Chapter 36

Disorders of the blood coagulation-fibrinolytic system

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Most human cells have no direct contact with the external environment and their living conditions are ensured by blood. An essential criterion for normal blood circulation is the ability of the blood to arrest bleeding at sites of tissue injury and to prevent thrombus formation inside the blood vessels under normal conditions. The balance between these two extreme states is rigorously controlled by cellular and molecular components such as the endothelial cells, hepatocytes, platelets and the blood coagulation-fibrinolytic system, and is designated as normal hemostasis. Any disorganization in hemostasis may lead to hemorrhagic or thrombotic disease; the latter is the most serious medical problem of our time in developed countries.

The purpose of this chapter is to survey the role of the blood coagulation-fibrinolytic system in hemostasis, its pathological alterations and treatment of the disorders.

36.1 Blood coagulation

An important feature of circulating blood is the ability to lose its fluidity. A molecular component, fibrinogen, and a cellular element, the platelet, play crucial roles in this process.

36.1.1 Conversion of fibrinogen to fibrin

Fibrinogen, a water phase soluble molecule (340 kDa) synthesized and secreted by the liver, is present in the blood circulation at a concentration of approximately 10 μM. When its two short N-terminal portions (fibrinopeptide-A and -B) are released by thrombin, the remaining part of the molecule (fibrin monomer) loses its solubility and several fibrin monomers bind together through noncovalent interactions (denoted as fibrin polymerization). In a subsequent step, crosslinks (isopeptide bonds) are formed among polymerized fibrin monomers by the action of a transglutaminase (activated Factor XIII; F XIIIa), whereby fibrin is stabilized.

The fibrin formation is initiated by thrombin, which, exists in the blood in a proenzyme form (prothrombin). Thus, the conversion of fibrinogen to fibrin is determined by the amount and activity of thrombin formed during prothrombin activation. The amount of thrombin depends on the rate of prothrombin activation, whereas its activity is limited by its inhibitor system (mainly by antithrombin; a blood plasma protein also synthesized by the liver).

36.1.2 Prothrombin activation

Prothrombin (a 70 kDa protein, synthesized by the liver) is activated by a serine protease, Factor Xa. The rate of activation is slow. When other components, such as Ca²⁺, Factor Va (a protein without any enzyme activity) and the phospholipid surface of activated platelets are also present, there is an approximately 300000-fold rate-enhancement. In this complex (prothrombinase complex) both Factor Xa and prothrombin are bound to the phospholipid structure via Gla-domains (Figure 36.1). Inhibition of Gla formation in the proteins inhibits the rate of conversion of prothrombin to thrombin; inhibition of the vitamin K cofactor function with coumarin derivatives (Dicumarol, Warfarin, Synumar, etc.) is frequently used in clinical practice for prevention of thrombus formation and growth (Figure 36.1, inset).

Factor Xa, however, also exists in a proenzyme form (Factor X), which is activated by either Factor VIIa or Factor IXa (Figure 36.2). The reactions occur in complexes, thus, an extremely high amount of thrombin is formed in a short
Figure 36.1 The prothrombinase complex. Prothrombin (ProT) is converted to thrombin (T) by activated Factor X (Xa) in the presence of activated Factor V (Va) and phospholipid membrane of activated platelet (PL). Ca²⁺ and Gla-domains of the proteins are prerequisites for the binding of prothrombin and Factor Xa to phospholipid. Gla (γ-carboxyglutamic acid) is formed from CO₂ and Glu (glutamate) in the protein by a carboxylase enzyme using the reduced form (hydroquinone) of vitamin K as a cofactor. During the reaction, vitamin K is oxidized (epoxide form) and thereafter is reduced in two steps (not shown here) by NADH dependent reductase and dehydrogenase enzymes. Coumarin derivatives (vitamin K analogs), inhibit the enzymes of the vitamin K cycle. Wide arrow denotes conversion, narrow arrow action, crossed arrow inhibition, throughout figures in this chapter.

