode of transmission, and the majority of patients are males or homozygous females. Thirty percent of patients have no family history of hemophilia A and presumably represent spontaneous mutations. The severity of the disease depends on the type of mutation in the factor VIII gene. Patients with 6% to 50% of normal factor VIII activity manifest a mild form of the disease; those with 2% to 5% activity manifest moderate disease; patients with less than 1% activity develop severe disease. All symptomatic patients demonstrate easy bruisability and can develop massive hemorrhage after trauma or surgery. Spontaneous hemorrhage can occur in body areas that are normally subjected to minor trauma, including joint spaces, where spontaneous hemorrhage leads to the formation of hemarthroses. Petechiae (microhemorrhages involving capillaries and small vessels, especially in mucocutaneous areas), which are usually an indication of platelet disorders, are absent in patients with hemophilia.

Patients with hemophilia A are currently treated with infusions of factor VIII that is either recombinant or derived from human plasma. Factor VIII infusion therapy is sometimes complicated in patients who develop antibodies against factor VIII. HIV infection was a serious complication of infusion therapy in patients who received factor VIII products before the institution of routine screening of blood for HIV infection (before the mid-1980s). Some sources suggest that the entire cohort of hemophiliacs who received factor VIII concentrates (factor VIII concentrated from the blood of many individuals) between 1981 and 1985 has been infected with HIV. With current blood screening practices and the development of recombinant factor VIII, the risk of contracting HIV through factor VIII infusions is now virtually zero.

PHARMACOLOGIC CLASSES AND AGENTS

Drugs have been developed to prevent and/or reverse thrombus formation. These drugs fall into three classes: antiplatelet agents, anticoagulants, and thrombolytic agents. Hemostatic agents, discussed at the end of the chapter, are occasionally used to reverse the effects of anticoagulants or to inhibit endogenous fibrinolysis.

ANTIPLATELET AGENTS

As described above, formation of a localized platelet plug in response to endothelial injury is the initial step in arterial thrombosis. Therefore, inhibition of platelet function is a useful prophylactic and therapeutic strategy against myocardial infarction and stroke caused by thrombosis in coronary and cerebral arteries, respectively. The classes of antiplatelet agents in current clinical use include cyclooxygenase (COX) inhibitors, phosphodiesterase inhibitors, ADP receptor pathway inhibitors, and GPIIb–IIIa antagonists.

Cyclooxygenase Inhibitors

Aspirin inhibits the synthesis of prostaglandins, thereby inhibiting the platelet release reaction and interfering with normal platelet aggregation.

The biochemistry of prostaglandin synthesis in platelets and endothelial cells provides a basis for understanding the mechanism of action of aspirin as an antiplatelet agent. [Figure 22-12](#) depicts the prostaglandin synthesis pathway, which is discussed in more detail in Chapter 41, Pharmacology of Eicosanoids. Briefly, activation of both platelets and endothelial cells induces phospholipase A₂ (PLA₂) to cleave membrane phospholipids and release arachidonic acid. Arachidonic acid is then transformed into a cyclic endoperoxide (also known as **prostaglandin G₂** or **PGG₂**) by the enzyme COX. In platelets, the cyclic endoperoxide is converted into thromboxane A₂ (TxA₂). Acting through cell surface TxA₂ receptors, TxA₂ causes localized vasoconstriction and is a potent inducer of platelet aggregation and the platelet release reaction. In endothelial cells, the cyclic endoperoxide is converted into prostacyclin (PGI₂). PGI₂, in turn, causes localized vasodilation and inhibits platelet aggregation and the platelet release reaction.

Aspirin acts by *covalently* acetylating a serine residue near the active site of the COX enzyme, thereby inhibiting the synthesis of the cyclic endoperoxide and the various metabolites of the cyclic endoperoxide. In the absence of TxA₂, there is a marked decrease in platelet aggregation and the platelet release reaction ([Fig. 22-13A](#)). *Because platelets do not contain DNA or RNA, these cells cannot regenerate new COX enzyme once aspirin has permanently inactivated all of the available COX enzyme.* That is, the platelets become irreversibly “poisoned” for the lifetime of these cells (7–10 days). Although aspirin also inhibits the COX enzyme in endothelial cells, its action is not permanent in endothelial cells because these cells are able to synthesize new COX molecules. Thus, the endothelial cell production of prosta-

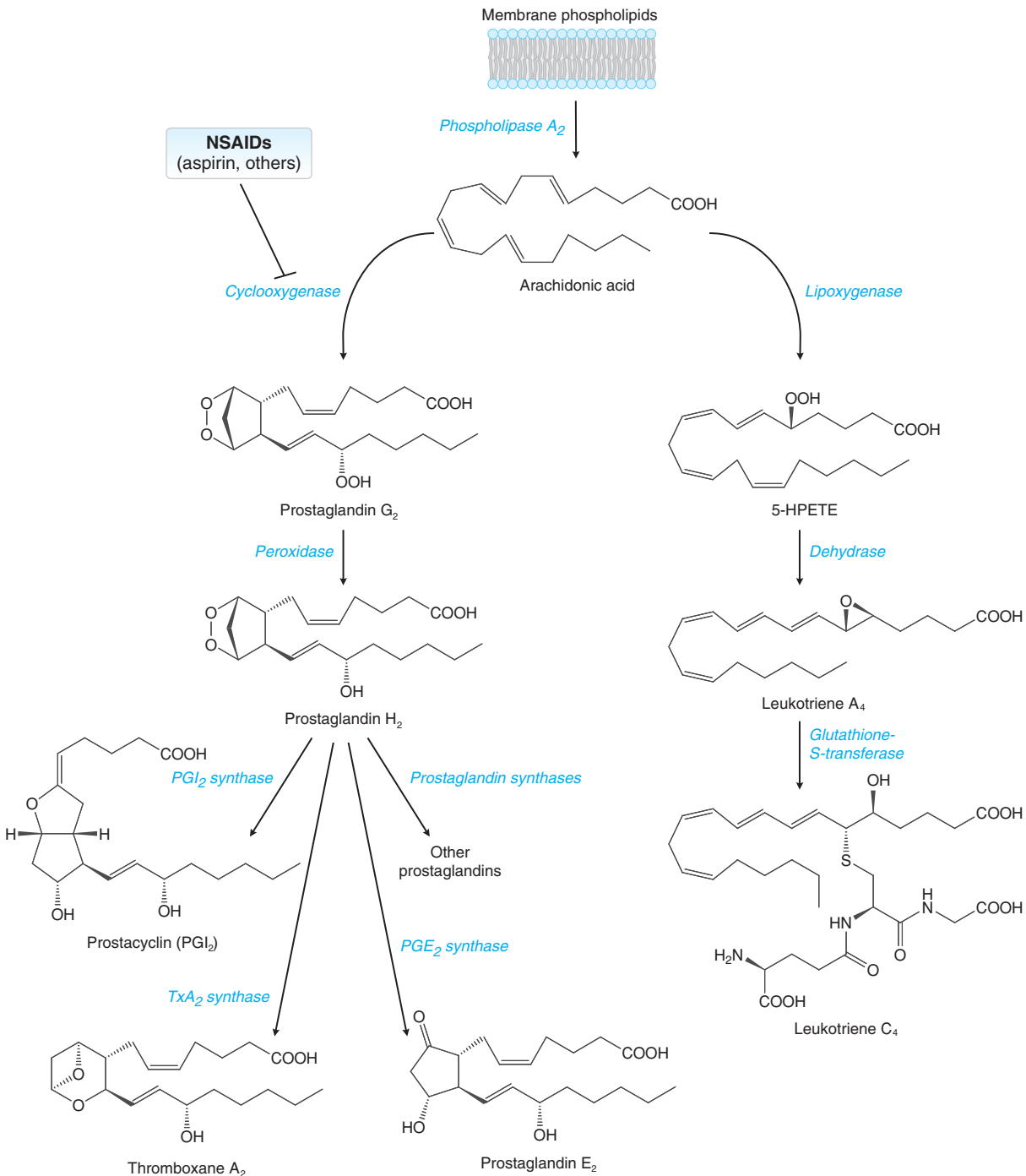


Figure 22-12. Overview of Prostaglandin Synthesis. Membrane phospholipids are cleaved by phospholipase A₂ to release free arachidonic acid. Arachidonic acid can be metabolized through either of two major pathways, the cyclooxygenase pathway or the lipoxygenase pathway. The cyclooxygenase pathway, which is inhibited by aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), converts arachidonic acid into prostaglandins and thromboxanes. Platelets express TxA₂ synthase and synthesize the pro-aggregatory mediator thromboxane A₂; endothelial cells express PGI₂ synthase and synthesize the anti-aggregatory mediator prostacyclin. The lipoxygenase pathway converts arachidonic acid into leukotrienes, which are potent inflammatory mediators. (See Chapter 41, Pharmacology of Eicosanoids, for a detailed discussion of the lipoxygenase and cyclooxygenase pathways.) Aspirin inhibits cyclooxygenase by covalent acetylation of the enzyme near its active site. Because platelets lack the capability to synthesize new proteins, aspirin inhibits thromboxane synthesis for the life of the platelet.

