BLOOD COAGULATION AND ANTICOAGULANT DRUGS

1. Introduction

Cardiovascular disease, including intravascular clot formation, represents the primary cause of death in the Western world. Blood coagulation is essential to our health; however, when it proceeds abnormally, myocardial infarction (heart attack), stroke, or pulmonary embolism can result. Pharmacologic interventions to control and correct these thromboembolic disorders have recently made much progress as the mechanisms of blood clotting have become better understood. This chapter will review the components of the hemostatic system

Table 1. **Components of the Hemostatic System**

The function of the hemostatic system is to maintain blood flow throughout the body and to react immediately to repair vascular damage to avoid blood loss. This is accomplished by an integrated balance between several cellular elements and plasma-based components.

- cellular elements
  - blood vessel
    - endothelial cells
  - platelets
  - leukocytes
  - erythrocytes
- plasma-based components
  - coagulation system
    - activators
    - cofactors
    - inhibitors
  - fibrinolytic system
    - activators
    - inhibitors
- blood flow–viscosity

and the process of blood coagulation. The anticoagulant and antiplatelet drugs used to treat thrombotic conditions as well as research trends will be reviewed.

To maintain blood in a fluid state is vital in order to deliver oxygen, nutrients and physiological messengers throughout the body. When vascular damage occurs the body reacts with an immediate response to preserve normal physiology. The hemostatic system achieves this balance between the fluid and solid states of blood. The components of the hemostatic system include blood flow, blood vessels, platelets, the coagulation system and the fibrinolytic system (Table 1) (1,2).

When the integrity of the vascular system has been compromised, the blood clots to preserve the continuity of the vasculature and the blood supply (Fig. 1). The initial response is the formation of the platelet aggregate. Platelets in the flowing blood rapidly adhere to the exposed subendothelial vessel wall matrix and become activated at the site where the endothelial cells have been damaged. During this activation process, products from the platelets are released causing further platelet activation and platelet aggregation. The platelet plug initially arrests the loss of blood. This, however, is not a permanent block. Negatively charged phospholipids on the outer membrane of activated platelets create a procoagulant surface on which coagulation activation takes place. The formation of a fibrin clot stabilizes the platelet plug.

The coagulation system is a network of proteins that work together to ultimately form fibrin, the physical structure of the blood clot (Fig. 2). Traditionally, coagulation has been viewed as having two distinct branches, the intrinsic and the extrinsic pathways depending on the initiating source of activation. The two pathways are linked at the level of factor Xa.

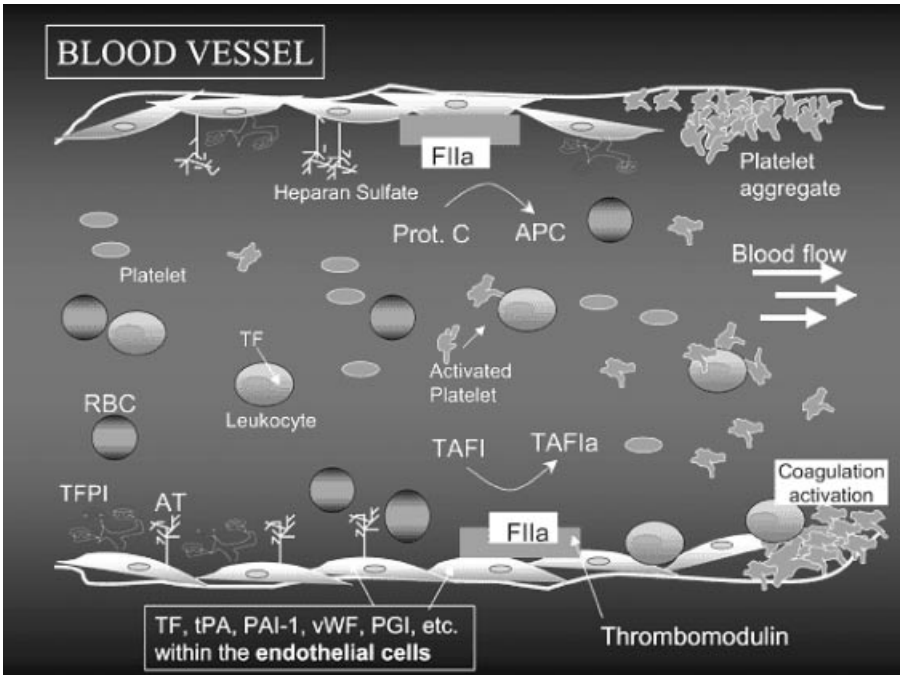


Fig. 1. Illustration of the cellular components of the hemostatic system: endothelial cells on the blood vessel wall, platelets (quiescent and activated), leukocytes, and erythrocytes. These cells normally express surface mediators that regulate coagulation, fibrinolysis, and platelet activation. Upon activation the cells express and/or release substances that modulate the physiological responses of cells and proteins in their environment and cause cell-cell interactions. These dynamic reactions take place under the physical conditions of flowing blood with vasoconstriction and relaxation of the blood vessel wall.

The extent to which each component of the hemostatic system contributes to the final clot is dependent on where in the circulation the clot is formed. In the venous circulation, where blood flow is relatively sluggish, clots contain a higher proportion of fibrin and fewer trapped blood cells. In the arterial circulation, where flow rates are higher and the presence of a stenosis leading to areas of high shear stress is more likely, clots tend to be richer in platelets. When a blood clot is no longer needed, it is broken down (lysed) by activated components of the fibrinolytic system.

Both the coagulation system and the fibrinolytic system are composed of several activators and inhibitors that provide for efficient physiological checks and balances. If any one component is over- or underactivated due to congenital or acquired abnormalities, pathologic blood clotting (thrombosis) occurs. As the components of hemostasis are many, there are multiple targets for therapeutic intervention. Targeting the mechanism that initiated the cardiovascular disorder will enhance the efficacy of the antithrombotic treatment. Bleeding complications can arise if the balance is pushed to the other extreme with drug treatment or due to physiologic abnormalities.

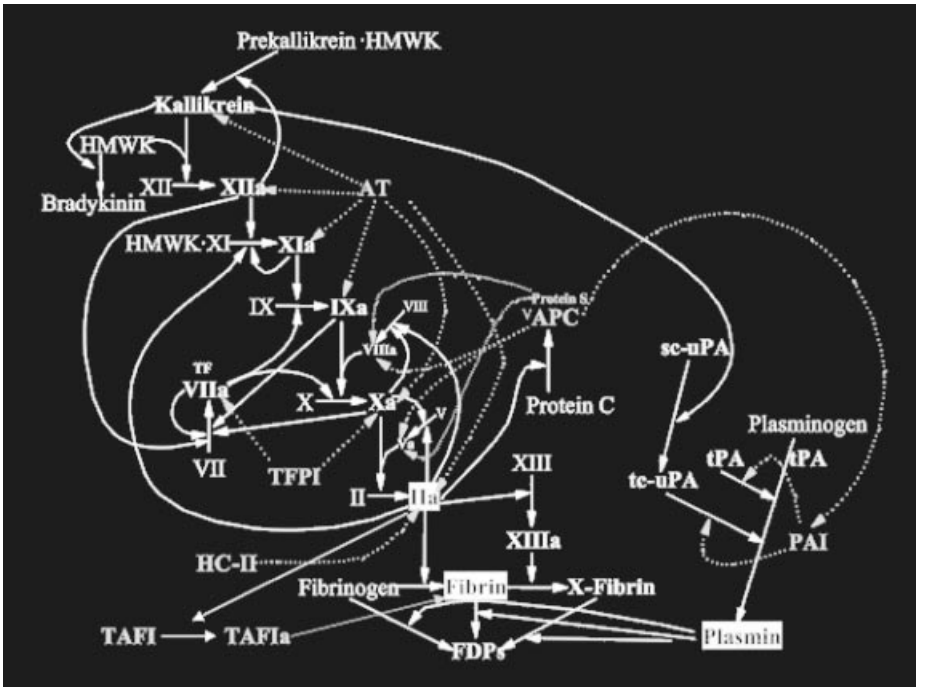


Fig. 2. Illustration of the plasma-based components of the hemostatic system: coagulation system, fibrinolytic system, and major inhibitors. Each system has its own series of activators, enzymes, inhibitors and feedback cycles. These systems interact intimately with each other. Platelets provide the phospholipid needed to activate factor X and factor II. This figure may be viewed in color online: http://www.interscience.wiley.com/cgi-bin/mrwhome/104554789/HOME

2. The Hemostatic System

2.1. Vascular Endothelium. The vascular endothelium plays an important role in hemostasis in that quiescent endothelial cells act as a barrier separating the flowing blood from subendothelial components such as tissue factor (activation of coagulation) and collagen (activation of platelets) (Fig. 1). More than just a passive barrier, the endothelium also produces a variety of substances that modulate platelet, coagulation, fibrinolytic, and vascular contraction processes (Figs. 1 and 2). Endothelial cells play a regulatory role to balance cellular and plasmatic reactions. The functional interactions of endothelial cells can be either procoagulant or anticoagulant in nature, as summarized in the following. These actions can lead to either maintenance of normal hemostasis or to pathologic occlusive disorders (stenosis, thrombosis).

Antithrombotic actions of endothelium:

1. Release of prostaglandin derivatives to control platelet activation.
2. Synthesis and release of TFPI to control coagulation activation.
3. Regulation of thrombin function through thrombomodulin.