Figure 36.2 Initiation, amplification and control of blood coagulation. Blood coagulation is initiated by tissue factor (TF), a protein present on the surface of cells which are not in direct contact with blood. When Factor VII (VII) in blood plasma meets tissue factor, it is activated and the active form (VIIa) efficiently activates Factor X (X) directly and/or indirectly via factor IX (IX) activation. Both reactions are accelerated by TF. Finally Factor Xa (Xa) activates prothrombin (ProT) in the prothrombinase complex (see also Figure 36.1). All these reactions occur in complexes, where Ca²⁺, phospholipids and cofactor proteins (tissue factor, Factor V and Factor VIII) are also present. In the complexes extremely high rate enhancements occur and the enzymes formed are protected against the endogenous inhibitor system (except one; not detailed here). Since a single enzyme catalyzes the activation of several zymogens, and the generated enzymes act on further zymogens, the system serves as amplifier of the blood coagulation, called a cascade. The cascade is controlled by positive (⊕) and negative (⊖) feedback mechanisms; thrombin may convert Factor XI (XI) to activated Factor XI (XIa), which in turn, activates Factor IX. Furthermore, thrombin directly activates Factor V (V) and Factor VIII (VIII), and inactivates activated Factor V (Va) and activated Factor VIII (VIIIa) via the Protein C-thrombomodulin system (PC, see also Figure 36.3). Tissue factor may be expressed on the monocyte and endothelial cell surface during Gram-negative infection (the bacterial endotoxin, a lipopolysaccharide induces tissue factor mRNA synthesis) and when TF appears in the blood a generalized thrombosis is induced (Disseminated Intravascular Coagulopathy; DIC). Blood coagulation may be initiated by activated Factor XII (XIIa), which activates Factor XI directly, this reaction, however, does not seem to play a role in vivo, at least, for thrombus formation; rather it is fibrinolytic via initiation of plasminogen activation. Patients deficient in Factor XII do not bleed.

period after initiation of the blood coagulation cascade. It is useful in external tissue damage to prevent blood loss but is dangerous inside the blood circulation provoking thrombosis.

The key to initiation of blood coagulation is a protein, the tissue factor, present on the surface of all cells which are not in direct contact with blood, while cellular elements of blood circulation (endothelial cells, platelets, red blood cells, neutrophils, etc.) do not express tissue factors under normal conditions. Under some pathological circumstances, however,
tissue factor expression may occur on endothelial cells and/or monocytes leading to generalized thrombosis and, as a consequence, fibrinolysis. Factor V and Factor VIII are proteins without any known biological function present in blood circulation, however, when one peptide bond in the molecule is hydrolyzed by thrombin, they become a cofactor (Factor Va and Factor VIIIa, without enzyme activity) accelerating activation rates in the complexes (see Figure 36.1 for Factor Va in prothrombinase complex; Factor IXa and Factor IXa complex is not shown). After additional degradation by activated Protein C (aPC), these cofactor functions of Factor Va and Factor VIIIa are diminished (Figure 36.3). Activated Protein C, however, exists in the blood in a proenzyme form (Protein C), which is activated by thrombin. The rate of activation of Protein C is enhanced by several orders of magnitude when thrombin is bound to thrombomodulin, present on the endothelial cell surface (Figure 36.3).

36.1.3 Thrombin inactivation

A crucial step in the blood coagulation cascade is its termination. Several endogenous protease inhibitors exist in the blood circulation, among them antithrombin (antithrombin III) seems to be the most important, at least from a medical viewpoint. Antithrombin forms an equimolar complex with thrombin, in which all its enzyme activities are blocked. The rate of inactivation is accelerated by heparin (Figure 36.4).

36.1.4 Platelet activation

As mentioned earlier, blood coagulation takes place in transiently formed compartments (in complexes), where activated platelet membrane surface (a phosphatidyl-serine rich surface, formerly called platelet factor 3) plays an essential role. Under normal conditions, native platelets (enucleated
cell fragments of megakaryocytes with a volume of approximately 7 \mu m^3 and a blood count of about 300,000 per microliter are distributed in a homogeneous suspension. When activated by thrombin, collagen, platelet activating factor (PAF), ADP or thromboxane A_2 (TXA_2), disk-shaped platelets (3 \mu m in diameter) develop filopodia extensions, release granulum contents and translocate phosphatidylserine to the outer layer of the cell membrane. Activated platelets may adhere (platelet adhesion) to a foreign surface (e.g. glass, damaged blood vessel wall) if von Willebrand factor (synthesized and secreted by endothelial cells) is present and/or they may associate with each other (platelet aggregation) in the presence of Ca^{2+} and fibrinogen. Platelet aggregation contributes to plug formation, preventing blood loss or causing stasis of blood circulation (e.g. myocardial infarction, stroke). Activation of platelet is initiated by thrombin via thrombin receptors; when the N-terminal portion of the protein is released by the enzyme, a conformational change of the remnant molecule coupled to a G-protein activates phospholipase C resulting in inositol triphosphate and cytosolic Ca^{2+} increase. Thereafter Ca^{2+} activates both the platelet contractile system and phospholipase A_2; as a result, the cytoskeleton of the platelet is rearranged (filopodia expression), granule content is secreted, phosphatidyl-serine is translocated, and the activated phospholipase A_2 induces thromboxane A_2 (TXA_2) synthesis from arachidonic acid. Both ADP (released from granules) and TXA_2 (secreted) activate neighboring platelets, serving as an amplification of platelet aggregation. ADP, binding to its platelet receptor coupled to G protein, decreases the cAMP synthesis via inhibition of adenylate cyclase. As a result, the platelet becomes more sensitive to activation. In contrast, prostacycline (PGI_2) binds to G protein and activates adenylate cyclase, and the elevated cAMP level protects platelets against activation.