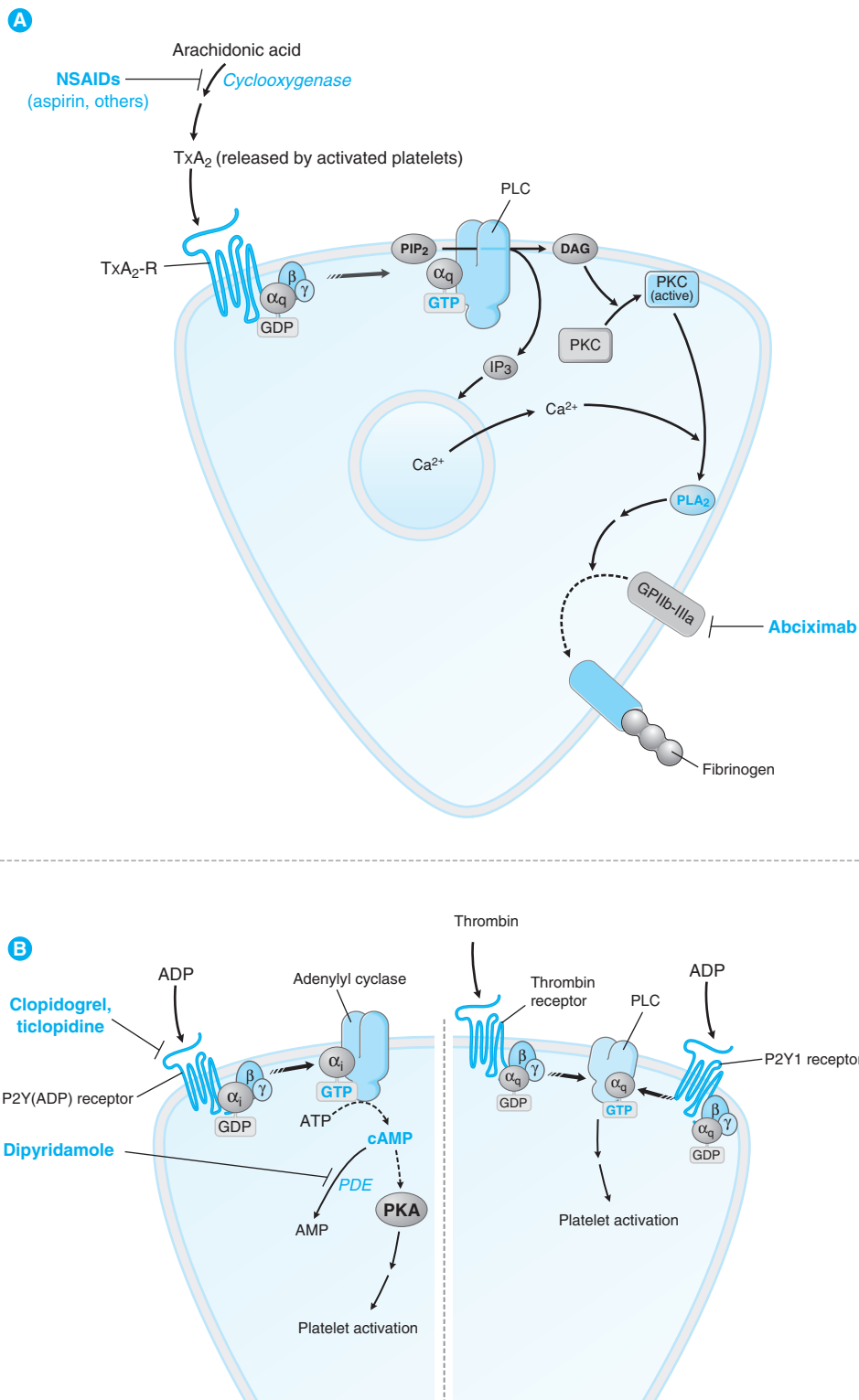


Figure 22-13. Mechanism of action of antiplatelet agents. **A.** NSAIDs and GPIIb-IIIa antagonists inhibit steps in thromboxane A₂ (TxA₂)-mediated platelet activation. Aspirin inhibits cyclooxygenase by covalent acetylation of the enzyme near its active site, leading to decreased TxA₂ production. The effect is profound because platelets lack the ability to synthesize new enzyme molecules. GPIIb-IIIa antagonists, such as the monoclonal antibody abciximab and the small-molecule antagonists eptifibatid and tirofiban (*not shown*), inhibit platelet aggregation by preventing activation of GpIIb-IIIa (*dashed line*), leading to decreased platelet crosslinking by fibrinogen. **B.** Clopidogrel, ticlopidine, and dipyridamole inhibit steps in ADP-mediated platelet activation. Clopidogrel and ticlopidine are antagonists of the P2Y(ADP) receptor. Dipyridamole inhibits phosphodiesterase (PDE), thereby preventing the breakdown of cAMP and increasing cytoplasmic cAMP concentration.

cyclin is relatively unaffected by aspirin at pharmacologically low doses (see below).

Aspirin is most often used as an antiplatelet agent to prevent arterial thrombosis leading to stroke, transient ischemic attack, and myocardial infarction. Because the action of aspirin on platelets is permanent, it is most effective as a selective antiplatelet agent when taken *in low doses and/or at infrequent intervals*. For example, aspirin is often used as an antiplatelet agent at a dose of 81 mg once daily, while a typical anti-inflammatory dose of this agent could be 650 mg three to four times daily. Taken at high doses, aspirin can inhibit prostacyclin production without increasing the effectiveness of the drug as an antiplatelet agent. A more extended discussion of the uses and toxicities of aspirin is found in Chapter 41. Compared with aspirin, other nonsteroidal anti-inflammatory drugs (NSAIDs) are not as widely used in the prevention of arterial thrombosis because the inhibitory action of these drugs on cyclooxygenase is not permanent.

COX-1 is the predominant COX isoform in platelets, but endothelial cells appear to express both COX-1 and COX-2 under physiologic conditions. Because aspirin inhibits COX-1 and COX-2 nonselectively, this drug serves as an effective antiplatelet agent. In contrast, the newer selective COX-2 inhibitors cannot be used as antiplatelet agents because they are poor inhibitors of COX-1. Furthermore, use of the selective COX-2 inhibitors appears to be associated with increased cardiovascular risk, most likely because these agents inhibit endothelial production of PGI₂ without inhibiting platelet generation of TxA₂. The adverse impact of the selective COX-2 inhibitors on cardiovascular risk has resulted in the recent withdrawal of most of these agents from the market (see Chapter 41).

Phosphodiesterase Inhibitors

In platelets, an *increase* in the concentration of intracellular cAMP leads to a *decrease* in platelet aggregability. Platelet cAMP levels are regulated physiologically by TxA₂ and PGI₂, among other mediators (see above). The mechanism by which increased intracellular cAMP concentration leads to decreased platelet aggregability is not well understood. cAMP activates protein kinase A, which, through incompletely elucidated mechanisms, decreases availability of the intracellular Ca²⁺ necessary for platelet aggregation (Fig. 22-13B). Inhibitors of platelet phosphodiesterase decrease platelet aggregability by inhibiting cAMP degradation, while activators of platelet adenylyl cyclase decrease platelet aggregability by increasing cAMP synthesis. (There are currently no direct adenylyl cyclase activators in clinical use.)

Dipyridamole is an inhibitor of platelet phosphodiesterase that decreases platelet aggregability (Fig. 22-13B). Dipyridamole by itself has only weak antiplatelet effects, and is therefore usually administered in combination with warfarin or aspirin. The combination of dipyridamole and warfarin can be used to inhibit embolization from prosthetic heart valves, while the combination of dipyridamole and aspirin can be used to reduce the likelihood of thrombosis in patients with a thrombotic diathesis. Dipyridamole also has vasodilatory properties. It may paradoxically induce angina in patients with coronary artery disease by causing the coronary

steal phenomenon, which involves intense dilation of coronary arterioles (see Chapter 21, Pharmacology of Vascular Tone).

ADP Receptor Pathway Inhibitors

Both **ticlopidine** and **clopidogrel** are derivatives of thienopyridine. These agents, which irreversibly inhibit the ADP-dependent pathway of platelet activation, have antiplatelet effects *in vitro* and *in vivo*. Ticlopidine and clopidogrel are thought to act by covalently modifying and inactivating the platelet P2Y(ADP) receptor (also called P2Y₁₂), which is physiologically coupled to the inhibition of adenylyl cyclase (Fig. 22-13B). Ticlopidine is a prodrug that requires conversion to active thiol metabolites in the liver. Maximal platelet inhibition is observed 8 to 11 days after initiating therapy with the drug; used in combination with aspirin, 4 to 7 days are needed to achieve maximal platelet inhibition. Administration of a loading dose can produce a more rapid antiplatelet response. Ticlopidine is approved in the U.S. for two indications: (1) secondary prevention of thrombotic strokes in patients intolerant of aspirin, and (2) in combination with aspirin, prevention of stent thrombosis for up to 30 days after placement of coronary artery stents. In general, ticlopidine is considered to be less safe than clopidogrel. The use of ticlopidine has occasionally been associated with neutropenia, thrombocytopenia, and thrombotic thrombocytopenic purpura (TTP); for this reason, blood counts must be monitored frequently when using ticlopidine.

Clopidogrel, a thienopyridine closely related to ticlopidine, has been used widely in combination with aspirin for improved platelet inhibition during and after elective percutaneous coronary intervention. Clopidogrel is a prodrug that must undergo oxidation by hepatic P450 3A4 to the active drug form; it may therefore interact with statins and other drugs metabolized by this P450 enzyme. Clopidogrel is approved for secondary prevention in patients with recent myocardial infarction, stroke, or peripheral vascular disease. It is also approved for use in acute coronary syndromes that are treated with either percutaneous coronary intervention or coronary artery bypass grafting. Like ticlopidine, clopidogrel requires a loading dose to achieve a maximal antiplatelet effect rapidly. For this reason, Mr. Soprano was given an intravenous loading dose of clopidogrel in the context of his myocardial infarction. The adverse effect profile of clopidogrel is more favorable than that of ticlopidine: the gastrointestinal side effects of clopidogrel are similar to those of aspirin, and clopidogrel lacks the significant bone marrow toxicity associated with ticlopidine.