4. Release of fibrinolytic mediators to regulate the fibrinolytic system.
5. Release of nitric oxide to promote vascular dilatation.
6. Presence of antithrombotic glycosaminoglycans (heparin-like molecules).

Prothrombotic actions of endothelium:

1. Release of tissue factor to initiate the clotting process.
2. Release of PAI-1 to inhibit the fibrinolytic response.
3. Generation of procoagulant proteins.
4. Expression of von Willebrand factor to promote platelet adhesion.

2.2. Platelets. Platelets are disk-shaped, anuclear cells that contain a contractile system, storage granules, and cell surface receptors. Platelets normally circulate in a nonactivated state in the blood but are extremely reactive to changes in their environment. Platelet membranes contain receptors for a variety of agonists including adenosine diphosphate (ADP), thromboxane A_2, platelet activating factor, immune complexes, and thrombin. Serotonin and epinephrine synergistically promote aggregation induced by other agents.

Upon activation the expression of cell receptors and procoagulant phospholipids on the platelet surface is upregulated (Figs. 1 and 2). A number of glycoproteins (GP) present on the membrane serve as receptors for collagen (GPIa/IIa), fibrinogen (GPIIb/IIIa), and von Willebrand factor (GPIb). These receptors belong to the superfamily of adhesive protein receptors known as integrins as they integrate cell–cell and cell–matrix interactions. Stimulation of these processes allows for bidirectional signaling between the intracellular and extracellular compartments of the platelet. Of the platelet-associated integrins, GPIIb/IIIa is the most abundant. Lack of GPIIb/IIIa receptors leads to the congenital bleeding disorder known as Glanzmann's thrombasthenia. Platelet GPIb binding to von Willebrand factor, which acts as a bridge to collagen binding in the blood vessel, is important as it serves to anchor the platelets to the blood vessel. Lack of the GPIb receptor leads to the congenital bleeding disorder known as Bernard-Soulier syndrome.

Platelet aggregation is another of the fundamental platelet functions. Fibrin(ogen) binding to platelet GPIIb/IIIa receptors is important as it serves as a bridge that links individual platelets together to form a large platelet aggregate. During the activation process, there is a morphologic shape change in the overall platelet structure as pseudopods are formed. This change facilitates the platelet aggregation process. Platelet aggregates serve to plug the damage to the vascular wall. Platelet granule release products promote vasoconstriction. In normal pathology, this decreases blood loss; in abnormal pathology, this causes stenosis of a blood vessel that can result in downstream tissue ischemia.

An increase in cytosolic calcium levels leads to activation of internal platelet enzymes with the subsequent release of platelet granule contents. The α-storage granules contain platelet factor 4 (PF4), β-thromboglobulin, platelet-derived growth factor, fibrinogen, factor V, von Willebrand factor, and plasminogen activator inhibitor-1 (PAI-1). The dense or β-granules contain adenosine triphosphate (ATP), ADP, and serotonin. The release of platelet granule contents

leads to further platelet activation and aggregation as well as coagulation activation.

Platelet activation also leads to the formation of platelet-derived microparticles. These are small pieces of the platelet membrane cleaved off from the platelet surface. Platelet microparticles promote activation of the coagulation system and further platelet activation (Figs. 1 and 2). The role of platelets bound to leukocytes in coagulation activation is under study.

Of particular interest in the study of thrombosis and antithrombotic drugs is the acute coronary syndrome (ACS), which encompasses unstable angina, non-ST segment myocardial infarction (MI), and acute ST elevation (transmural) myocardial infarction (AMI). The ACS stems from rupture of atherosclerotic plaque leading to intravascular thrombosis. Disruption of the protective cap exposes procoagulant materials (tissue factor, thrombin) that activate platelets and coagulation (factor Xa and thrombin generation). The role of platelets in ACS, in addition to the role of thrombin and the coagulation system, has been the focus of extensive drug development.

2.3. The Coagulation System. The plasma proteins that comprise the coagulation system are referred to as coagulation factors. Most coagulation proteins are zymogens (nonactivated enzymes) that upon activation are converted into active serine proteases. A schematic of the coagulation cascade is depicted in Figure 2. Several of the coagulation factors are dependent on vitamin K for structural formation required for activity.

In the intrinsic pathway of the coagulation system, activation occurs when the complex of factor XII, factor XI, prekallikrein, and high molecular weight kininogen come together on a negatively charged surface. This is referred to as contact activation. Factor XII is converted to its active form, factor XIIa, which in turn converts prekallikrein to kallikrein. Kallikrein can convert factor XII to its active form thereby setting up a positive feedback loop that amplifies the activation of the coagulation system. Kallikrein also activates urokinase, an activator of the fibrinolytic system.

Factor XIIa converts factor XI to factor XIa, which, in turn, activates factor IX. Factor IXa bound to the negatively charged phospholipid (on activated platelet membranes) along with its cofactor factor VIIIa and calcium ions form the "tenase" complex. Through this complex, factor X is converted to factor Xa initiating activation of the common pathway of the coagulation system.

The extrinsic pathway of coagulation is activated when circulating factor VII comes into contact with tissue factor. Tissue factor is a transmembrane glycoprotein that is expressed by subendothelial cells that surround the blood vessel. Tissue factor expression can also be induced on activated monocytes and activated endothelial cells.

Factor VII exhibits a weak procoagulant activity on its own, typically accounting for $\sim$1–2% of the total factor VII/VIIa activity. Upon binding to tissue factor, a 10,000,000-fold increase in factor VIIa enzymatic activity occurs. Both factor VII and factor VIIa bind to tissue factor with equal affinity. The factor VIIa-tissue factor complex can then activate factor X. The tissue factor-factor VIIa complex also activates factor IX to factor IXa.

The small amounts of factor Xa initially generated are sufficient to cleave prothrombin and generate a small amount of thrombin. In a feedback loop,

thrombin activates factors V, VIII, and possibly XI, thereby sustaining continued activation of the coagulation cascade. Factors V and VIII are activated through direct proteolytic cleavage by factor Xa or thrombin; they are not active proteases as are the other coagulation factors.

The majority of factor Xa joins with its cofactor factor Va, calcium ions and phospholipid (on surface membranes of activated platelets) to form the "prothrombinase" complex. The prothrombinase complex acts to convert prothrombin (factor II) into the active enzyme thrombin. Thrombin (factor IIa) serves many functions in coagulation as well as in various physiological processes. In the coagulation cascade, thrombin holds the key position in that it cleaves soluble fibrinogen to generate an insoluble fibrin clot (thrombus).

Fibrinogen circulates as a disulfide-linked dimer containing two A-α chains, two B-β chains, and two γ chains. Cleavage of fibrinogen by thrombin results in the release of fibrinopeptides A and B and the exposure of charged domains at opposite ends of the molecule. Exposure of these charged domains leads to polymerization of the fibrin monomers. These monomers are cross-linked by the transaminase factor XIIIa and calcium to form the physical meshwork of the fibrin clot.

Thrombin augments its own generation through several feedback loops in the coagulation cascade activating factors XII, XI, VIII, and V. Thrombin also activates platelets, it activates the coagulation inhibitor protein C through binding with thrombomodulin, and it stimulates activated endothelial cells to release the profibrinolytic enzyme tissue plasminogen activator (see Figs. 1 and 2).

The outcome of activation of the various factors that comprise the coagulation system is to generate thrombin. Excessive thrombin generation (a hypercoagulable state) results in unwanted blood clots that cause tissue ischemia. Depending on the location of the thrombus, skeletal muscle, heart, lung, brain, or other organs are affected. There are several anticoagulant drugs that target one or another of the coagulation factors to reduce thrombin generation. Inhibition of one or more of the coagulation factors that excessively reduces thrombin generation, such as by a congenital factor deficiency or overdose of anticoagulant treatment, may result in bleeding.

2.4. Natural Inhibitors of Coagulation. Antithrombin (AT) is a single chain glycoprotein with a molecular weight of ~58,000 Da. Normal plasma levels of AT are ~2–3 μM. AT is the primary inhibitor of coagulation and targets most coagulation factors as well as trypsin, plasmin and kallikrein (Fig. 2). Inhibition takes place when a stoichiometric complex between the active site serine of the enzyme and the Arg393-Ser394 bond of AT forms.

The efficient inhibition of proteases by AT requires heparin as a cofactor. In the presence of heparin, the inhibition rate constants for thrombin and factor Xa have been estimated to be accelerated 1000-fold to 3×10^7 and 4×10^6 L mol^{-1} s^{-1}, respectively. Deficiency of AT, due to low protein levels or to functionally abnormal molecules predisposes an individual to thrombotic complications.

Heparin cofactor II (HCII) is another plasma inhibitor that resembles AT in that it is activatable by glycosaminoglycan binding. HCII has a molecular weight of ~68,000 Da. The normal plasma level of HCII is ~1.0–1.4 μM. Two patients to date have been described as having HCII deficiency related to thrombosis.

HCII has a higher protease specificity than AT. Of the coagulation enzymes, it only inhibits thrombin (Fig. 2). However, it has also been shown to inhibit chymotrypsin and leukocyte cathepsin G. Like AT, HCII inhibits proteases by forming a 1:1 stoichiometric complex with the enzyme. Whereas AT contains an Arg-Ser bond as its active site, HCII is unique in containing a Leu-Ser bond suggesting that another portion of the HCII molecule may be required for protease binding.

