

Part IV

Antithrombotics, Anticoagulants and Fibrinolytics

"The meaning of good and bad, of better and worse, is simply helping or hurting"

Emerson

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CHAPTER 16

Blood Coagulation and Fibrinolytic System

- Introduction
- Extrinsic Pathway of Coagulation
- Intrinsic Pathway of Coagulation
- Thrombin Formation
- Thrombin Actions
- Natural Anticoagulation Mechanisms
- Fibrinolytic System
- Some Components of Coagulation Cascade

INTRODUCTION

- Process of blood coagulation involves a series of enzymatic reactions that end in the formation of insoluble fibrin clot.
- Blood coagulation serves to prevent blood loss whenever there is injury of a blood vessel in skin or mucous membranes.
- Depending upon the initiating event and the first steps in coagulation cascade two pathways are identified:
 - *Intrinsic pathway* is initiated by contact of blood with a foreign material e.g., prosthetic tissue or artificial valve or physiologic surface rich in collagen or sulfatides.
 - *Extrinsic pathway* is activated by tissue factor exposed at the site of injury.
- Both pathways converge on a common pathway after activation of factor X (figure 16-1).
- Coagulation factors are normally present as plasma proteins in inactive forms of enzyme precursors (zymogen).
- Process of blood coagulation requires besides the initiating event and coagulation factors additional factors:
 - Cofactor proteins.
 - Calcium ions.
 - Cell membrane phospholipid.