GPIIb–IIIa Antagonists

As noted above, platelet membrane GPIIb–IIIa receptors are important because they constitute the final common pathway of platelet aggregation, serving to bind fibrinogen molecules that bridge platelets to one another. A variety of stimuli (e.g., TxA₂, ADP, epinephrine, collagen, and thrombin), acting through diverse signaling molecules, are capable of inducing the expression of functional GPIIb–IIIa on the platelet surface. It could therefore be predicted that antagonists of GPIIb–IIIa would prevent fibrinogen binding to the GPIIb–IIIa receptor and thus serve as powerful inhibitors of

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platelet aggregation. **Eptifibatide**, the GPIIb–IIIa receptor antagonist used in the opening case, is a highly efficacious inhibitor of platelet aggregation. A synthetic peptide, eptifibatide antagonizes the platelet GPIIb–IIIa receptor with high affinity. This drug has been used to reduce ischemic events in patients undergoing percutaneous coronary intervention and to treat unstable angina and non-ST elevation myocardial infarction.

Abciximab is a chimeric mouse–human monoclonal antibody directed against the human GPIIb–IIIa receptor. Experiments in vitro have shown that occupation of 50% of platelet GPIIb–IIIa receptors by abciximab significantly reduces platelet aggregation. The binding of abciximab to GPIIb–IIIa is essentially *irreversible*, with a dissociation half-time of 18 to 24 hours. In clinical trials, adding abciximab to conventional antithrombotic therapy reduces both long-term and short-term ischemic events in patients undergoing high-risk percutaneous coronary intervention.

Tirofiban is a nonpeptide tyrosine analogue that reversibly antagonizes fibrinogen binding to the platelet GPIIb–IIIa receptor. Both in vitro and in vivo studies have demonstrated the ability of tirofiban to inhibit platelet aggregation. Tirofiban has been approved for use in patients with acute coronary syndromes.

Because of their mechanism of action as antiplatelet agents, all of the GPIIb–IIIa antagonists can cause bleeding as an adverse effect. In the opening case, Mr. Soprano developed a hematoma in his right thigh near the arterial access site at which eptifibatide was being infused. The expanding hematoma was caused by the excessive antiplatelet effect of a very high local concentration of eptifibatide at the infusion site. Importantly, the ability to reverse the effect of GPIIb–IIIa receptor antagonists differs for the different agents. Because abciximab is an irreversible inhibitor of platelet function, and all the abciximab previously infused is already bound to platelets, infusion of fresh platelets after the drug has been stopped can reverse the antiplatelet effect. In contrast, because the two small-molecule antagonists (eptifibatide and tirofiban) bind the receptor reversibly and are infused in great stoichiometric excess of receptor number, infusion of fresh platelets simply offers new sites to which the drug can bind, and it is not practical to deliver a sufficient number of platelets to overwhelm the vast excess of drug present. Therefore, one must stop the drug infusion and wait for platelet function to return to normal as the drug is cleared. In the case of Mr. Soprano, no other measure could have been taken to reverse the effect of eptifibatide at the time his hematoma was recognized.

ANTICOAGULANTS

As with antiplatelet agents, anticoagulants are used both to prevent and treat thrombotic disease. There are four classes of anticoagulants: warfarin, unfractionated and low molecular weight heparins, selective factor Xa inhibitors, and direct thrombin inhibitors. Anticoagulants target various factors in the coagulation cascade, thereby interrupting the cascade and preventing the formation of a stable fibrin meshwork (secondary hemostatic plug). In this section, the four classes of anticoagulants are discussed in order of selectivity, from the least selective agents (warfarin and unfractionated heparin)

to the most selective agents (selective factor Xa inhibitors and direct thrombin inhibitors). Recombinant activated protein C also has anticoagulant activity, although its clinical indication is severe sepsis. Because of the mechanisms of action of these drugs, bleeding is an adverse effect common to all anticoagulants.

Warfarin

In the early 1900s, farmers in Canada and the North Dakota plains adopted the practice of planting sweet clover instead of corn for fodder. In the winter months of 1921 to 1922, a fatal hemorrhagic disease was reported in cattle that had foraged on the sweet clover. In almost every case, it was found that the affected cattle had foraged on sweet clover that had been spoiled by the curing process. After an intensive investigation, scientist K. P. Link reported that the spoiled clover contained the natural anticoagulant 3,3'-methylene-bis-(4-hydroxycoumarin) or “dicumarol.” Dicumarol and **warfarin** (a potent synthetic congener) were introduced during the 1940s as rodenticides and as oral anticoagulants. Because the oral anticoagulants act by affecting vitamin K-dependent reactions, it is important to understand how vitamin K functions.

Mechanism of Action of Vitamin K

Vitamin K (“K” is derived from the German word “Koagulation”) is required for the normal hepatic synthesis of four coagulation factors (II, VII, IX, and X), protein C, and protein S. The coagulation factors, protein C, and protein S are biologically inactive as unmodified polypeptides following protein synthesis on ribosomes. These proteins gain biological activity by post-translational carboxylation of their 9 to 12 amino-terminal glutamic acid residues. The γ -carboxylated glutamate residues (but not the unmodified glutamate residues) are capable of binding Ca^{2+} ions. Ca^{2+} binding induces a conformational change in these proteins that is required for efficient binding of the proteins to phospholipid surfaces. The ability of the γ -carboxylated molecules to bind Ca^{2+} increases the enzymatic activity of coagulation factors IIa, VIIa, IXa, Xa, and protein C by approximately 1,000-fold. Thus, vitamin K-dependent carboxylation is crucial for the enzymatic activity of the four coagulation factors and protein C, and for the cofactor function of protein S.

The carboxylation reaction requires (1) a precursor form of the target protein with its 9 to 12 amino-terminal glutamic acid residues, (2) carbon dioxide, (3) molecular oxygen, and (4) *reduced* vitamin K. The carboxylation reaction is schematically presented in [Figure 22-14](#). During this reaction, vitamin K is oxidized to the inactive 2,3-epoxide. An enzyme, epoxide reductase, is then required to convert the inactive 2,3-epoxide into the active, reduced form of vitamin K. *Thus, the regeneration of reduced vitamin K is essential for the sustained synthesis of biologically functional clotting factors II, VII, IX, and X, all of which are critical components of the coagulation cascade.*

Mechanism of Action of Warfarin

Warfarin acts on the carboxylation pathway, not by inhibiting the carboxylase directly, but by blocking the epoxide

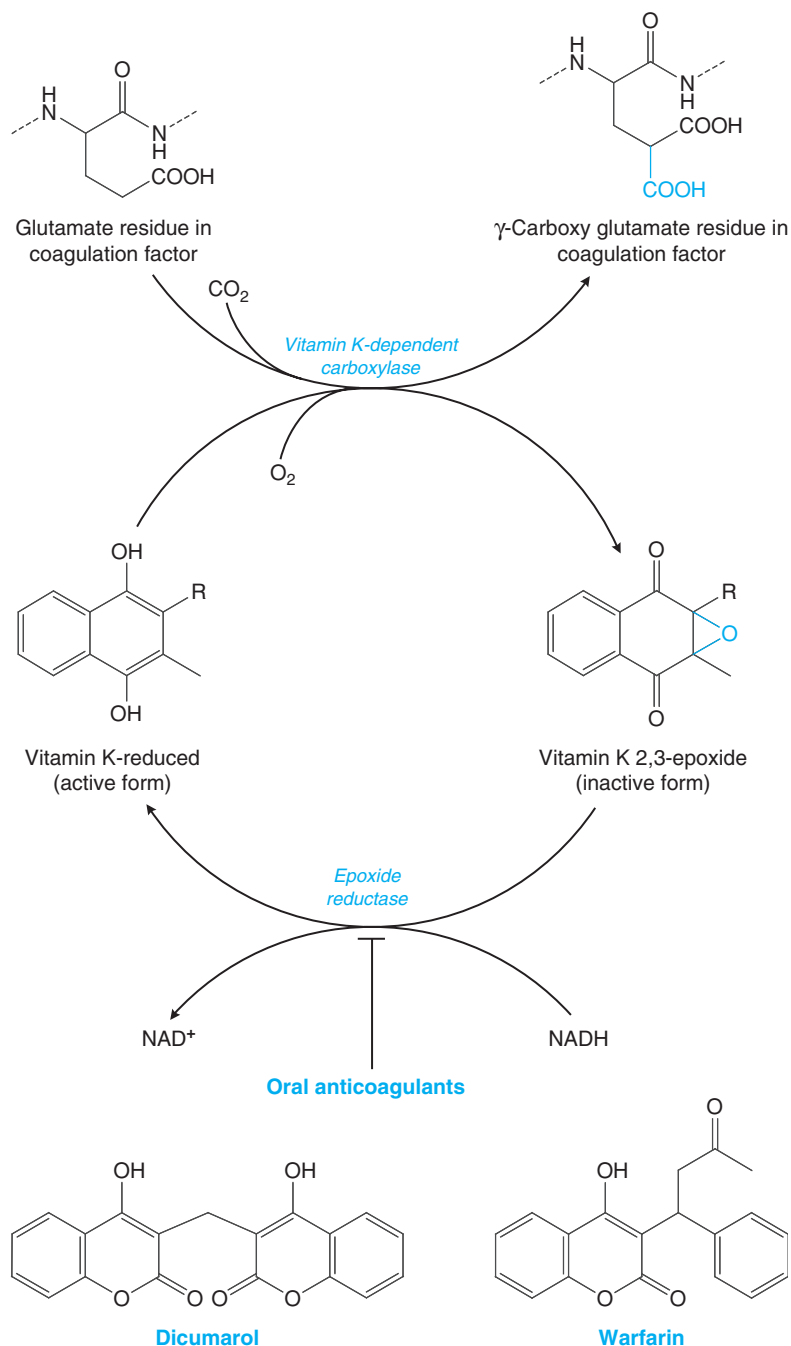


Figure 22-14. Mechanism of action of warfarin.