Although the inhibition of protease activity by HCII is promoted by glycosaminoglycan binding, it can be activated by a wide variety of agents unlike AT, which is dependent on the presence of a specific heparin chain sequence. Heparins, heparans, and dermatan sulfate all bind to HCII and promote thrombin inhibition. Agents with relatively little sulfation such as chondroitin 4-O- or 6-O-sulfate, keratan sulfate, or hyaluronic acid do not activate HCII.

Tissue factor pathway inhibitor (TFPI) is a 42-kDa inhibitor that contains three Kunitz domains tandemly linked between a negatively charged amino terminus and a positively charged carboxy terminus. It serves an important function to control coagulation activation. The active site of the first Kunitz domain binds to the active site of the VIIa-tissue factor complex; the active site of the second Kunitz domain binds to the active site of factor Xa. The second domain appears to facilitate the inhibitory action of the first domain, and the carboxy-terminus appears to facilitate the action of the second domain. The third Kunitz domain has been shown to contain a heparin-binding site. Mutation of the active site of the third Kunitz domain has no effect on the inhibition of either factor VIIa or factor Xa.

TFPI is produced by megakaryocytes and the endothelium (Fig. 1). Small amounts of TFPI are stored in platelets (<2.5%) and can be released upon platelet activation. Plasma TFPI accounts for 10–50% of the total pool. Most plasma TFPI is bound to lipoproteins, only ~5% of the plasma pool of TFPI circulates in the free form. Lipoprotein bound TFPI is of relatively low inhibitory activity. The largest pool of TFPI is bound to the endothelial surface. The TFPI bound to the endothelium can be released into the plasma by heparin and low molecular weight heparin treatment.

Protein C is another important natural anticoagulant. Circulating thrombin can bind to a high affinity receptor on the endothelium known as thrombomodulin (Fig. 1). The complex of thrombin bound to thrombomodulin is a 20,000-fold better activator of protein C than is free thrombin. Thrombomodulin-bound thrombin no longer cleaves fibrinogen, is not able to activate other coagulation proteases such as factors V and VIII and does not activate platelets.

Protein C is a vitamin K-dependent zymogen. It is made up of disulfide linked heavy and light chains and has a molecular weight of approximately 62,000 Da. Protein C derives its anticoagulant properties from its ability to cleave and inactivate membrane bound forms of factors Va and VIIIa. Protein C requires two cofactors to express its anticoagulant activity, protein S and factor V.

2.5. The Fibrinolytic System. The fibrinolytic system keeps the formation of blood clots in check. Like the coagulation cascade, this system consists of a number of serine protease activators and inhibitors (Fig. 2). The zymogen plasminogen normally circulates in the blood in micromolar concentrations.

Two endogenous activators of plasminogen, tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA), are produced primarily by the endothelium and circulate in sub-picomolar amounts. Both tPA and uPA convert plasminogen to the active fibrinolytic enzyme plasmin. Plasmin ultimately cleaves fibrin into smaller fibrin degradation products.

Regulation of the fibrinolytic pathway occurs at the level of several inhibitors. Plasminogen activator inhibitor-1 (PAI-1) inhibits the enzymatic activity of the activators tPA and uPA. PAI-1 covalently binds to the active site of these plasminogen activators thereby preventing the generation of plasmin. Activated platelets are an important source of PAI-1. Plasmin also can be directly inhibited by the serine protease inhibitor α_2-antiplasmin.

Thrombin activatable fibrinolytic inhibitor (TAFI) is a third recently identified inhibitor that has a different type of inhibitory function. TAFI is a procarboxypeptidase that is activated by the thrombin/thrombomodulin complex. Activated TAFI (TAFIa) catalyzes the cleavage of carboxy-terminal basic amino acids (such as arginine and lysine) from fibrin, plasmin, and other proteins. Without these end structures plasmin loses it ability to digest fibrin. Thus, fibrinolytic activity is suppressed leaving procoagulant activity to proceed unopposed. New studies have revealed that certain antithrombotic drugs in addition have a pro-fibrinolytic effect mediated by the drug's interaction with and blockade of TAFIa.

2.6. Leukocytes. Recent studies suggest more and more that the line between coagulation and inflammation is less distinct (3). Studies have indicated that leukocytes, alone or bound to platelets, play a role in coagulation activation (Fig. 1). Cytokines elicit the expression of tissue factor (extrinsic coagulation system activator) on mononuclear cells, and procoagulant activity associated with leukocytes is not limited to the expression of tissue factor (Fig. 2). Several monocyte/macrophage derived procoagulant activities have been characterized including factor VII, factor XIII, factor V/Va, and binding sites for factor X and for the factor IXa/VIII complex. Prothrombin can be activated on the cell surface of monocytes and lymphocytes. Monocyte procoagulant activity is also induced by endotoxin, complement and prostaglandins.

Coagulation that takes place on the surface of endothelial cells is affected by the inflammatory process. Cytokines released from activated leukocytes, such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor (TNF), upregulate the procoagulant and down regulate the fibrinolytic nature of endothelial cells.

In addition, products of the coagulation process such as thrombin, fibrinopeptides, and fibrin degradation products have chemotactic and mitogenic properties.

2.7. Autonomic Nervous System. Although limited research has been undertaken in this area, there is supportive evidence that the autonomic nervous system may impart control on the regulation of hemostasis and activation mechanisms leading to thrombogenesis. Circadian variations with peak incidences of coronary events in the morning hours has been known. This has been shown to be associated with an increase in blood pressure, heart rate, platelet aggregability, and a decrease in fibrinolytic activity. These physiological responses reflect sympathetic activity largely induced by increased levels of plasma noradrenaline (4,5). In combination with an increase in sympathetic

mediated vasoconstriction, these factors can lead to atherosclerotic plaque rupture. During hemorrhage the hemostatic mechanisms controlling hemostasis are also partly controlled by the autonomic nervous system (6,7).

3. Therapeutic Intervention of Thromboembolic Disorders

Thrombosis is associated with a high degree of morbidity and mortality. There are numerous risk factors for thrombosis (Table 2). Anticoagulant drugs (heparin, and warfarin-derivatives) have been used clinically since the late 1930s. These drugs are not specific in mechanism; they target the inhibition of thrombin, thrombin generation and the initiation of coagulation, among other factors. Historically, heparin and warfarin have been rather easily monitored as they prolong the time to clot of global clotting assays [activated partial thromboplastin time (aPTT) and prothrombin time (PT), respectively] in a dose-dependent manner. Thus, these have been called anticoagulant drugs.

With an increased understanding of the mechanisms involved in the pathogenesis of thrombosis, specific plasma, and cellular sites within the hemostatic network are now targeted by a host of newly developed anticoagulant, antithrombotic and antiplatelet drugs (Table 3). These drugs are collectively referred to as antithrombotic drugs since their mechanisms and their effect on coagulation lab assays differ from the anticoagulant drugs heparin and warfarin. There is now a division between *in vitro*/*ex vivo* clot inhibition per se (as determined by traditional coagulation assays) and control of thrombogenesis *in vivo*. The pharmacology of heparin has also advanced. In this section, the growing area of antithrombotic agents will be reviewed.

The category of fibrinolytic agents, which differ from antithrombotic drugs in their targets and mechanisms of action, will not be covered.

3.1. Heparin. Heparin, discovered in 1916 by Jay McLean, is "... a family of polysaccharide species whose chains are made up of alternating 1–4 linked and variously sulfated residues of uronic acid and D-glucosamine" (8). It is a

Table 2. **Risk Factors of Thrombosis (Partial List)**[a]

congenital deficiencies/abnormalities of the hemostatic components (eg, factor V Leiden, prothrombin 20210, mutations of the AT molecule)
antiphospholipid antibodies/lupus anticoagulant
hyper-homocysteinemia
heparin-induced thrombocytopenia
heart failure
malignancy
burn
previous thrombosis
smoking
oral contraceptives
obesity
age
surgery
physical inactivity/stasis/immobilization

[a] Usually two or more risk factors need to be present for thrombosis to occur.