Prostacycline, however, is synthesized and secreted by endothelial cells. The signal transduction mechanism is similar to platelet thromboxane A_2 synthesis; when phospholipase A_2 is activated in endothelial cells (e.g. via endothelial thrombin receptor), prostacycline is synthesized from arachidonic acid and released. In turn, PGI_2 inhibits platelet aggregation.

### 36.2 Fibrinolysis

Almost parallel with the insoluble fibrin formation, it is solubilized by fibrinolytic enzymes under normal conditions. Although several proteases degrade fibrin, plasmin is considered as the main fibrinolytic enzyme.

#### 36.2.1 Plasminogen activation

Plasmin exists in a zymogen form in the blood, as plasminogen. Thus, a prerequisite of plasmin action is its formation, the activation of plasminogen.

Plasminogen (92 kDa) is synthesized by hepatocytes and secreted into the blood circulation, where its concentration is fairly stable, approximately 2 \mu M. It is converted to plasmin by plasminogen activators when its conformation is changed (e.g. it is bound to fibrin or its N-terminal portion is hydrolyzed by plasmin or elastase). The activation is a simple reaction; a peptide bond between Arg_561 and Val_562 is hydrolyzed by plasminogen activators (Figure 36.5). There are endogenous plasminogen activators, e.g. urokinase-type (uPA) and tissue-type (tPA) and exogenous, e.g streptokinase. All of them are used in clinical practice. While tPA and uPA, synthesized by endothelial cells and tumor cells, act directly on plasminogen, streptokinase, a product of Streptococcus haemolyticus, binds to human plasminogen, and in the complex plasminogen becomes a plasminogen activator and activates free plasminogen. The catalytic efficiency of uPA is approximately an order of magnitude higher than that of tPA, but the latter is extremely stimulated in the presence of cofactors, such as fibrin, endothelial cell membrane, actin, myosin, some extracellular matrix components and denatured proteins (Figure 36.6). Since endothelial cells synthesize plasminogen activator inhibitors as well, it is an open question at present how the levels of activators and inhibitors are controlled.

![Figure 36.5 Plasminogen activation. Native plasminogen (nPg) is not a susceptible substrate to plasminogen activators (PA); modification of its N-terminal portion either conformationally or proteolytically (mPg) makes peptide bond Arg_561-Val_562 sensitive to PA, and the rate of plasmin formation is enhanced (inset). After hydrolysis of a single peptide bond a two-chain protein (held together with S-S bridges) is formed and the active center (AC) of the serine protease plasmin (P) is expressed on the small chain. Plasmin, among several proteins, digests fibrin(ogen).](image_url)
When formed, plasmin may digest fibrin and several other proteins. Its activity on fibrin degradation is determined by the rate of plasminogen activation and by the rate of its inactivation. There are several types of protease inhibitors present in the blood, among them plasmin inhibitor (formerly named α₂-antiplasmin) is one of the most efficient inhibitors in the mammalian system (second-order rate constant for inactivation of plasmin in solution is $4 \times 10^6 \text{M}^{-1}\text{s}^{-1}$). It is remarkable that this inhibitor can be bound covalently by Factor XIIIa to fibrin, where it retains its function (see later).

### 36.2.2 Fibrin degradation

While polymerized fibrin monomers are easily digested by plasmin, the rate of degradation of crosslinked fibrin crosslinked with plasmin inhibitor by Factor XIIIa is almost zero (Figure 36.7). Thus, "old" fibrin (crosslinked) is relatively resistant to plasmin digestion.

Plasmin, on the other hand, is rapidly inactivated in the blood plasma by plasma protease inhibitors, primarily by plasmin inhibitor. If plasminogen activation occurs on the fibrin surface, the plasmin formed binds to its substrate and the rate of its inactivation by plasmin inhibitor slows down; plasmin is "protected" against the inhibitor system (Figure 36.8).