Vitamin K is a necessary cofactor in the post-translational carboxylation of glutamate residues on factors II, VII, IX, and X. During the carboxylation reaction, vitamin K is oxidized to the inactive 2,3-epoxide. The enzyme epoxide reductase converts the inactive vitamin K 2,3-epoxide into the active, reduced form of vitamin K. The regeneration of reduced vitamin K is essential for the sustained synthesis of biologically functional coagulation factors II, VII, IX, and X. Warfarin acts on the carboxylation pathway by inhibiting the epoxide reductase that is required for the regeneration of reduced (active) vitamin K. Dicumarol is the natural anticoagulant formed in spoiled clover. Both warfarin and dicumarol are orally bioavailable, and are often termed "oral anticoagulants."

reductase that mediates the regeneration of reduced vitamin K (Fig. 22-14). Because depletion of reduced vitamin K in the liver prevents the γ -carboxylation reaction that is required for the synthesis of biologically active coagulation factors, the onset of action of the oral anticoagulants parallels the half-life of these coagulation factors in the circulation. Of the four affected clotting factors (II, VII, IX, and X), factor VII has the shortest half-life (6 hours). Thus, the pharmacologic effect of a single dose of warfarin is not manifested for approximately 18 to 24 hours (i.e., for 3 to 4 factor VII half-lives). This *delayed action* is one pharmacologic property that distinguishes the warfarin class of anticoagulants from all the other classes of anticoagulants.

Evidence from studies of long-term rodenticide use supports the hypothesis that the epoxide reductase is the molecular target of oral anticoagulant action. The use of oral anticoagulants as rodenticides has been a widespread practice in farming communities. In some areas of the United States, heavy rodenticide use has selected for a population of wild rodents that is resistant to 4-hydroxycoumarins. In vitro studies of tissues from these rodents have demonstrated a mutation in the rodent epoxide reductase that renders the enzyme resistant to inhibition by the anticoagulant. Similarly, a small population of patients is genetically resistant to warfarin because of mutations in their epoxide reductase gene. These patients require 10

TABLE 22-2A Examples of Drugs That Diminish Warfarin's Anticoagulant Effect

DRUG OR DRUG CLASS	MECHANISM
Cholestyramine	Inhibits warfarin absorption in the GI tract
Barbiturates, carbamazepine, phenytoin, rifampin	Accelerate warfarin metabolism by inducing hepatic P450 enzymes (especially P450 2C9)
Vitamin K (reduced)	Bypasses warfarin's inhibition of epoxide reductase

GI, gastrointestinal.

to 20 times the usual dose of warfarin to achieve the desired anticoagulant effect.

Clinical Uses of Warfarin

Warfarin is often administered to complete a course of anticoagulation that has been initiated with heparin (see below) and to prevent thrombosis in predisposed patients. Orally administered warfarin is nearly 100% bioavailable, and its levels in the blood peak at 0.5 to 4 hours after administration. *In the plasma, 99% of racemic warfarin is bound to plasma protein (albumin).* Warfarin has a relatively long elimination half-life (approximately 36 hours). The drug is hydroxylated by the cytochrome P450 system in the liver to inactive metabolites that are subsequently eliminated in the urine.

Drug–drug interactions must be carefully considered in patients taking warfarin. Because warfarin is highly albumin-bound in the plasma, co-administration of warfarin with other albumin-bound drugs can increase the free (unbound) plasma concentrations of both drugs. In addition, because warfarin is metabolized by P450 enzymes in the liver, co-administration of warfarin with drugs that induce and/or compete for P450 metabolism can affect the plasma concentrations of both drugs. [Table 22-2](#) lists some of the major interactions between warfarin and other drugs.

Among the adverse effects of warfarin, bleeding is the most serious and predictable toxicity. Withdrawal of the drug may be recommended for patients who suffer from repeated bleeding episodes at otherwise therapeutic drug

concentrations. For severe hemorrhage, patients should promptly receive fresh frozen plasma, which contains biologically functional clotting factors II, VII, IX, and X. *Warfarin should never be administered to pregnant women* because it can cross the placenta and cause a hemorrhagic disorder in the fetus. In addition, newborns exposed to warfarin in utero may have serious congenital defects characterized by abnormal bone formation (certain bone matrix proteins are γ -carboxylated). Rarely, warfarin causes skin necrosis as a result of widespread thrombosis in the microvasculature. The fact that warfarin can cause thrombosis may seem paradoxical. Recall that, in addition to inhibiting the synthesis of biologically active coagulation factors II, VII, IX, and X, warfarin also prevents the synthesis of biologically active proteins C and S, which are natural anticoagulants. In patients who are genetically deficient in protein C or protein S (most commonly, patients who are heterozygous for protein C deficiency), an imbalance between warfarin's effects on coagulation factors and its effects on proteins C and S may lead to microvascular thrombosis and skin necrosis.

Because warfarin has a narrow therapeutic index and participates in numerous drug–drug interactions, the pharmacodynamic (functional) effect of chronic warfarin therapy must be monitored regularly (on the order of every 2 to 4 weeks). Monitoring is most easily performed using the **prothrombin time (PT)**, which is a simple test of the extrinsic and common pathways of coagulation. In this test, the patient's plasma is added to a crude preparation of tissue factor (called

TABLE 22-2B Examples of Drugs That Enhance Warfarin's Anticoagulant Effect

DRUG OR DRUG CLASS	MECHANISM
Chloral hydrate	Displaces warfarin from plasma albumin
Amiodarone, clopidogrel, ethanol (intoxicating dose), fluconazole, fluoxetine, metronidazole, sulfamethoxazole	Decrease warfarin metabolism by inhibiting hepatic P450 enzymes (especially P450 2C9)
Broad-spectrum antibiotics	Eliminate gut bacteria and thereby reduce availability of vitamin K in the GI tract
Anabolic steroids (testosterone)	Inhibit synthesis and increase degradation of coagulation factors

GI, gastrointestinal.

thromboplastin), and the time for formation of a fibrin clot is measured. Warfarin prolongs the PT mainly because it decreases the amount of biologically functional factor VII in the plasma. (Recall that factor VII is the vitamin K-dependent coagulation factor with the shortest half-life.) Measurement of the PT has been standardized worldwide, and is expressed as the **International Normalized Ratio (INR)** of the prothrombin time in the patient sample to that in a control sample, normalized for the international sensitivity index (ISI) of the laboratory's thromboplastin preparation compared to the World Health Organization's reference thromboplastin preparation. The formula used to calculate the INR is as follows: $INR = [PT_{\text{patient}} / PT_{\text{control}}]^{ISI}$.

Unfractionated and Low Molecular Weight Heparins

Structure of Heparin

Heparin is a sulfated mucopolysaccharide stored in the secretory granules of mast cells. It is a highly sulfated polymer of alternating uronic acid and D-glucosamine. Heparin molecules are highly negatively charged; indeed, endogenous heparin is the strongest organic acid in the human body. Commercial preparations of heparin are quite heterogeneous, with molecular weights ranging from 1 to 30 kDa. Conventionally, commercially prepared heparins have been categorized into unfractionated (standard) heparin and low molecular weight (LMW) heparin. **Unfractionated heparin**, which is often prepared from bovine lung and porcine intestinal mucosa, ranges in molecular weight from 5 to 30 kDa. **LMW heparins** are prepared from standard heparin by gel filtration chromatography; their molecular weights range from 1 to 5 kDa.

Mechanism of Action of Heparin

Heparin's mechanism of action depends on the presence of a specific plasma protease inhibitor, antithrombin III. Antithrombin III is actually a misnomer because, in addition to inactivating thrombin, antithrombin III inactivates other serine proteases including factors IXa, Xa, XIa, and XIIa. Antithrombin III can be considered as a stoichiometric "suicide trap" for these serine proteases. When one of the proteases encounters an antithrombin III molecule, the serine residue at the active site of the protease attacks a specific Arg-Ser peptide bond in the reactive site of the antithrombin. The result of this nucleophilic attack is the formation of a covalent ester bond between the serine residue on the protease and the arginine residue on the antithrombin III. This results in a stable 1:1 complex between the protease and antithrombin molecules, which prevents the protease from further participation in the coagulation cascade.

In the absence of heparin, the binding reaction between the proteases and antithrombin III proceeds slowly. Heparin, acting as a cofactor, accelerates the reaction by 1,000-fold. Heparin has two important physiologic functions: (1) it serves as a catalytic surface to which both antithrombin III and the serine proteases bind; and (2) it induces a conformational change in antithrombin III that makes the reactive site of this molecule more accessible to the attacking protease. The first step of the reaction involves the binding of the negatively charged heparin to a lysine-rich region (a region

of positive charge) on antithrombin III. Thus, the interaction between heparin and antithrombin III is partly electrostatic. During the conjugation reaction between the protease and the antithrombin, heparin may be released from antithrombin III and become available to catalyze additional protease-antithrombin III interactions (i.e., heparin is not consumed by the conjugation reaction [Fig. 22-9]). In practice, however, heparin's high negative charge often causes this "sticky" molecule to remain electrostatically bound to protease, antithrombin or another nearby molecule in the vicinity of a thrombus.