Table 3. **Antithrombotic Drugs**

heparin
low molecular weight heparin
synthetic heparin pentasaccharides (fondaparinux, idraparinux)
oral heparins
non-heparin glycosaminoglycans (eg, dermatan sulfates, intimatan)
vitamin K antagonists (oral)
direct thrombin inhibitors
oral thrombin inhibitors
factor Xa inhibitors (direct and indirect FXa inhibitors)
anti-tissue factor agents
other protease inhibitors (other than thrombin and Xa inhibitors, eg, FVIIa and FIXa inhibitors, protein Ca)
antiplatelet drugs

strongly anionic glycosaminoglycan that contains three functional side groups: $-OSO_3^-$, $-NHSO_3^-$, and $-COO^-$. The chemical structure of heparin is depicted in Figure 3. Heparin is largely derived from porcine intestinal mucosa. The average molecular weight of heparin is 15,000, but the individual molecules range from 3,000 to 30,000 Da. Thus, heparin is not one molecule but a heterogeneous

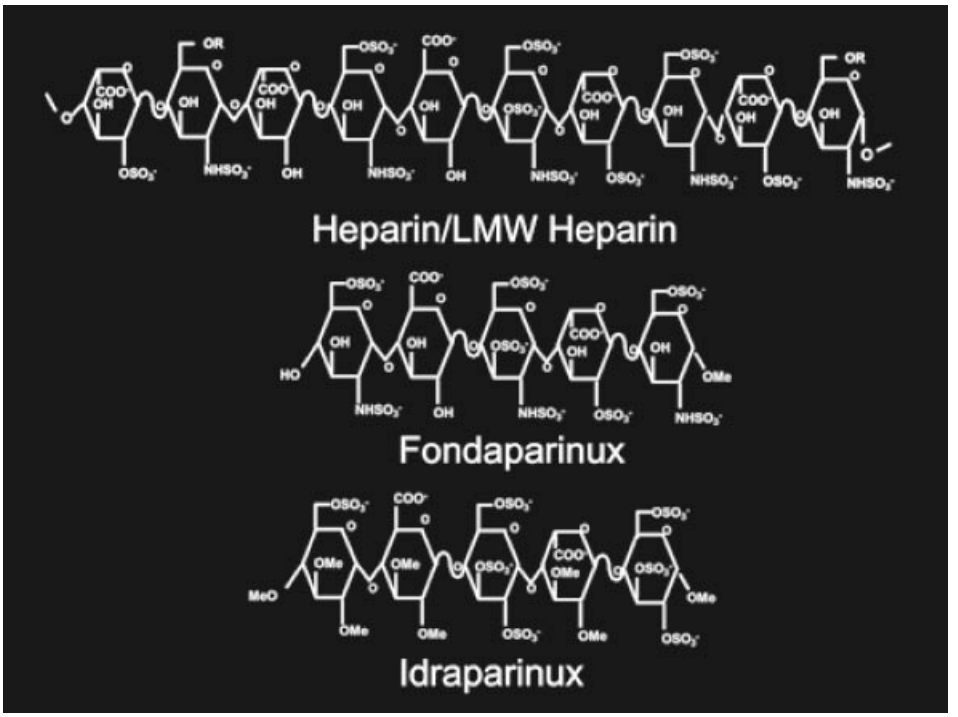


Fig. 3. Representative chemical structures of the anticoagulant heparin: the heterogeneous unfractionated heparin/low molecular heparin (differ by molecular weight and end groups), the synthetic heparin pentasaccharide (fondaparinux) with high affinity binding to AT that only produces inhibition of factor Xa, and a sulfated/methylated modification of pentasaccharide (idraparinux) designed for an extended half-life.

mixture of different molecules. Owing to its structural heterogeneity, heparin exhibits a number of pharmacologic properties. Among these are antilipemic and antiviral properties; it can also inhibit tumor growth (9,10).

Foremost among the actions of heparin is its ability to inhibit blood clotting. Heparin produces little anticoagulant or antithrombotic effect directly. Rather, its effects are mediated through specific saccharide sequences that bind to one of several endogenous plasma proteins that include AT, HCII, and TFPI. The AT–heparin complex inhibits several of the coagulation factors. The major antithrombotic activity of heparin, that which is used for pharmacologic evaluation, is the ability of heparin to inhibit thrombin (anti-thrombin or anti-factor IIa activity) and factor Xa (anti-factor Xa activity). Administration of heparin causes an increase in the plasma levels of TFPI that adds to its antithrombotic action. In addition, heparin has numerous antithrombotic properties derived from its components that have low or no affinity to AT.

Heparin is administered either by intravenous infusion or subcutaneous injection. Heparin binds to a variety of plasma proteins in the blood, thereby lowering its bioavailability and producing a variable anticoagulant response. These proteins include histidine rich glycoprotein, platelet factor 4 (PF4), vitronectin and von Willebrand factor. Heparin is eliminated by receptor-mediated internalization into endothelial cells and macrophages and by a nonsaturable renal mechanism. The anticoagulant effect of heparin is, therefore, not linearly related to dose when in the therapeutic range. The biologic half-life of heparin increases from 30 min following an IV bolus dose of 25 U kg^{-1} to 150 min following a dose of 400 U kg^{-1} (9). Subcutaneous bioavailability of heparin is limited to only 20–30%. Heparin administered by inhalation exhibits a prolonged elimination half-life, but does not exhibit significant bioavailability following oral administration.

Clinical Uses of Heparin. Heparin is the drug of choice for effective treatment of venous thrombosis and pulmonary embolism (PE) (Table 4) (11,12). Mortality is reduced in patients receiving heparin for the treatment of PE. Heparin is also used for prophylaxis in patients at risk of developing deep venous

Table 4. **Clinical Uses of Anticoagulant/Antithrombotic Drugs**

prophylaxis of venous thrombosis
treatment of established venous thromboembolic events
treatment of acute coronary syndromes
adjunct to atrial fibrillation treatment
treatment of thrombotic stroke
alternative anticoagulant for heparin compromised patients
disseminated intravascular coagulation associated with sepsis
adjunct to chemotherapy (cancer associated thrombosis)
posttransplant veno-occlusive disease
interventional cardiology procedures
surgical anticoagulation
anticoagulation for extracorporeal devices (eg, heart–lung and dialysis machines)
surface coating of biomedical devices
adjunct to anti-inflammatory agents
modulatory agent for growth factors

thrombosis (DVT) and PE (11). The risk of developing DVT and PE is reduced by 60–70% compared to patients not receiving prophylaxis. Both congenital and acquired risk factors associated with the development of thrombosis are many (Table 2). Postsurgical patients constitute the largest single group that routinely receives thrombosis prophylaxis. General medicine patients have risk factors for thrombosis that include cancer, bed rest, heart failure, and severe lung disease.

Heparin is also used to anticoagulate patients with ACS. This includes unstable angina, non-Q wave MI and AMI with or without thrombolytic therapy (11). Heparin can prevent AMI and recurrent refractory angina in patients with unstable angina. In patients with a previous MI, heparin significantly reduces reinfarction and death. Heparin as an adjunct to thrombolytic therapy increases patency during the initial stages of recanalization by preventing rethrombosis. Heparin is also used to treat thrombotic stroke.

ACS is now and treated more frequently by percutaneous coronary intervention (PCI). Since these procedures can cause intraarterial thrombus formation at the site of vessel wall damage, intravenous administration of unfractionated heparin is most frequently used to inhibit such a complication (13).

Extracorporeal devices are used in multiple clinical situations. Blood in contact with a foreign surface will clot within minutes if left without anticoagulant. Heparin has been used as a flush solution for most catheters inserted in hospitalized patients. Heparin-coated devices are now being produced that eliminate the need for heparin administration directly to the patient. Heparin is used with renal dialysis. The most extreme case where the highest level of anticoagulation is needed is with the heart–lung machine used for cardiopulmonary bypass in cardiac surgery. Heparin is used to prevent blood clotting here.

Heparin can be used safely in pregnancy because it does not cross the placental barrier and does not cause unwanted effects on the fetus (14). Heparin is effectively used in the pediatric population for the same indications as in the adult, but dosing regimens are different (15).

Part of heparin's attractiveness for use as an anticoagulant in surgical situations relates to the relative ease in which it can be neutralized upon completion of the procedure or in the event of an overdose (10). The anticoagulant actions of heparin are neutralized with a protamine salt that binds heparin in a charge-dependent manner. Protamine reduces both the anti-thrombin and anti-factor Xa activities of heparin. Heparinase, a bacterial enzyme that can cleave heparin chains into components as small as disaccharides, is being developed as a heparin-neutralizing agent. Heparinase effectively neutralizes the anti-thrombin activity of heparin but is less effective against its anti-factor Xa activity.

Monitoring heparin levels is necessary in order that drug concentrations remain in the safe and effective therapeutic range. This minimizes bleeding from overdosing or clotting from underdosing. Therapeutic intravenous heparin is monitored by the aPTT assay. This assay is performed in a laboratory using patient's blood plasma. In situations where high concentrations of heparin are required such as in surgery, the activated clotting time (ACT) is used. This assay can be done at the point of patient care on whole blood. For both the aPTT and the ACT, several reagents and instruments are commercially available. Results from each system can vary.

Heparin has been considered by some to be an old, inefficient, and clinically suboptimized drug. This statement is not justified. Heparin has provided reliable thromboprophylaxis for many years and it remains a useful and very effective drug that is easily dosed, monitored and neutralized. Had it not been for heparin, it would not have been feasible to have such surgical procedures as open-heart surgery, organ transplantation, and medical treatments of heart attack, deep vein thrombosis, and pulmonary embolism. As a polypharmaceutical, heparin is a unique drug with multiple beneficial effects. Whether the new antithrombotic agents or the newer versions of heparin (represented today by the low molecular weight heparins, and the synthetic pentasaccharide), as discussed below, will prove to be better clinical options than the original (unfractionated) heparin remains to be determined for different clinical settings.

Side Effects of Heparin Therapy. The most common side effect of heparin therapy is hemorrhage (16). The hemorrhagic effect associated with heparin therapy can range from minor to life threatening and is related to the total administered dose and the degree of prolongation of the aPTT.