### 36.3 Pathological changes in the blood coagulation-fibrinolytic system

Diseases of hemostasis may be hereditary or acquired with clinical symptoms of bleeding or thrombosis or both. In this review only defects are discussed, the treatment of which is clear and explicit on the basis of the mechanisms described in this chapter.
Defects of blood coagulation factors (except their inhibitors and the Protein C system) and of inhibitors of the fibrinolytic enzymes generally cause bleeding problems. Blood transfusion or isolated factor administration is used for treatment (not detailed in this survey).

When the blood coagulation proenzymes are activated (especially when tissue factor expression is facilitated either locally or in a generalized form), or the inhibitor system of blood coagulation proteases, the Protein C system or enzymes of the fibrinolytic system are deficient, thrombophilia occurs. In many cases, however, the cause of thrombosis is not known, only clinical symptoms indicate increased thrombus formation (e.g. myocardial infarction, stroke, deep vein thrombosis, pulmonary embolism). Most frequently endothelial damage underlies the problems, but it remains hidden until serious clinical symptoms appear.

36.3.1 Treatment of thrombosis

Thrombosis treatment generally means prevention; inhibition of thrombus growth and thrombus formation using vitamin K antagonists, heparin, hirudin and/or inhibitors of platelet activation. A real therapy is the initiation of fibrinolysis with tPA, uPA or streptokinase to dissolve the thrombus. The most frequent thrombotic diseases and their treatments are summarized in Table 36.1.

Vitamin K antagonists, coumarins, inhibit thrombin formation via decreasing rate-enhancements in the blood coagulation complexes (Figure 36.1). It is important to keep in mind that coumarins inhibit amino acid modification in proteins, therefore efficient treatment is achieved with a delay in the range of days and in the case of overdose bleeding may depend on the synthesis rate of blood coagulation factors containing Gla-domain. Thus, monitoring vitamin K-dependent anticoagulation is mandatory (not detailed here).

Clinical use of heparin is based on its accelerating effect on the inactivation of thrombin by antithrombin (Figure 36.4). It may be administered alone or in combination with coumarins or fibrinolytic agents in the treatment of acute myocardial infarction (AMI), deep vein thrombosis (DVT), pulmonary embolism (PE) and stroke. A mini-dose heparin administered subcutaneously is used during surgery to prevent AMI and DVT. Heparin action is very rapid, its half-life in blood circulation is short, and in addition, when overdosed, protamin, as antidote, neutralizes it. In the future, perhaps hirudin will be used because it inactivates thrombin directly, it is as efficient as heparin, there are no immunological complications and recombinant forms of the protein are available. It seems to be promising especially in antithrombin deficiency.

Therapeutically, inhibition of platelet activation is a frequently used tool in the management of thrombosis. The most popular drug is aspirin, which inhibits cyclooxygenase, thereby preventing thromboxane A₂ synthesis and the amplification of platelet aggregation. A new trend is to create compounds which interfere with platelet receptors participating in platelet adhesion and aggregation (e.g. thrombin receptor, fibrinogen receptor, ADP receptor, etc.).

36.3.2 Fibrinolytic therapy

When tPA, uPA or streptokinase is administered to a patient, plasminogen present in the blood is activated (Figure 36.6), but plasmin forms randomly and is inactivated immediately by plasmin inhibitor present in high concentration in the blood plasma. Plasmin formed on fibrin networks, however, binds to its substrate and escapes rapid inactivation (Figure 36.8). An
apparent advantage of tPA is that it also binds to fibrin, whereby plasminogen activation occurs on the fibrin surface where plasmin is protected against the inhibitor. This mechanism is logical, but in practice tPA has no essential advantage in efficiency over other activators. Streptokinase, although efficient, may cause immunological problems, anaphylactic reactions, etc. In the future, perhaps a fibrin-dependent form of streptokinase will be used. If the N-terminal residues (59 amino acids) are removed, the remaining portion of streptokinase in complex with plasminogen becomes fibrin-dependent plasminogen activator. It seems to be an efficient, and not expensive, drug. Fibrinolytic therapy is generally efficient with dose to 50% of plasminogen activated (concentration of plasminogen is 2 nM, whereas the plasmin inhibitor is 1 nM in blood), that is, the consumption of the fibrinolytic inhibitor is a prerequisite for thrombus dissolution. An additional clinical aspect of fibrinolysis is that cross-linked fibrin and especially fibrin cross-linked with plasmin inhibitor are dissolved at a slower rate than polymerized fibrin (Figure 36.7), indicating the need for urgent (within a few hours) initiation of the fibrinolytic therapy.

Acknowledgments

This work was supported by Grants OTKA T-031891, ETT 529/96 and FKFP 006/97. I am grateful to Dr. Krasimir Kolev and Dr. István Léránt for helpful criticism.

References