Interestingly, heparins of different molecular weights have divergent anticoagulant activities. These divergent activities derive from the differential requirements for heparin binding exhibited by the inactivation of thrombin and factor Xa by antithrombin III (Fig. 22-15). To catalyze most efficiently the inactivation of thrombin by antithrombin III, a single molecule of heparin must bind simultaneously to both thrombin and antithrombin. This "scaffolding" function is required in addition to the heparin-induced conformational change in antithrombin III that renders the antithrombin susceptible to conjugation with thrombin. In contrast, to catalyze the inactivation of factor Xa by antithrombin III, the heparin molecule must bind only to the antithrombin, because the conformational change in antithrombin III induced by heparin binding is sufficient by itself to render the antithrombin susceptible to conjugation with factor Xa. Thus, *LMW heparins*, which have an average molecular weight of 3 to 4 kDa and contain fewer than 18 monosaccharide units, *efficiently catalyze the inactivation of factor Xa by antithrombin III but less efficiently catalyze the inactivation of thrombin by antithrombin III*. In contrast, *unfractionated heparin*, which has an average molecular weight of 20 kDa and contains more than 18 monosaccharide units, is of sufficient length to bind simultaneously to thrombin and antithrombin III, and therefore *efficiently catalyzes the inactivation of both thrombin and factor Xa by antithrombin III*. Quantitatively, LMW heparin has a threefold higher ratio of anti-Xa to anti-thrombin (anti-IIa) activity than does unfractionated heparin. LMW heparin is therefore a more selective therapeutic agent than unfractionated heparin. Both LMW heparin and unfractionated heparin use a pentasaccharide structure of high negative charge to bind antithrombin III and to induce the conformational change in antithrombin required for the conjugation reactions. This pentasaccharide has recently been approved for use as a highly selective inhibitor of factor Xa (**fondaparinux**; see below).

Clinical Uses of Heparins

Heparins are used for both prophylaxis and treatment of thromboembolic diseases. Both unfractionated and LMW heparins are used to prevent propagation of established thromboembolic disease such as deep vein thrombosis and pulmonary embolism. For prophylaxis against thrombosis, heparins are used at much lower doses than those indicated for the treatment of established thromboembolic disease. Because the enzymatic coagulation cascade functions as an amplification system (e.g., 1 unit of factor Xa generates 40 units of thrombin), the administration of relatively small amounts of circulating heparin at the first generation of factor Xa is highly effective. Heparins are highly negatively charged, and

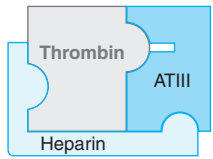
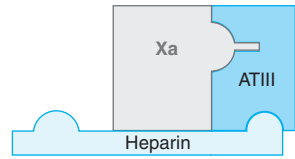
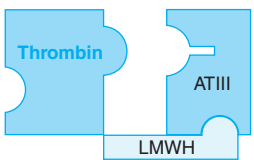
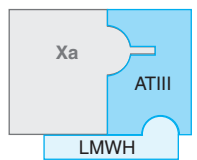
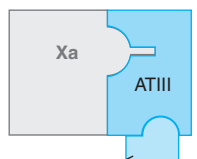
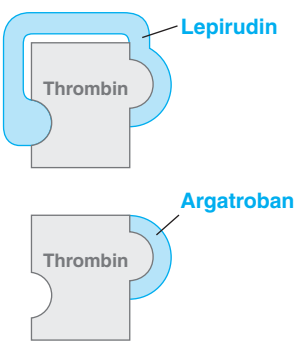
Anticoagulant class	Effect on Thrombin	Effect on Factor Xa
Unfractionated heparin (about 45 saccharide units, MW ~ 13,500)	 <p>Binds to antithrombin III (ATIII) and thrombin (inactivates thrombin)</p>	 <p>Binds to antithrombin III (ATIII) via pentasaccharide (sufficient to inactivate Xa)</p>
Low molecular weight (LMW) heparins (about 15 saccharide units, MW ~ 4,500)	 <p>Binds to antithrombin III (ATIII) but not to thrombin (poorly inactivates thrombin)</p>	 <p>Binds to antithrombin III (ATIII) via pentasaccharide (sufficient to inactivate Xa)</p>
Selective factor Xa inhibitors	No effect on thrombin	 <p>Fondaparinux Binds to antithrombin III (ATIII) via pentasaccharide (sufficient to inactivate Xa)</p>
Direct thrombin inhibitors	 <p>Lepirudin Argatroban</p> <p>Selectively inactivate thrombin</p>	No effect on Xa

Figure 22-15. Differential effects of unfractionated heparin and low molecular weight heparin on coagulation factor inactivation. *Effect on thrombin:* To catalyze the inactivation of thrombin, heparin must bind both to antithrombin III via a high-affinity pentasaccharide unit (5) and to thrombin via an additional 13 saccharide unit (here labeled “Heparin”). Low molecular weight heparin (LMWH) does not contain a sufficient number of saccharide units to bind thrombin, and therefore is a poor catalyst for thrombin inactivation. *Effect on factor Xa:* Inactivation of factor Xa requires only the binding of antithrombin III to the high-affinity pentasaccharide unit (5). Both unfractionated heparin and low molecular weight heparin (and fondaparinux; *not shown*) are therefore able to catalyze the inactivation of factor Xa.

neither unfractionated heparin nor LMW heparin can cross the epithelial cell layer of the gastrointestinal tract. Hence, heparin must be administered parenterally, usually via intravenous or subcutaneous routes.

Unfractionated heparin is often used in combination with antiplatelet agents in the treatment of acute coronary syndromes. For example, Mr. Soprano was treated with the antiplatelet agents aspirin and eptifibatid and with unfractionated heparin in an attempt to limit the extent of his myocardial infarction. Monitoring of unfractionated heparin therapy is important for maintaining the anticoagulant effect within the therapeutic range, because excessive heparin administration significantly increases the risk of bleeding. Monitoring is usually performed using the **activated partial thromboplastin time (aPTT)** assay. The aPTT is a simple test of the intrinsic and common pathways of coagulation. The patient's plasma is added to an excess of phospholipid, and fibrin forms at a normal rate only if the factors in the intrinsic and common pathways are present at normal levels. Increasing amounts of unfractionated heparin in the plasma prolong the time required for the formation of a fibrin clot.

As is true of the other anticoagulants, the major adverse effect of heparin is bleeding. Thus, it is critical to maintain the anticoagulant effect of unfractionated heparin within the therapeutic range in order to prevent the rare, devastating adverse effect of intracranial hemorrhage. In addition, a small fraction of patients taking heparin develop **heparin-induced thrombocytopenia (HIT)**. In this syndrome, patients develop antibodies to a hapten created when heparin molecules bind to the platelet surface. In HIT type 1, the antibody-coated platelets are targeted for removal from the circulation, and the platelet count decreases by 50% to 75% approximately 5 days into the course of heparin therapy. The thrombocytopenia in HIT type 1 is transient and rapidly reversible upon heparin withdrawal. In HIT type 2, however, the heparin-induced antibodies not only target the platelets for destruction but also act as agonists to *activate* the platelets, leading to platelet aggregation, endothelial injury, and potentially fatal thrombosis. There is a higher incidence of HIT in patients receiving unfractionated heparin than in those receiving LMW heparin.

The LMW heparins **enoxaparin**, **dalteparin** and **tinzaparin** are each fractionated heparins of low molecular weight. As discussed above, these agents are relatively selective for anti-Xa compared to anti-IIa (anti-thrombin) activity. All LMW heparins are approved for use in the prevention and treatment of deep vein thrombosis. Additionally, enoxaparin and dalteparin have been studied in the treatment of acute myocardial infarction and as adjuncts to percutaneous coronary intervention. LMW heparins have a higher therapeutic index than unfractionated heparin, especially when used for prophylaxis. For this reason, it is generally not necessary to monitor blood activity levels of LMW heparins. Accurate measurement of the anticoagulant effect of LMW heparins requires a specialized assay for anti-factor Xa activity. Because LMW heparins are excreted via the kidneys, care should be taken to avoid excessive anticoagulation in patients with renal insufficiency.

Selective Factor Xa Inhibitors

Fondaparinux is a synthetic pentasaccharide molecule that contains the sequence of five essential carbohydrates neces-

sary for binding to antithrombin III and inducing the conformational change in antithrombin required for conjugation to factor Xa (see above). This agent is therefore a specific inhibitor of Xa, with negligible anti-IIa (anti-thrombin) activity. Fondaparinux is approved for prevention and treatment of deep vein thrombosis, and is available as a once-daily subcutaneous injection. It is excreted via the kidneys and should not be administered to patients with renal insufficiency.

Direct Thrombin Inhibitors

As discussed above, thrombin plays a number of critical roles in the hemostatic process (Fig. 22-8). Among other effects, this clotting factor (1) proteolytically converts fibrinogen to fibrin, (2) activates factor XIII, which crosslinks fibrin polymers to form a stable clot, (3) activates platelets, and (4) induces endothelial release of PGI₂, t-PA, and PAI-1. Thus, direct thrombin inhibitors would be expected to have profound effects on coagulation. The currently approved direct thrombin inhibitors include lepirudin, desirudin, bivalirudin, and argatroban. These agents are specific inhibitors of thrombin, with negligible anti-factor Xa activity.

Lepirudin, a recombinant 65-amino acid polypeptide derived from the medicinal leech protein **hirudin**, is the prototypical direct thrombin inhibitor. For years, surgeons have used medicinal leeches to prevent thrombosis in the fine vessels of reattached digits. Lepirudin binds with high affinity to two sites on the thrombin molecule — the enzymatic active site and the “exosite,” a region of the thrombin protein that orients substrate proteins. Lepirudin binding to thrombin prevents the thrombin-mediated activation of fibrinogen and factor XIII. Lepirudin is a highly effective anticoagulant because it can inhibit both free and *fibrin-bound* thrombin in developing clots, and because lepirudin binding to thrombin is essentially irreversible. It is approved for use in the treatment of heparin-induced thrombocytopenia. Lepirudin has a short half-life, is available parenterally, and is renally excreted. It can be administered with relative safety to patients with hepatic insufficiency. As with all direct thrombin inhibitors, bleeding is the major side effect of lepirudin, and clotting times must be monitored closely. A small percentage of patients may develop anti-hirudin antibodies, limiting the long-term effectiveness of this agent as an anticoagulant. Another recombinant formulation of hirudin, **desirudin**, has been approved for prophylaxis against deep vein thrombosis in patients undergoing hip replacement.