Heparin-induced thrombocytopenia (HIT), which occurs in ~3% of patients exposed to heparin, is perhaps the worst of all drug-induced allergic reactions (17). HIT Type I occurs early in heparin treatment, causes a transient reduction in platelet count and patients remain asymptomatic. This is due to a direct effect of heparin on platelets. HIT Type II is a more severe thrombocytopenia and typically occurs with a delayed onset. This form of HIT often results in thrombosis and is associated with an increased mortality. While the mechanism of HIT has not been completely identified, it is known that antibodies are generated against the heparin–PF4 complex. Antibodies bind to the FcγRIIA receptor on the platelet surface resulting in platelet activation. Due to the severity of the clinical events in patients with HIT Type II, all exposure to heparin must be stopped including heparin for catheter flushes, etc. Alternative antithrombotic agents have recently become available for treatment of patients with HIT (see thrombin inhibitors).

Heparin therapy is associated with transient elevations in serum transaminase levels. Whether this is of clinical importance in terms of liver dysfunction is unknown. Long-term heparin therapy has been shown to produce osteoporosis. Heparin-induced skin necrosis is a rare complication of subcutaneously administered heparin (18).

3.2. Low Molecular Weight Heparin. The depolymerization of heparin (the original unfractionated heparin) either chemically (nitrous acid degradation, benzylation-alkaline hydrolysis, peroxidative cleavage), enzymatically (heparinase), or by physicochemical means (γ irradiation) results in the production of clinically useful drugs known as low molecular weight (LMW) heparins. This depolymerization process produces a material whose molecular weight is approximately one-third that of the parent heparin (average 5000 Da; range 2000–6000 Da), and also modifies some structural elements (19). Chemical depolymerization results in partial desulfation, reduction in charge density, a reduction in the number of AT binding sites and other changes in the consensus sequences. End-residues of fragments are typical of the specific depolymerization method.

The bioavailability of LMW heparin is nearly 80% as determined by anti-factor Xa activity. The LMW heparin is dosed subcutaneously once or twice

Table 5. **Low Molecular Weight Heparins**

Generic name	Trade name	Currently approved indications in the United States
enoxaparin	Lovenox	DVT/PE prophylaxis, extended outpatient use, DVT/PE treatment, ACS
dalteparin	Fragmin	DVT/PE prophylaxis, ACS
ardeparin	Normiflo	DVT/PE prophylaxis (inpatient use only)
tinzaparin	Innohep	DVT/PE treatment
Synthetic ultra-low molecular weight heparin		
fondaparinux (pentasaccharide)	Arixtra	DVT/PE prophylaxis
idraparinux (methylated derivative of pentasaccharide)		DVT prophylaxis

daily. The specific activity of LMW heparins ranges from 35 to 45 anti-factor IIa units/mg; anti-factor Xa activity ranges from 80 to 120 units mg^{-1}. Thus, as heparin exhibits a 1:1 ratio of anti-thrombin (160 units mg^{-1}): anti-factor Xa activity (160 units mg^{-1}), the ratio for LMW heparins range from 1:2 to 1:4 depending upon the molecular composition of the given LMW heparin (19). The LMW heparins have a lower anticoagulant potency (aPTT activity) than unfractionated heparin as a reflection of the lower anti-thrombin activity. Reduced protein binding of LMW heparin results in a more predictable dose-response. The LMW heparins cause the release of TFPI as does heparin.

Clinical Uses of LMW Heparins. It is important to know that each LMW heparin is a different chemical entity, as well each has different pharmacological behaviors (20). It is for these reasons that their dosing regimens differ. Therefore, each individual LMW heparin should only be used as described in its corresponding package insert.

Four LMW heparins have been approved for use in the United States (Table 5). These include enoxaparin (Lovenox, Aventis), ardeparin (Normiflo, Wyeth-Ayerst), dalteparin (Fragmin, Pharmacia & Upjohn) and tinzaparin (Innohep, Pharmion). Clinical trials have established their safety and efficacy in a number of indications: prevention of venous thrombosis in patients undergoing abdominal surgery, hip/knee repair/replacement (21) or medically ill patients with restricted mobility; treatment of existing venous thrombosis with or without PE (12); and prevention of ischemic complications in patients with unstable angina/non-Q-wave MI (11).

LMW heparins can also be used as anticoagulants in patients with end-stage renal disease requiring extracorporeal hemodialysis treatment. They can be used in children (15) and are the drug of choice in pregnant women requiring anticoagulation (14).

Because LMW heparins are safe and effective as thromboprophylactic agents, routine monitoring is not required for this clinical use. If monitoring is requested, a special chromogenic anti-factor Xa assay has to be used since the

aPTT does not detect these drugs. The chromogenic assay is not routine, but can be found in clinical–research laboratories associated with medical centers.

There is a debate regarding the duration of postsurgical thrombotic risk and the appropriate duration of prophylaxis. Several studies have suggested that prolonged prophylaxis results in improved clinical outcome (22). At-home dosing with LMW heparin is as safe and effective as in-hospital treatment by heparin infusion. Another debate focuses on the timing for the initiation of therapy in surgical patients (23). There are arguments both for beginning before and after surgery.

Newer indications for possible uses of LMW heparins are in the management of thrombotic stroke and in cancer patients. LMW heparins may not only decrease the incidence of cancer associated thrombosis but they may also positively impact all-cause mortality.

In cardiology, LMW heparins are effective for the reduction of restenosis after interventional cardiologic procedures, maintenance of peripheral arterial and coronary graft patency, and as adjunct anticoagulants in stenting and other interventional cardiologic procedures (24). In this setting where drug levels are higher, the ACT as used for heparin, has also been used for LMW heparins. However, a definitive monitoring system with optimal performance characteristics for all LMW heparins is not available.

LMW heparins will not become the drug of choice in surgical settings where a short half-life anticoagulant is required. Additional disadvantages of LMW heparins in surgery are that reversal agents such as protamine do not completely block the antithrombotic activity of LMW heparin, and there are no commonly available devices/assays to effectively monitor the high drug levels required.

Side Effects of LMW Heparin Therapy. Data from clinical trials has shown that LMW heparins are less likely to cause hemorrhagic complications than unfractionated heparin during treatment of venous thrombosis (16).

Retrospective data suggests that LMW heparins are less likely to cause clinical symptoms of HIT Type II (17). However, the generation of the antibody to heparin-PF4 that causes HIT occurs and LMW heparins can cross-react with a pre-formed antibody (25,26). Thus, LMW heparin should not be given to a patient suspected of having HIT.

Other side effects of heparin, such as osteoporosis are reduced with LMW heparins.

3.3. Synthetic Heparins. Heparin exerts its antithrombotic activity mainly via binding to AT thereby inhibiting thrombin and factor Xa. Investigations into the structure–activity relationships of heparin revealed a molecular weight dependence of heparin–AT on the inhibition of the coagulation proteins. Of particular interest was the finding that a saccharide sequence of 18 units or longer was necessary to produce thrombin inhibition. Inhibition of factor Xa could be produced with heparin chains of smaller length. Eventually studies focused on a decasaccharide and an octasaccharide possessing high anti-factor Xa activity with no detectable inhibitory action against thrombin. Careful study of the structures by ^{13}C NMR revealed that a pentasaccharide was the minimal heparin sequence that would bind AT and elicit a high anti-factor Xa activity.

The original pentasaccharide sequence was identified from natural heparin by fractionation procedures (27). A specific pentasaccharide of a predetermined

sequence was subsequently synthesized (28). This synthetic pentasaccharide was composed of a regular region (units G and H) and an irregular region of heparin (units D, E and F) (Fig. 3). The relative positioning of the sulfated monosaccharides was of critical importance. Four specific sulfate groups within the pentasaccharide were also shown to be critical for optimal binding to AT, ie, the 6-O sulfate on the D unit, the 3-O sulfate on the F unit, and 2-N sulfates on the F and H units (Fig. 3). Particularly important for binding to AT, was a unique 3-O sulfate group within the glucosamine residues in the irregular region.

This pentasaccharide is the first synthetic heparin (29). However, unlike heparin and LMW heparins derived from natural material, the chemically synthesized pentasaccharide is free of viral or other animal contaminants. It represents a homogeneous, single targeting entity. It does not bind to plasma proteins. Because of its minimal size, it possesses only the ability to inhibit factor Xa, via binding to AT. It has a high specific activity of $\sim$650 anti-factor Xa units mg^{-1}. It is devoid of other therapeutic effects of heparins such as the release of TFPI, anti-thrombin activity, pro-fibrinolytic actions and antiinflammatory actions. It has 100% subcutaneous bioavailability and a half-life of $\sim$18 h. These characteristics may make this drug useful for long-term prophylaxis such as for home therapy.

In clinical evaluation, pentasaccharide (fondaparinux, Arixtra; Sanofi-Organon) was well tolerated in healthy individuals (30). The PT and aPTT were not significantly prolonged even at excessive doses. Clinical studies have been performed with once daily subcutaneous dosing of fondaparinux in patients undergoing hip fracture repair or hip/knee replacement. These studies revealed that fondaparinux is more effetive but comparable to the safety of enoxaparin or unfractionated heparin for the treatment of DVT or PE, respectively, in these surgical populations (31).

Whether fondaparinux produces enhanced clinical efficacy compared to LMW heparin treatment remains a point of debate as the study endpoints may have been influenced by the timing of test drug administration. A potential limiting factor in the use of fondaparinux is the somewhat higher rate of hemorrhage compared to LMW heparin observed in some studies. The long half-life, lack of an effective antidote and lack of an easy to perform monitoring assay may be additional limitations. Because of the high affinity of pentasaccharide for AT and the limited amount of AT in plasma, decreased levels of AT with some disease states and congenital deficiencies, studies will be needed to relate the effects of pentasaccharide to endogenous AT plasma concentrations.