Bivalirudin is a synthetic 20-amino acid peptide that, like lepirudin and desirudin, binds to both the active site and exosite of thrombin and thereby inhibits thrombin activity. Thrombin slowly cleaves an arginine–proline bond in bivalirudin, leading to reactivation of the thrombin. Bivalirudin is approved for anticoagulation in patients undergoing coronary angiography and angioplasty, and may have reduced rates of bleeding relative to heparin for this indication. The drug is excreted renally and has a short half-life (25 minutes).

Argatroban is a small-molecule inhibitor of thrombin that is approved for the treatment of patients with heparin-induced thrombocytopenia. Unlike other direct thrombin inhibitors, argatroban binds only to the active site of thrombin

(i.e., it does not interact with the exosite). Also unlike other direct thrombin inhibitors, argatroban is excreted by biliary secretion, and can therefore be administered with relative safety to patients with renal insufficiency.

Recombinant Activated Protein C (r-APC)

As described above, endogenously activated protein C (APC) exerts an anticoagulant effect by proteolytically cleaving factors Va and VIIIa. APC also reduces the amount of circulating plasminogen activator inhibitor 1, thereby enhancing fibrinolysis. Finally, APC reduces inflammation by inhibiting the release of tumor necrosis factor α (TNF- α) by monocytes. Because enhanced coagulability and inflammation are both hallmarks of septic shock, APC has been tested both in animal models of this disorder and in humans. **Recombinant activated protein C (r-APC)** has been found to significantly reduce mortality in patients at high risk of death from septic shock, and the U. S. Food and Drug Administration (FDA) has approved r-APC for the treatment of patients with severe sepsis who demonstrate evidence of acute organ dysfunction, shock, oliguria, acidosis, and hypoxemia. r-APC is not indicated for the treatment of patients with severe sepsis and a lower risk of death, however. As is the case with other anticoagulants, r-APC increases the risk of bleeding. This agent is therefore contraindicated in patients who have recently undergone a surgical procedure and in those with chronic liver failure, kidney failure, or thrombocytopenia.

THROMBOLYTIC AGENTS

Although warfarin, unfractionated and low molecular weight heparins, selective factor Xa inhibitors, and direct thrombin inhibitors are effective in preventing the formation and propagation of thrombi, these agents are generally ineffective against pre-existing clots. Thrombolytic agents are used to lyse already-formed clots, and thereby to restore the patency of an obstructed vessel before distal tissue necrosis occurs. Thrombolytic agents act by converting the inactive zymogen plasminogen to the active protease plasmin (Fig. 22-10). As noted above, plasmin is a relatively nonspecific protease that digests fibrin to fibrin degradation products. Unfortunately, thrombolytic therapy has the potential to dissolve not only pathologic thrombi, but also physiologically appropriate fibrin clots that have formed in response to vascular injury. Thus, the use of thrombolytic agents can lead to hemorrhage of varying severity.

Streptokinase

Streptokinase is a protein produced by β -hemolytic streptococci as a component of that organism's tissue-destroying machinery. The pharmacologic action of streptokinase involves two steps — complexation and cleavage. In the complexation reaction, streptokinase forms a stable, noncovalent 1:1 complex with plasminogen. The complexation reaction produces a conformational change in plasminogen that exposes this protein's proteolytically active site. Streptokinase-complexed plasminogen, with its active site exposed and available, can then proteolytically cleave *other* plasminogen

molecules to plasmin. In fact, the thermodynamically stable streptokinase:plasminogen complex is the most catalytically efficient plasminogen activator in vitro.

Although streptokinase exerts its most dramatic and potentially beneficial effects in fresh thrombi, its use has been limited by two factors. First, streptokinase is a foreign protein that is capable of eliciting antigenic responses in humans upon repeated administration. Previous administration of streptokinase is a contraindication to its use, because of the risk of anaphylaxis. Second, the thrombolytic actions of streptokinase are relatively nonspecific and can result in systemic fibrinolysis. Currently, streptokinase is approved for treatment of ST elevation myocardial infarction and for treatment of life-threatening pulmonary embolism.

Recombinant Tissue Plasminogen Activator (t-PA)

An ideal thrombolytic agent would be nonantigenic and would cause local fibrinolysis only at the site of a pathologic thrombus. Tissue plasminogen activator (t-PA) approximates these goals. t-PA is a serine protease produced by human endothelial cells; therefore, t-PA is not antigenic. t-PA binds to newly formed (fresh) thrombi with high affinity, causing fibrinolysis at the site of a thrombus. Once bound to the fresh thrombus, t-PA undergoes a conformational change that renders it a potent activator of plasminogen. In contrast, t-PA is a poor activator of plasminogen in the absence of fibrin-binding.

Recombinant DNA technology has allowed the production of **recombinant t-PA**, generically referred to as **alteplase**. Recombinant t-PA is effective at recanalizing occluded coronary arteries, limiting cardiac dysfunction, and reducing mortality following an ST elevation myocardial infarction. At pharmacologic doses, however, recombinant t-PA can generate a systemic lytic state and (as with other thrombolytic agents) cause unwanted bleeding, including cerebral hemorrhage. Thus, its use is contraindicated in patients who have had a recent hemorrhagic stroke. Like streptokinase, t-PA is approved for use in the treatment of patients with ST elevation myocardial infarction or life-threatening pulmonary embolism. It is also approved for the treatment of acute ischemic stroke.

Tenecteplase

Tenecteplase is a genetically engineered variant of t-PA. The molecular modifications in tenecteplase increase its fibrin specificity relative to t-PA and make tenecteplase more resistant to plasminogen activator inhibitor 1. Large trials have shown that tenecteplase is identical in efficacy to t-PA, with similar (and possibly decreased) risk of bleeding. Additionally, tenecteplase has a longer half-life than t-PA. This pharmacokinetic property allows tenecteplase to be administered as a single weight-based bolus, thus simplifying administration.

Retepase

Similar to tenecteplase, **reteplase** is a genetically engineered variant of t-PA with longer half-life and increased specificity

for fibrin. Its efficacy and adverse effect profile are similar to those of streptokinase and t-PA. Because of its longer half-life, reteplase can be administered as a “double bolus” (two boluses, 30 minutes apart).

INHIBITORS OF ANTICOAGULATION AND FIBRINOLYSIS

Protamine

Protamine, a low molecular weight polycationic protein, is a chemical antagonist of heparin. This agent rapidly forms a stable complex with the negatively charged heparin molecule through multiple electrostatic interactions. Protamine is administered intravenously to reverse the effects of heparin in situations of life-threatening hemorrhage or great heparin excess (for example, at the conclusion of coronary artery bypass graft surgery). Protamine is most active against the large heparin molecules in unfractionated heparin and it can partially reverse the anticoagulant effects of low molecular weight heparins, but it is inactive against fondaparinux.

Serine-Protease Inhibitors

Aprotinin, a naturally occurring polypeptide, is an inhibitor of the serine proteases plasmin, t-PA, and thrombin. By inhibiting fibrinolysis, aprotinin promotes clot stabilization. Inhibition of thrombin may also promote platelet activity by preventing platelet hyperstimulation. At higher doses, aprotinin may also inhibit kallikrein and thereby (paradoxically) inhibit the coagulation cascade. Clinical trials have demonstrated decreased perioperative bleeding and erythrocyte transfusion requirement in patients treated with aprotinin during cardiac surgery. However, these positive findings have been tempered by recent evidence suggesting that, compared to other antifibrinolytic agents, aprotinin may increase the risk of postoperative acute renal failure.

Lysine Analogues

Aminocaproic acid and **tranexamic acid** are analogues of lysine that bind to and inhibit plasminogen and plasmin. Like aprotinin, these agents are used to reduce perioperative bleeding during coronary artery bypass grafting. Unlike aprotinin, these agents may not increase the risk of postoperative acute renal failure.

Conclusion and Future Directions

Hemostasis is a highly regulated process that maintains the fluidity of blood in normal vessels and initiates rapid formation of a stable fibrin-based clot in response to vascular injury. Pathologic thrombosis results from endothelial injury, abnormal blood flow, and hypercoagulability. Antiplatelet agents, anticoagulants, and thrombolytic agents target different stages of thrombosis and thrombolysis. Antiplatelet agents interfere with platelet adhesion, the platelet release reaction, and platelet aggregation; these agents can provide powerful prophylaxis against thrombosis in susceptible indi-

viduals. Anticoagulants primarily target plasma coagulation factors and disrupt the coagulation cascade by inhibiting crucial intermediates. After a fibrin clot has been established, thrombolytic agents mediate dissolution of the clot by promoting the conversion of plasminogen to plasmin. These classes of pharmacologic agents can be administered either individually or in combination, to prevent or disrupt thrombosis and to restore the patency of blood vessels occluded by thrombus.

Future development of new antiplatelet, anticoagulant, and thrombolytic agents will be forced to contend with two major constraints. First, for many clinical indications in this field, highly effective, orally bioavailable, and inexpensive therapeutic agents are already available: these include the antiplatelet drug aspirin and the anticoagulant warfarin. Second, virtually every antithrombotic and thrombolytic agent is associated with the mechanism-based toxicity of bleeding, and this side effect is likely to plague new agents under development. Nonetheless, opportunities remain for the development of safer and more effective therapies. It is likely that pharmacogenomic techniques (see Chapter 52, Pharmacogenomics) will be capable of identifying individuals in the population who carry an elevated genetic risk of thrombosis, and such individuals may benefit from long-term antithrombotic treatment. Combinations of antiplatelet agents, low molecular weight heparins, orally bioavailable direct thrombin inhibitors (such as the snake-venom-derived prodrug **ximelagatran**, which is not approved for use in the U.S.), and new agents that target currently unexploited components of hemostasis (such as inhibitors of the factor VIIa/tissue factor pathway) could all be useful in these settings. At the other end of the spectrum, there remains a great need for new agents that can achieve rapid, noninvasive, convenient, and selective lysis of acute thromboses associated with life-threatening emergencies such as ST elevation myocardial infarction and stroke. Carefully designed clinical trials will be critical to optimize the indications, dose, and duration of treatment for such drugs and drug combinations.

Suggested Readings

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- Brass LF. The molecular basis for platelet activation. In: Hoffman R, Benz EJ, Shatill SJ, et al, eds. *Hematology: basic principles and practice*. 4th ed. Philadelphia: Churchill Livingstone; 2004. (Detailed and mechanistic description of platelet activation.)
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Drug Summary Table Chapter 22 Pharmacology of Hemostasis and Thrombosis

Drug	Clinical Applications	Serious and Common Adverse Effects	Contraindications	Therapeutic Considerations
ANTIPLATELET AGENTS				
Cyclooxygenase Inhibitors				
<i>Mechanism—Inhibit platelet cyclooxygenase, thereby blocking thromboxane A2 generation and inhibiting platelet release reaction and platelet aggregation</i>				
Aspirin	<p>Prophylaxis against transient ischemic attack, myocardial infarction, and thromboembolic disorders</p> <p>Treatment of acute coronary syndromes</p> <p>Prevention of reocclusion in coronary revascularization procedures and stent implantation</p> <p>Arthritis, juvenile arthritis, rheumatic fever</p> <p>Mild pain or fever</p>	<p>GI bleeding, acute renal insufficiency, thrombocytopenia, hepatitis, angioedema, asthma, Reye's syndrome</p> <p>Tinnitus, dyspepsia, occult bleeding, prolonged bleeding time, rash</p>	<p>NSAID-induced sensitivity reactions</p> <p>Children with chickenpox or flu-like syndromes</p> <p>G6PD deficiency</p> <p>Bleeding disorders such as hemophilia, von Willebrand's disease, or immune thrombocytopenia</p>	<p>Inhibits COX-1 and COX-2 nonselectively. Use cautiously in patients with GI lesions, impaired renal function, hypothermia, vitamin K deficiency, thrombotic thrombocytopenic purpura, or hepatic impairment</p> <p>Co-administration with aminoglycosides, bumetanide, capreomycin, cisplatin, erythromycin, ethacrynic acid, furosemide, or vancomycin may potentiate ototoxic effects</p> <p>Co-administration with ammonium chloride or other urine acidifiers may lead to aspirin toxicity</p> <p>Aspirin antagonizes uricosuric effects of phenylbutazone, probenecid, and sulfinpyrazone; avoid co-administration with these agents</p>
Phosphodiesterase Inhibitors				
<i>Mechanism—Inhibit platelet cAMP degradation and thereby decrease platelet aggregability</i>				
Dipyridamole	<p>Prophylaxis against thromboembolic disorders;</p> <p>Alternative to exercise in thallium myocardial perfusion imaging</p>	<p>Exacerbation of angina (IV route), rare myocardial infarction, rare ventricular arrhythmia, rare bronchospasm</p> <p>Abnormal ECG, hypotension (IV route), abdominal discomfort (oral route), dizziness, headache</p>	<p>Hypersensitivity to dipyridamole</p>	<p>Weak antiplatelet effect</p> <p>Usually administered in combination with warfarin or aspirin</p> <p>Has vasodilatory properties; may paradoxically induce angina by causing the coronary steal phenomenon</p>
ADP Receptor Pathway Inhibitors				
<i>Mechanism—Covalently modify platelet ADP receptor, thereby preventing receptor signaling and irreversibly inhibiting ADP-dependent platelet activation pathway</i>				
Ticlopidine	<p>Secondary prevention of thrombotic strokes in patients intolerant of aspirin</p> <p>Prevention of stent thrombosis (in combination with aspirin)</p>	<p>Aplastic anemia, neutropenia, thrombotic thrombocytopenic purpura</p> <p>Pruritus, rash, dyspepsia, abnormal liver function tests, dizziness</p>	<p>Active bleeding disorder</p> <p>Neutropenia, thrombocytopenia</p> <p>Severe liver dysfunction</p>	<p>Use is limited by associated myelotoxicity</p> <p>Requires a loading dose to achieve immediate antiplatelet effect</p>
Clopidogrel	<p>Secondary prevention of atherosclerotic events in patients with recent myocardial infarction, stroke, or peripheral vascular disease</p> <p>Acute coronary syndromes</p> <p>Prevention of stent thrombosis (in combination with aspirin)</p>	<p>Atrial fibrillation, heart failure, erythema multiforme, GI hemorrhage (in combination with aspirin), very rare anemia or neutropenia, rare intracranial hemorrhage, abnormal renal function</p> <p>Chest pain, edema, hypertension, purpura, rare abnormal liver function tests, GI discomfort, arthralgia, dizziness</p>	<p>Active bleeding disorder</p>	<p>More favorable adverse effect profile than ticlopidine; significantly less myelotoxicity than ticlopidine</p> <p>Requires a loading dose to achieve immediate antiplatelet effect</p>

(Continued)

Drug Summary Table Chapter 22 Pharmacology of Hemostasis and Thrombosis (Continued)

Drug	Clinical Applications	Serious and Common Adverse Effects	Contraindications	Therapeutic Considerations
<p>GPIIb–IIIa Antagonists <i>Mechanism—Bind to platelet receptor GPIIb–IIIa and thereby prevent binding of fibrinogen and other adhesive ligands</i></p>				
<p>Eptifibatid</p>	<p>Acute coronary syndromes Percutaneous coronary intervention</p>	<p>Major bleedings, intracerebral hemorrhage, thrombocytopenia Hypotension, bleeding</p>	<p>History of bleeding diathesis or recent abnormal bleeding Concomitant administration of a second glycoprotein IIb–IIIa antagonist Recent major surgery Recent stroke or history of hemorrhagic stroke Intracranial hemorrhage, mass, or arteriovenous malformation Severe uncontrolled hypertension</p>	<p>Avoid co-administration with a second GPIIb–IIIa antagonist Minimize use of arterial and venous punctures, urinary catheters, and nasotracheal and nasogastric tubes Eptifibatid is a synthetic peptide delivered via parenteral administration</p>
<p>Abciximab</p>	<p>Adjunct to percutaneous coronary intervention or atherectomy to prevent acute cardiac ischemic complications Unstable angina not responding to conventional therapy in patients scheduled for percutaneous coronary intervention</p>	<p>Same as eptifibatid</p>	<p>Same as eptifibatid</p>	<p>Same therapeutic considerations as eptifibatid, except abciximab is a chimeric mouse–human monoclonal antibody Adding abciximab to conventional antithrombotic therapy reduces both long-term and short-term ischemic events in patients undergoing high-risk coronary angioplasty</p>
<p>Tirofiban</p>	<p>Acute coronary syndromes in patient undergoing angioplasty or atherectomy or managed medically</p>	<p>Same as eptifibatid; Additionally, coronary artery dissection is rarely observed</p>	<p>Same as eptifibatid</p>	<p>Same therapeutic considerations as eptifibatid, except tirofiban is a nonpeptide tyrosine analogue</p>
<p>ANTICOAGULANTS</p>				
<p>Warfarin</p>	<p><i>Mechanism—Inhibit hepatic epoxide reductase that catalyzes the regeneration of reduced vitamin K, which is required for synthesis of biologically active coagulation factors II, VII, IX, and X and anticoagulant proteins C and S</i></p>			
<p>Warfarin</p>	<p>Prophylaxis and treatment of pulmonary embolism Prophylaxis and treatment of deep vein thrombosis systemic embolism after myocardial infarction Prophylaxis and treatment of systemic embolism associated with atrial fibrillation, rheumatic heart disease with heart valve damage, or prosthetic mechanical heart valve</p>	<p>Pregnancy Hemorrhagic tendency or blood dyscrasia Bleeding tendency associated with active ulceration or bleeding due to mucosal lesions, cerebrovascular hemorrhage, cerebral or aortic aneurysm, pericarditis and pericardial effusion, bacterial endocarditis Recent eye, brain, or spinal surgery Severe uncontrolled hypertension</p>	<p>Monitoring is required by using the prothrombin time (PT), expressed as the international normalized ratio (INR) Drug–drug interactions must be carefully considered with warfarin (refer to Table 22-2 for examples of important interactions); Co-administration of warfarin with other albumin-bound drugs can increase the free (unbound) plasma concentrations of both drugs; Co-administration of drugs that induce and/or compete for P450 metabolism can affect the plasma concentrations of both drugs</p>	

Threatened abortion, eclampsia, preeclampsia
 Regional or lumbar block anesthesia
 History of warfarin-induced necrosis
 Unsupervised patients with psychosis, senility, alcoholism, or lack of cooperation, and especially those with risk of falling

Warfarin should never be given to pregnant women because it can cause a hemorrhagic disorder and/or congenital defects in the fetus
 Warfarin can cause skin necrosis as a result of widespread thrombosis in the microvasculature
 For severe hemorrhage due to warfarin, patients should promptly receive fresh frozen plasma

Unfractionated Heparin and Low Molecular Weight Heparins

Mechanism—Unfractionated heparin: combines with antithrombin III and inhibits secondary hemostasis via nonselective inactivation of thrombin (factor IIa), factor Xa, factor IXa, factor XIa, and factor XIIa. LMW heparins: combine with antithrombin III and inhibit secondary hemostasis via relatively (3-fold) selective inactivation of factor Xa.