The synthesis of the pentasaccharide has opened the door for the possibility of synthesizing other heparin-like agents that exhibit specific pharmacologic profiles. Modified pentasaccharides with varying degrees of sulfation and/or methylation have been described. Idraparinux is one such agent that is in clinical trial (32). Such agents exhibit higher affinity to AT, more potent anti-factor Xa activity and extended half-life. In addition, larger molecules have been synthesized that incorporate the high AT affinity pentasaccharide with a thrombin-binding domain.

3.4. Oral Heparin. Recent attempts to produce heparin formulations that exhibit oral bioavailability have met with varying degrees of success. The use of diamine salts as counterions, bile acids, and surfactants promotes heparin

absorption in various animal species. More recently, oral administration of heparin-loaded biodegradable nanoparticles has been shown to produce increases in plasma anti-factor Xa levels and to prolong the aPTT. An absolute bioavailability of 23% was observed. The most studied means of delivering heparin orally is through the coadministration of *N*-[8-(2-hydroxybenzoyl)amino] caprylate (SNAC) (Emisphere) (33). It was recently shown in patients undergoing elective hip surgery that antithrombotic protection could be achieved with the administration of SNAC–heparin. The incidence of DVT/PE in patients receiving SNAC–heparin was comparable to the incidence in patients treated with subcutaneous LMW heparin; however, further studies need to be conducted to better assess the clinical usefulness of this compound. Several issues remain at this time that limit the development of SNAC–heparin including safety and patient compliance (taste).

3.5. Non-Heparin Glycosaminoglycans. Dermatans, heparans and chondroitin sulfates represent non-heparin glycosaminoglycans (GAGs) that are used mainly in the intravenous management of DVT prophylaxis. These drugs can be given to patients who are heparin compromised.

Dermatan Sulfate. Dermatan sulfate is a glycosaminoglycan polymer of iduronic acid and *N*-acetylated galactosamine. Due to a difference in the molecular backbone, dermatan sulfate is unable to interact with AT, but rather complexes with HCII to mediate thrombin inhibition. Dermatan sulfate inhibits thrombin as it is formed rather than preventing its generation. It has been shown that thrombin generation inhibition by dermatan sulfate is much less than for an equigravimetric amount of heparin.

Dermatan sulfate is active *in vivo* as an antithrombotic agent in the rabbit stasis thrombosis model, but to a lesser extent than heparin. The advantage dermatan sulfate has over heparin as an antithrombotic agent is a lower risk of bleeding complications.

Intimatan. Intimatan, iduronate → *N*-acetyl-D-galactosamine 4,6-O-disulfate, is a newly developed semisynthetic dermatan sulfate prepared by site-specific sulfation of a highly purified dermatan sulfate derived from porcine mucosa. Due to its unique structure, Intimatan has higher anti-thrombin potency than the naturally occurring, parent dermatan sulfate. It has been suggested that a unique benefit of Intimatan as an antithrombotic agent is its ability to inhibit surface-bound thrombin (eg, on extracorporeal devices) more effectively than heparin–AT (34). Since surface-bound thrombin contributes to coagulant activity and thrombus formation as fluid-phase thrombin does, and clot-bound thrombin remains active and catalyzes the generation of systemic thrombin promoting further clot growth, this agent may prove to be better than other antithrombotics. However, Intimatan has not been studied in humans yet.

3.6. Vitamin K Antagonists. Long-term prophylaxis against thrombosis is typically achieved using vitamin K antagonists (VKA). In the United States, warfarin (Coumadin) is most commonly used. Acenocoumarol and phenprocoumon with shorter and longer half-lives than warfarin, respectively, are used in other countries. Warfarin was first isolated as the substance from moldy sweet clover that induced hemorrhage in cattle. The VKAs interfere with blood coagulation by inhibiting vitamin K reductase and vitamin K epoxide reductase (Fig. 4). These enzymes are involved in the recycling of vitamin K

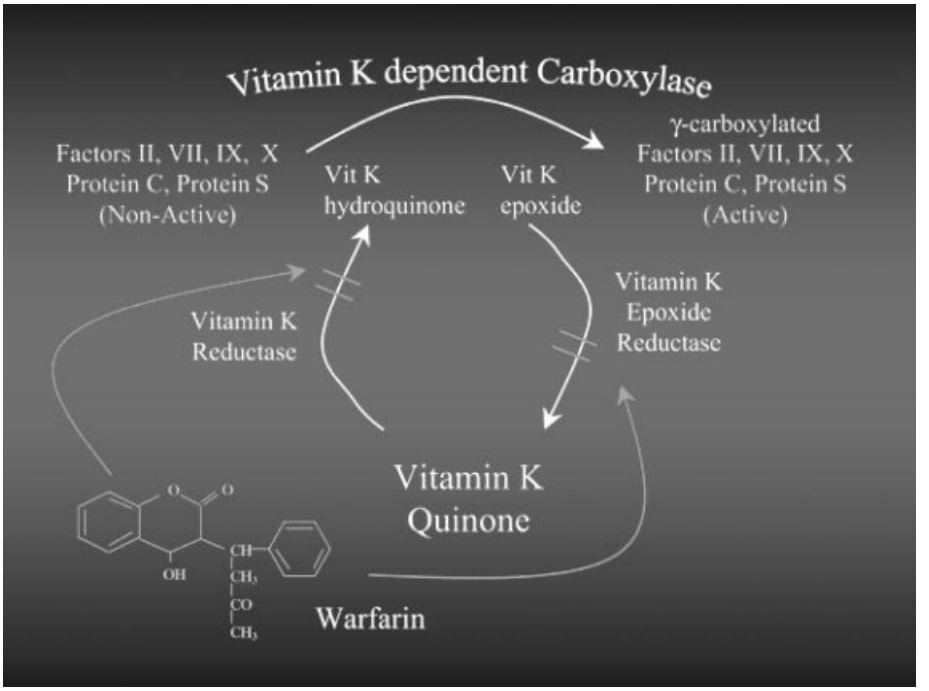


Fig. 4. The oral vitamin K antagonists: chemical structure of warfarin, the conversion of the coagulation factors from an inactive to an active enzymatic state, and the mechanism of action of warfarin to inhibit the γ-carboxylation of the coagulation factors, thus producing a physiologic anticoagulant effect. The γ-carboxylation of vitamin K-dependent coagulation factors makes possible the binding of calcium that promotes the attachment of the molecules to a lipid surface where coagulation activation takes place.

(quinone) to vitamin KH_2 (hydroquinone), which is a required cofactor for the specific carboxylation of glutamic acid residues on vitamin K-dependent coagulation factors (factors II, V, VII, IX, X, protein C, and protein S). Inhibition of this γ-carboxylation of glutamic acid residues results in a loss of calcium-binding ability with resultant decreases in binding to phospholipid surfaces (9–11).

The primary benefit of VKAs over the heparins is their ability to be administered orally. As such, VKAs are commonly used for the prophylaxis and treatment of DVT (in particular when long-term treatment is needed) (35), for anticoagulation of patients with atrial fibrillation (36), and for anticoagulation of patients with mechanical heart valves (37,38). Patients experiencing AMI may also benefit from anticoagulant treatment with VKAs.

The use of oral anticoagulants is associated with several limitations and management of patients requires a knowledgeable care provider (39). Unlike with heparin, the onset of anticoagulation with VKAs takes several days as the half-lives of affected coagulation factors range from 6 to 72 h. Plasma VKA levels are altered by a number of factors including diet, gastrointestinal and metabolic factors, vitamin K levels, coadministration of a wide variety of different drugs, and patient compliance. Hemorrhage is by far the most frequent complication of VKA therapy (16). As this class of drugs has a relatively narrow

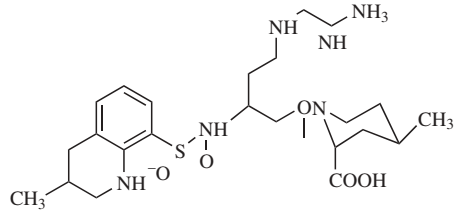
Table 6. **Direct Thrombin Inhibitor Drugs**

Generic name	Trade name	Molecular weight	Clinical uses
lepirudin (hirudin)	Refludan	6980	anticoagulation for HIT
argatroban	Argatroban	527	anticoagulation for HIT; in PCI
bivalirudin (hirulog)	Angiomax	2180	PCI
ximelagatran/melagatran	Exanta	474/430	DVT/PE prophylaxis

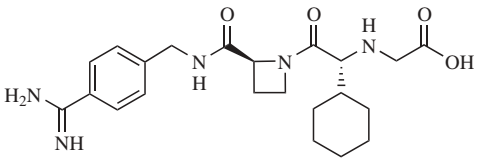
safety–efficacy margin, frequent monitoring of drug levels using the prothrombin time test and the calculated international normalized ratio (PT/INR) is necessary.

The anticoagulant actions of VKAs are not easily reversed, complicating the anticoagulant management of treated patients who require surgical intervention. Vitamin K given with fresh frozen plasma or prothrombin complex concentrate if the bleeding is severe is used to reverse over-anticoagulation. Skin necrosis can occur during treatment in individuals with protein C deficiency, activated protein C resistance (factor V Leiden), and protein S deficiency. Teratogenic effects of VKAs on fetuses preclude its use in the anticoagulant management of pregnant patients.

3.7. Direct Thrombin Inhibitors. Direct thrombin inhibitors are a new class of antithrombotic agents that includes the following agents (Table 6, Fig. 5). The first thrombin inhibitor to be developed was hirudin, a leech-derived protein of 65 amino acids (6980 Da). The synthetic peptide inhibitor hirulog (bivalirudin) is a 20 amino acid polypeptide bioengineered as an analogue of hirudin. It consists specifically of the two thrombin binding sites within hirudin (2180 Da). There are two small molecule inhibitors. Argatroban (527 Da) is a derivative of

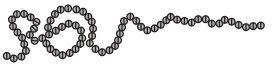


Argatroban



Melagatran

Bivalirudin



Hirudin

Fig. 5. The chemical structures of the new direct thrombin inhibitors: the arginine derivative argatroban that hinders the access of substrates to the catalytic pocket of thrombin (527 Da); melagatran a modified dipeptide that mimics the peptide sequence preceding the thrombin cleavage site in the αA-chain of fibrinogen (430 Da); bivalirudin a 20 amino acid polypeptide engineered as an analogue of hirudin consisting of its two binding sites to thrombin (2180 Da); and the leech-derived hirudin (65 amino acids; 6980 Da).

arginine. It acts by hindering the access of substrates to the catalytic pocket of thrombin. Melagatran (430 Da) is a modified dipeptide that mimics the peptide sequence preceding the thrombin cleavage site in the αA-chain of fibrinogen. Melagatran bound thrombin is blocked from producing its biological effects.

Thrombin inhibitors offer advantages over heparin in that they selectively inhibit thrombin. They directly inhibit thrombin without the need for plasmatic cofactors. They can inhibit both fluid-phase and clot-bound thrombin. Because they are not neutralized by plasma proteins, they have predictable and consistent pharmacodynamics, which can translate into fast therapeutic control and fewer treatment failures.

While all direct thrombin inhibitors have high affinity for thrombin they differ in their mechanisms of binding, with some binding only the active site of the enzyme, whereas others also bind to various exosites on thrombin. In addition, the binding to thrombin is so strong that it is nearly irreversible in some cases (hirudin) but quickly reversible in others (bivalirudin, argatroban, melagatran). As a class, direct thrombin inhibitors exhibit a short half-life; however, the specific half-lives differ among the drugs. There are reports of antibodies developing to hirudin in up to 40% of treated patients. These affect the pharmacokinetics of the drug, increasing the anticoagulant effect in the majority of cases. In several patients, anaphylactic reactions upon reexposure have resulted in death.

Thrombin inhibitors have a relatively narrow therapeutic window, and there is no known effective means of neutralizing their anticoagulant activity. Direct thrombin inhibitors not only inhibit the procoagulant actions of thrombin, but also thrombin's many physiological regulatory actions. The clinical relevance of this, however, has not been shown yet.

Three direct thrombin inhibitors have been approved by the FDA for use in United States, Canada, and Europe (clinical indications vary by country). These include argatroban (Argatroban), hirudin (lepirudin, Refludan) and hirulog (bivalirudin, Angiomax). Both argatroban (for prophylaxis and treatment) and lepirudin (for treatment) are approved for managing thrombotic complications in patients with HIT Type II (40–42). The approval of thrombin inhibitors was an extremely important achievement for providing treatment to these patients in whom heparin is contraindicated yet require anticoagulation.

Argatroban and bivalirudin are also approved for use as an anticoagulant during percutaneous coronary interventions in patients with ACS.

For prophylaxis and treatment of thrombosis in patients with HIT, the aPTT can be effectively used to monitor the thrombin inhibitor treatment. In the setting of PCI, the ACT can be used to monitor the higher doses of these drugs. In some settings and with some thrombin inhibitors, the aPTT and ACT do not provide an optimal dose-response (particularly with higher doses of the thrombin inhibitors). The ecarin clotting time assay (ECT) has been proposed as a replacement. This test is not standardized and is not widely available for purchase.

These inhibitors are currently limited to use by parenteral administration. Patients treated with thrombin inhibitors who require long-term anticoagulation are switched to a VKA. Bridging between a thrombin inhibitor and an oral anticoagulant is complicated by the fact that thrombin inhibitors prolong all

clot-based assays including an artificial increase in the PT/INR level used to monitor dosing of the oral anticoagulant. Specific dosing protocols are provided by the manufacturers to guide one through this process.

Ongoing clinical studies are evaluating additional indications for the use of direct thrombin inhibitors. Some of these indications include prevention of DVT following orthopedic surgery, as an anticoagulant for coronary interventions, for the treatment of ACS, as an adjunct anticoagulant to thrombolysis for the treatment of AMI, in patients undergoing peripheral and cerebral vascular procedures, for patients with intermittent claudication and during renal dialysis. Several large clinical trials using thrombin inhibitors for treatment of ACS have been completed with mixed results (43). Although cardiac events have been reduced, significant bleeding occurred. Some have suggested that thrombin inhibitors may be used for anticoagulation in cardiopulmonary bypass surgery. This is not an approved indication and must be used with extreme caution as dosing regimens and monitoring systems have not been established and bleeding has been reported.

Oral Thrombin Inhibitors. Orally administered drugs facilitate long-term prophylaxis of venous thrombosis, PE, stroke in patients with atrial fibrillation, as well as treatment of acute venous thrombosis, PE, AMI, and ACS. Today VKAs are the only oral antithrombotics. The high risk of bleeding with VKAs and the need for frequent monitoring, however, have prompted the development of new oral antithrombotic agents. Agents developed to date have not had sufficient activity. Thrombin inhibitors may prove otherwise.

The agent that has progressed the farthest in development is the prodrug ximelagatran (Exanta, AstraZeneca). The active form of the drug, melagatran, is a competitive, reversible, selective inhibitor of thrombin. This agent is a modified dipeptide mimicking the peptide sequence preceding the thrombin cleavage site on the αA-chain of fibrinogen. When first pass metabolism is taken into consideration, ximelagatran exhibits an ∼20% bioavailability. As this drug exhibits low protein binding, no active transport during absorption or excretion and is not metabolized by the cytochrome P450 system, ximelagatran may produce a predictable anticoagulant response to fixed doses of the drug. If this is true it may not require routine monitoring.

In patients undergoing total knee arthroplasty, a fixed dose of 24-mg ximelagatran twice daily started the morning of surgery was at least as effective as warfarin in preventing venous thromboembolism without the need for monitoring or dose adjustment (44). Additional studies on ximelagatran in comparison to either VKAs or subcutaneous enoxaparin are in progress in which increased doses are being studied, as well as a combination of subcutaneous melagatran and oral ximelagatran.

3.8. Factor Xa Inhibitors. Antithrombotic drugs that target factor Xa may have certain advantages over drugs that target thrombin. Factor Xa is a key enzyme involved in the generation of thrombin. It is common to both the extrinsic and intrinsic coagulation pathways. Factor Xa is formed at an earlier stage than thrombin in the coagulation pathway, and the procoagulant effect of factor Xa is strongly amplified by the prothrombinase complex. Factor Xa has no known activity other than as a procoagulant, in contrast to thrombin, which has multiple physiological roles. Factor Xa has relatively slow activation

kinetics, in contrast to thrombin. Opposing its function could result in easier management of the balance between the therapeutic and bleeding effects of a drug.

Factor Xa inhibitors are a diverse class of agents, each with distinct characteristics. It was learned from early experience that a drug that targets factor Xa requires high potency to produce an effective antithrombotic response. Several agents have been identified that are able to either directly bind to and inhibit factor Xa or indirectly inhibit factor Xa (45). Thus, these agents derive their antithrombotic activity from their ability to inhibit thrombin generation. Proteins and small peptides derived from natural sources including ticks, leeches, snakes, and hookworms, the original factor Xa inhibitors identified, have been discontinued from clinical development. The synthetic heparin pentasaccharide, fondaparinux, can be considered a factor Xa inhibitor. It differs from all other factor Xa inhibitors in that it has an indirect inhibitory effect requiring binding to AT to produce its anti-factor Xa activity.

A number of synthetic or recombinant, direct factor Xa inhibitors are in development. Unlike heparin, LMW heparin and fondaparinux, the direct factor Xa inhibitors can inhibit clot-bound factor Xa and factor Xa that has been incorporated within the prothrombinase complex. This is an advantage for drug efficacy. It may also explain why direct factor Xa inhibitors can be monitored by the aPTT, whereas fondaparinux cannot. The agents in Phase II clinical development planned for thrombosis prophylaxis or in cardiology include recombinant TFPI (tifacogin; Pharmacia/Chiron) and the synthetic agents DX-9065a (Daiichi), DPC-423 (DuPont), ZK-807834 (Berlex/Pfizer), and BAY 59-7939 (Bayer). The therapeutic potential and safety issues of all factor Xa inhibitors remain to be defined.

3.9. Other Inhibitors. Several other proteases of the coagulation system are also targeted for drug development. In addition to the thrombin and factor Xa inhibitors already discussed, agents that specifically target either factor VIIa or tissue factor are in development. The inhibitor to the factor VIIa/tissue factor complex, rNAPc2 (Corvas), is in phase II development planned for DVT prophylaxis in post hip and knee surgery and in arterial thrombosis treatment. Inhibitors to factor XIIa/XIa, factor IXa, and factor XIIIa are also under development as potential antithrombotic drugs.