Unfractionated heparin

Prevention and treatment of:
 —Pulmonary embolism
 —Deep vein thrombosis
 —Cerebral thrombosis
 —Left ventricular thrombus
 Prevention of systemic embolism associated with myocardial infarction
 Unstable angina
 Open-heart surgery
 Disseminated intravascular coagulation
 Maintain patency of IV catheters

Hemorrhage, heparin-induced thrombocytopenia, hypersensitivity reactions including anaphylactoid reactions
 Overly prolonged clotting time, mucosal ulceration, hematoma

Heparin-induced thrombocytopenia
 Active major bleeding
 Bleeding tendencies such as hemophilia, thrombocytopenia, or hepatic disease with hypoprothrombinemia
 Suspected intracranial hemorrhage
 Open ulcerative wounds, extensive denudation of skin
 Conditions that cause increased capillary permeability
 Bacterial endocarditis
 Severe hypertension

There is a higher incidence of heparin-induced thrombocytopenia in patients receiving unfractionated heparin than in those receiving LMW heparin
 Antihistamines, cardiac glycosides, nicotine, and tetracyclines may partially counteract anticoagulant effect
 Cephalosporins, penicillins, oral anticoagulants, and platelet inhibitors may increase anticoagulant effect
 Discourage concomitant use of herbs such as dong quai, garlic, ginger, ginkgo, motherwort, and red clover due to increased risk of bleeding

LMW heparins: Enoxaparin Dalteparin Tinzaparin

Prevention and treatment of deep vein thrombosis (all LMW heparins)
 Treatment of acute coronary syndromes and adjunct to percutaneous coronary intervention (enoxaparin and dalteparin)

Hemorrhage, thrombocytopenia, abnormal liver function tests, anaphylactoid reaction, spinal hematoma
 Edema, diarrhea, nausea, hematoma, normocytic hypochromic anemia, confusion, pain, dyspnea, fever, local irritation

Active major bleeding
 Heparin-induced thrombocytopenia
 Hypersensitivity to heparin or pork products
 Renal insufficiency (relative contraindication)

Administered as weight-based subcutaneous injection
 Avoid excessive anticoagulation in patients with renal insufficiency

Selective Factor Xa Inhibitors

Mechanism—Combine with antithrombin III and inhibits secondary hemostasis via highly selective inactivation of factor Xa

Fondaparinux

Prophylaxis and treatment of deep vein thrombosis
 Prophylaxis and treatment of pulmonary embolism

Hemorrhage, thrombocytopenia, abnormal liver function tests, anaphylactoid reaction, spinal hematoma
 Edema, diarrhea, nausea, hematoma, normocytic hypochromic anemia, confusion, pain, dyspnea, fever, local irritation

Active major bleeding
 Severe renal impairment
 Bacterial endocarditis

Fondaparinux is a pentasaccharide composed of the essential five carbohydrates necessary for binding antithrombin III: It is a specific indirect inhibitor of factor Xa, with negligible anti-thrombin (anti-IIa) activity
 Avoid excessive anticoagulation in patients with renal insufficiency
 Fondaparinux use has not been associated with heparin-induced thrombocytopenia

(Continued)

Drug Summary Table Chapter 22 Pharmacology of Hemostasis and Thrombosis (Continued)

Drug		Clinical Applications	Serious and Common Adverse Effects	Contraindications	Therapeutic Considerations
Direct Thrombin Inhibitors					
<i>Mechanism—Bind directly to thrombin and thereby inhibit secondary hemostasis</i>					
Hirudin-related agents: Lepirudin Desirudin Bivalirudin	Heparin-induced thrombocytopenia (lepirudin) Prophylaxis against deep vein thrombosis (desirudin) Anticoagulation in patients undergoing coronary angiography and angioplasty (bivalirudin)	Heart failure, gastrointestinal hemorrhage, bleeding, abnormal liver function tests, anaphylaxis, hypertension, hypotension, cerebral ischemia, intracranial hemorrhage, peripheral nerve paralysis, facial nerve paralysis, hematuria, renal failure, extrinsic allergic respiratory disease, pneumonia, sepsis Cutaneous hypersensitivity, anemia, fever	Active major bleeding Pregnancy Severe uncontrolled hypertension Severe renal impairment	Recombinant polypeptides based on the medicinal leech protein hirudin; bind to both active site and exosite of thrombin Lepirudin inhibits both free and fibrin-bound thrombin After bivalirudin binds to thrombin, the thrombin slowly cleaves an arginine–proline bond in bivalirudin, leading to reactivation of thrombin Dose adjustment is required in patients with renal insufficiency because these agents are excreted via the kidneys	
Argatroban	Coronary artery thrombosis Prophylaxis in percutaneous coronary intervention Heparin-induced thrombocytopenia	Cardiac arrest, cerebrovascular disorder, ventricular tachycardia, sepsis, hypotension	Active major bleeding Severe liver impairment	Binds to active site but not exosite of thrombin Dose adjustment is required in patients with liver disease because argatroban is excreted in the bile	
Recombinant Activated Protein C (r-APC)					
<i>Mechanism—Proteolytically inactivates factors Va and VIIIa; may also exert anti-inflammatory effect by inhibiting tumor necrosis factor production and blocking leukocyte adhesion to selectins</i>					
Recombinant activated protein C (r-APC)	Severe sepsis with organ dysfunction and high risk of death	Hemorrhage	Active internal bleeding Intracranial mass Hemorrhagic stroke within 3 months Recent intracranial or intraspinal surgery or severe head trauma within 2 months Presence of an epidural catheter Major trauma with an increased risk of life-threatening bleeding	Prolongs activated partial thromboplastin time (aPTT) but has little effect on prothrombin time (PT)	
THROMBOLYTIC AGENTS					
<i>Mechanism—Proteolytically activate plasminogen to form plasmin, which digests fibrin to fibrin degradation products</i>					
Streptokinase	ST elevation myocardial infarction Arterial thrombosis Deep vein thrombosis Pulmonary embolism Intra-arterial or intravenous catheter occlusion	Cardiac arrhythmia, cholesterol embolus syndrome, major bleeding, anaphylactoid reaction, polyneuropathy, non-cardiogenic pulmonary edema, hypotension Fever, shivering	Active internal bleeding or known bleeding diathesis Intracranial or intraspinal surgery or trauma within 2 months Cerebrovascular accident within 2 months Intracranial mass Severe uncontrolled hypertension	Streptokinase is a foreign bacterial protein that can elicit antigenic responses in humans upon repeated administration; prior administration of streptokinase is a contraindication to use due to the risk of anaphylaxis Thrombolytic actions of streptokinase are relatively nonspecific and can result in systemic fibrinolysis	

Recombinant tissue plasminogen activator (t-PA) (Alteplase)
 Acute myocardial infarction
 Acute cerebrovascular thrombosis
 Pulmonary embolism
 Central venous catheter occlusion

Cardiac arrhythmia, cholesterol embolus syndrome, gastrointestinal hemorrhage, rare allergic reaction, intracranial hemorrhage, sepsis

Same as streptokinase

Binds to newly formed (fresh) thrombi with high affinity, causing fibrinolysis at the site of a thrombus
 As with other thrombolytic agents, t-PA can generate a systemic lytic state and cause unwanted bleeding

Tenecteplase Reteplase
 Acute myocardial infarction

Cardiac arrhythmia, cholesterol embolus syndrome, major bleeding, allergic reaction, anaphylaxis, cerebrovascular accident, intracranial hemorrhage

Same as streptokinase

Genetically engineered variants of t-PA with increased specificity for fibrin
 Longer half-life than t-PA; Tenecteplase is administered as a single weight-based bolus; reteplase is administered as a double bolus

INHIBITORS OF ANTICOAGULATION AND FIBRINOLYSIS

Protamine

Mechanism—Inactivates heparin by forming a stable 1:1 protamine:heparin complex

Protamine
 Heparin overdose

Bradycardia, hypotension, anaphylactoid reaction, circulatory collapse, capillary leak, noncardiogenic pulmonary edema
 Flushing, nausea, vomiting, dyspepsia

Hypersensitivity to protamine

Protamine can also partially reverse the anticoagulant effect of low molecular weight heparin, but it cannot reverse the anticoagulant effect of fondaparinux

Serine-Protease Inhibitor

Mechanism—Inhibits serine proteases, including plasmin, t-PA, and thrombin

Aprotinin
 Reduce perioperative bleeding during coronary artery bypass graft surgery

Heart failure, myocardial infarction, shock, thrombotic disorder, anaphylaxis with reexposure, cerebral artery occlusion, renal failure

Hypersensitivity to aprotinin

At higher doses, aprotinin may also inhibit kallikrein and thereby paradoxically inhibit the coagulation cascade
 Aprotinin may increase the risk of postoperative acute renal failure relative to other antifibrinolytic agents

Lysine Analogues

Mechanism—Analogues of lysine that bind to and inhibit plasminogen and plasmin

Aminocaproic acid
Tranexamic acid
 Disorder involving the fibrinolytic system
 Hemorrhage from increased fibrinolysis

Bradycardia, hypotension, thrombotic disorder, drug-induced myopathy (aminocaproic acid), rare renal failure

Disseminated intravascular coagulation
 Hypersensitivity to aminocaproic acid

May cause less acute renal failure relative to aprotinin

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