The natural inhibitors of the coagulation system are also targets for potential drug development. In addition to TFPI already discussed, AT, HCII, C_1-esterase inhibitor, and PAI-1 are under development. Protein Ca concentrate, which targets the inhibition of factor Va and factor VIIIa, has shown successful outcomes in patients with sepsis and disseminated intravascular coagulation (DIC).

3.10. Antiplatelet Drugs. The ability to inhibit platelet activation or the aggregation of previously activated platelets is of significant importance in the treatment of coronary artery disease (43), cerebrovascular, peripheral vascular disease (46), following coronary interventions (13), and following cardiac surgery (47). A wide variety of antiplatelet agents that inhibit different aspects of the platelet activation response have proven clinical efficacy or are currently under development (Table 7) (48).

Aspirin, the most widely used antiplatelet agent, blocks platelet activation by inhibiting cyclooxygenase (COX) thereby limiting thromboxane (a potent

Table 7. **Antiplatelet Drugs**

Generic name	Trade name	Mechanism	Clinical uses
aspirin		COX inhibitor	ACS, PCI, stroke
clopidogrel	Plavix	ADP receptor blockers	aspirin substitute
abciximab	ReoPro	fibrinogen receptor blocker	ACS, PCI
tirofiban	Aggrastat	"	
eptifibatide	Integrilin	"	
dipyridamole	Persantine	phosphodiesterase inhibitor	stroke, aspirin adjunct
cilostazol	Pletaal	type III phosphodiesterase inhibitor	intermittent claudication

platelet aggregation activator) generation. Aspirin offers clinical benefit in both the primary and secondary prevention of cardiovascular events, prevention of DVT and PE, and is used in the treatment of AMI, stable and unstable angina, carotid artery stenosis, ischemic stroke, and placental insufficiency. Aspirin is also used in combination with other antiplatelet drugs during PCI and in the prophylaxis of thrombotic complications following PCI. After coronary bypass grafting (CABG surgery) patients are put on life-long aspirin therapy.

The minimum effective dose of aspirin ranges between 50 and 100 mg day^{-1}. For high risk patients, doses from 50 to 1500 mg day^{-1} have been shown to be effective. Aspirin has been shown in vascular patients to reduce the risk of stroke, MI and death by ~25%. However, it has been estimated that up to 40% of patients may experience thromboembolic events despite aspirin therapy. This has been referred to as aspirin resistance (49). The true significance of the problem remains unknown because of differences in the definition of resistance, variations in detection methods and lack of controlled trials. Multiple mechanisms have been proposed, including increased reactivity to platelet aggregating factors, genetic polymorphisms and alternate pathways for thromboxane synthesis. Strategies are needed to identify patients at risk for aspirin resistance who might benefit from alternative or combined antiplatelet therapy.

Another specific inhibitor of platelet activation is dipyridamole (Persantine). This drug is believed to work by inhibition of a phosphodiesterase enzyme that degrades cyclic adenosine monophosphate (AMP). An accumulation of cyclic AMP inhibits platelet activation. The clinical efficacy of dipyridamole has been questioned; however, it may be that doses have been too low.

The thienopyridines, ticlopidine and clopidogrel (Plavix), are ADP receptor antagonists. Platelets have two distinct ADP receptors, $P2X_1$ and $P2Y_1$. It is not clear, but suggested that these drugs block a third ADP receptor that mediates the inhibition of stimulated adenylyl cyclase activity ($P2T_{AC}$). These are prodrugs that require hepatic transformation into the active state. Ticlopidine has been associated with neutropenia, thrombocytopenia, aplastic anemia, and thrombotic thrombocytopenic purpura. Clopidogrel, a drug chemically similar to ticlopidine is associated with a lower incidence of these side effects and, therefore, is more commonly used. While initially approved for use in patients with symptomatic atherosclerosis, clopidogrel also has proven benefit in the treatment of unstable angina, AMI, and is used extensively in combination with aspirin for

the prevention of stent thrombosis. Clopidogrel is often used in combination with aspirin.

There are multiple activation pathways in platelets. Glycoprotein (GP) IIb/IIIa receptor blockers are potent antiplatelet agents that inhibit the final common pathway of platelet aggregation. The GPIIb/IIIa inhibitors are effective regardless of the platelet activation stimulus. Several such drugs are currently available. The Fab fragment of a monoclonal antibody against the GPIIb/IIIa receptor, abciximab (ReoPro), was the first GPIIb/IIIa inhibitor to be clinically developed. The development of synthetic small molecules and peptidomimetic inhibitors that compete with fibrinogen and other ligands for occupancy of the platelet receptor followed. Tirofiban (Aggrastat) is a non-peptide derivative of tyrosine. Eptifibatide (Integrilin) is a synthetic heptapeptide based on the amino acid sequence Lys-Gly-Asp found in the venom of the *Sistrurus m barbouri* snake. GPIIb/IIIa inhibitors have been shown to be effective in reducing late restenosis and preventing mortality in patients undergoing PCI and in preventing mortality in patients with ACS.

One of the main weaknesses of the current GPIIb/IIIa inhibitors is the need to administer them intravenously, thus precluding their use for long-term platelet inhibition. The optimal level of GPIIb/IIIa inhibition to maximize efficacy without inducing an enhanced risk of bleeding is unknown. Unlike aspirin and clopidogrel where complete inhibition of cyclooxygenase or ADP receptors, respectively, is desirable, complete inhibition of GPIIb/IIIa leads to an unacceptably high incidence of bleeding. To some degree, the lack of knowledge concerning the optimal degree of GPIIb/IIIa inhibition is related to a lack of efficient assays for monitoring the antiplatelet effects of these agents.

Antibodies that cause thrombocytopenia to $<50{,}000/\mu L$ platelets in up to 2% of treated patients is an important side effect of abciximab. In 1% of these cases, the thrombocytopenia is rapid beginning within 2 h of initiation of drug administration.

For chronic arterial insufficiency in the extremeties aspirin, dipyridamole and clopidogrel have been found useful. Cilostazol (Pletaal) is a type III phosphodiesterase inhibitor with both antiplatelet and vasodilatory properties. It has been recently approved for the treatment of intermittent claudication.

Trials with orally available GPIIb/IIIa inhibitors have not been successful to date. An oral P2T ADP receptor inhibitor, CS-747 (Sankyo/Lilly), is in phase I development planned for arterial thrombosis treatment. Other antiplatelet drugs under development include thromboxane and serotonin receptor antagonists.

3.11. Pharmacologic Considerations. The future holds promise for effective new antithrombotic drugs in individual and specific indications. It is likely that each drug will have a role in specific clinical indications and that one drug will not be optimal for all thrombotic situations as these drugs do not exhibit a polytherapeutic spectrum. As more is learned of the mechanisms of thrombosis, there will be drugs that target a patient's individual needs to combat the various type of clinical thrombosis that he/she is experiencing. Combination approaches may be more beneficial in the overall management of thrombotic disorders.

The development of LMW heparin began in the 1980s. Today these are the drugs of choice for several thrombotic indications. From identified structure–

Table 8. **Pharmacologic Considerations for New Antithrombotic Drugs**

mechanism of action (dependence on plasma factors, pro-drug)
subcutaneous bioavailability
oral bioavailability
endogenous modulations
endothelial/vascular interactions
metabolic transformations
patient-to-patient variability in dose-response
drug interactions
bleeding risk
ease of monitoring drug levels
ability to generate antibodies (heparin-induced thrombocytopenia, anti-platelet antibodies, antibodies that cause leukopenia, antibodies that alter the pharmacokinetics) other unwanted side effects

activity relationships, such heparinomimetics as fondaparinux have been developed. Additionally, studies to develop drugs with antithrombotic activities but without anticoagulant aspects are in progress at this time. Direct acting factor Xa inhibitors are currently in clinical development. However, many of these drugs do not have anticoagulant actions and may be limited in scope for different situations. The development of direct thrombin inhibitors has moved quickly. This was spurred by the obvious need for alternative antithrombotic treatment in patients with HIT. These patients have the highest risk of thrombosis but cannot receive heparin products and no other fast-acting, strong anticoagulant was available. There are potential advantages for factor Xa inhibitors over thrombin inhibitors, particularly a higher safety margin in prophylactic regimens and less frequent dosing requirements. On the other hand, factor Xa inhibitors may be less potent than thrombin inhibitors and could thus have limited clinical application.

As with any new drug there are certain issues to be considered during development (Table 8). Synthetic agents have certain advantages over naturally derived products, not the least of which is their specific chemical design to target desired biological effects. Drug interactions have to be considered as patients are often on multiple antithrombotic as well as other types of drugs. For example, heparin/antiplatelet drugs, antithrombin/antiplatelet drugs, and antiplatelet drugs of different mechanisms are often combined. In the future, antithrombin/anti-factor Xa/antiplatelet drugs may be combined. Cost is an obvious issue that can prevent widespread use of any new drug.

How and where each drug is used clinically, and how each will compete with the standard heparin, warfarin, and aspirin treatments remains to be determined. The newly developed drugs are mostly monotherapeutic and do not mimic the polytherapeutic actions of heparins. It is therefore, important to stress that heparin and its derived/modified forms will continue to play an important role in the management of thrombosis (50).

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