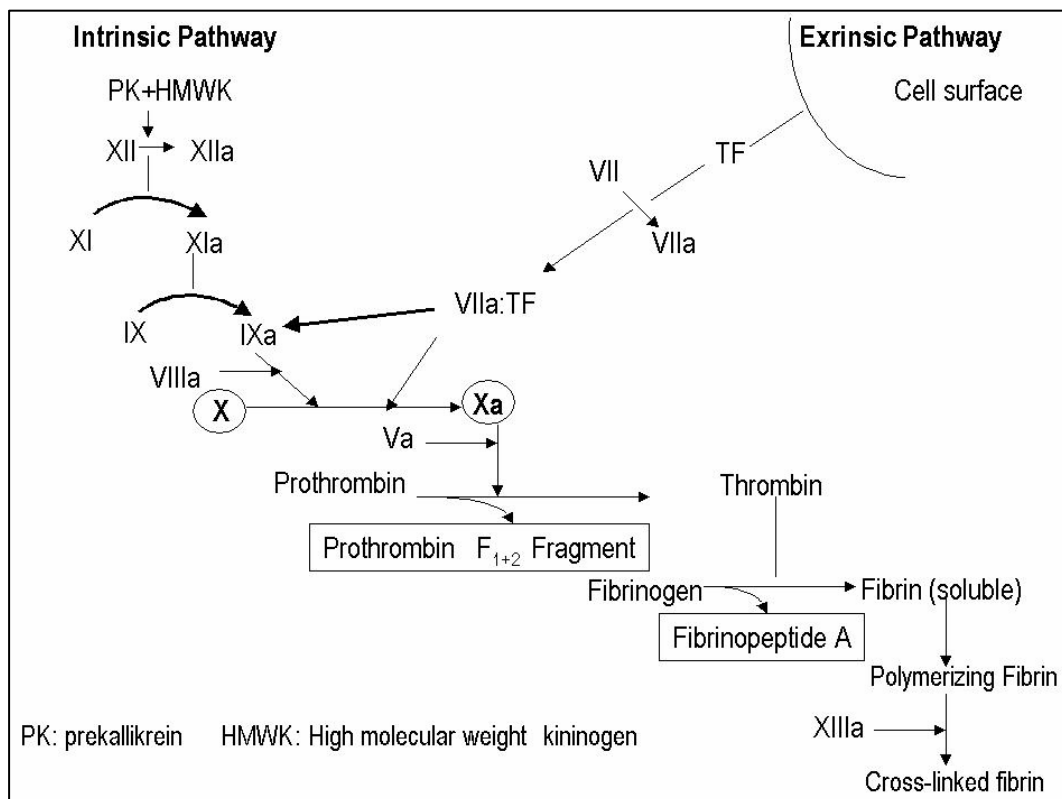


Figure (16-1): Blood Coagulation

EXTRINSIC PATHWAY OF COAGULATION

- Endothelial damage leads to exposure of tissue factor (TF) to blood flow.
- TF is a transmembrane glycoprotein normally expressed by sub-endothelial fibroblast cells, activated monocytes, activated endothelial cells, and atherosclerotic plaques.
- An intact endothelium normally shields the circulating blood from exposure to TF.
- In blood TF combines with coagulation factor VII and activated factor VII (VIIa) increasing its enzymatic activity.
- TF-F VIIa complex activates factors IX and X (figure 16-1).
- Activated factor X (Xa) in combination of activated factor V (Va) converts prothrombin (factor II) to its activated form, thrombin (factor IIa). The process occurs on activated platelet phospholipids membrane.
- Breakdown of prothrombin to thrombin results in the formation of prothrombin fragment, known as prothrombin activation fragment F 1+2.
- Thrombin converts fibrinogen to fibrin, which undergoes polymerization.
- Activated factor XIII (F XIIIa) stabilizes and cross links overlapping fibrin strands.

INTRINSIC PATHWAY OF COAGULATION

- The initial phase of intrinsic or contact activation pathway consists of several plasma proteins activation by contact with negatively charged surfaces.
- Activation of intrinsic pathway is initiated by exposure of collagen basement membrane or microfibrillar substance to the blood.
- Factor XII, a zymogen in plasma, binds to these subendothelial structures and is converted to an active enzyme XIIa.
- Generation of XIIa is enhanced by an autocatalytic circuit that involves the zymogens prekallikrein and plasminogen and the co factor high-molecular-weight kininogen.
- F XIIa rapidly activates F XI which then cleaves factor X zymogen to produce the active enzyme (protease) Xa.
- The conversion of factor X to factor Xa is enhanced by activated co-factor VIII (VIIIa) in conjunction with calcium ions and an activated platelet surface.
- Once the extrinsic and intrinsic pathways have generated sufficient factor Xa, this enzyme can convert prothrombin to thrombin.
- The generation of thrombin is accelerated by the action of co factor Va in conjunction with calcium ions and activated platelet surface.
- Thrombin activates a number of coagulation factors (zymogens), co factors and blood platelets.

Thrombin Formation

Requires six components:

1. Substrate: prothrombin.
2. Enzyme: activated factor X (Xa).
3. Co factor: factor Va.
4. Platelet factor 3.
5. Calcium ions.
6. Phospholipid surface

The enzymes thrombin, factors Xa, Va, VIIa and protein C and S are synthesized in liver cells by a vitamin K dependent process.

Thrombin Actions

- Cleaves the soluble protein fibrinogen to generate the insoluble fibrin with the release of fibrinopeptides A and B.
- Activates factors XII, XI, VIII and V. By activating these precursors to its own generation, thrombin greatly amplifies its own generation.
- Activates platelets.
- Activates protein C through binding with thrombomodulin.
- Stimulates endothelial cells to release tissue plasminogen activator.
- Activates factor XIII (XIIIa). XIIIa in presence of calcium ions renders the fibrin more stable and less amenable to lysis through polymerization and stabilization.

NATURAL ANTICOAGULATION MECHANISMS

Anti-coagulation is modulated by a number of mechanisms

- Removal of activated factors through the reticulo endothelial system.
- Natural antithrombotic pathways which include (Figure 16-2):
 1. Antithrombin-heparan sulfate mechanism.
 2. Protein C-thrombomodulin, protein S mechanism (Figure 16-3).
 3. Tissue factor pathway inhibitor (TFPI).
 4. Prostacyclin and thromboxane.
 5. Nitric oxide (No)

The aim of natural anticoagulant systems is to keep the blood in the fluid state.

- **Antithrombin-heparan sulfate mechanism**
 - Antithrombin (AT) is a circulating plasma protease inhibitor.

- It neutralizes most of the enzymes in the clotting cascade specially thrombin, factors Xa, IXa, XIIa and XIa by forming irreversible complexes.
- Heparan sulfate synthesized by endothelial cells of blood vessels contain a pentasaccharide that mediate part of the actions of AT and enhances several folds the rate of enzyme inhibitor action.

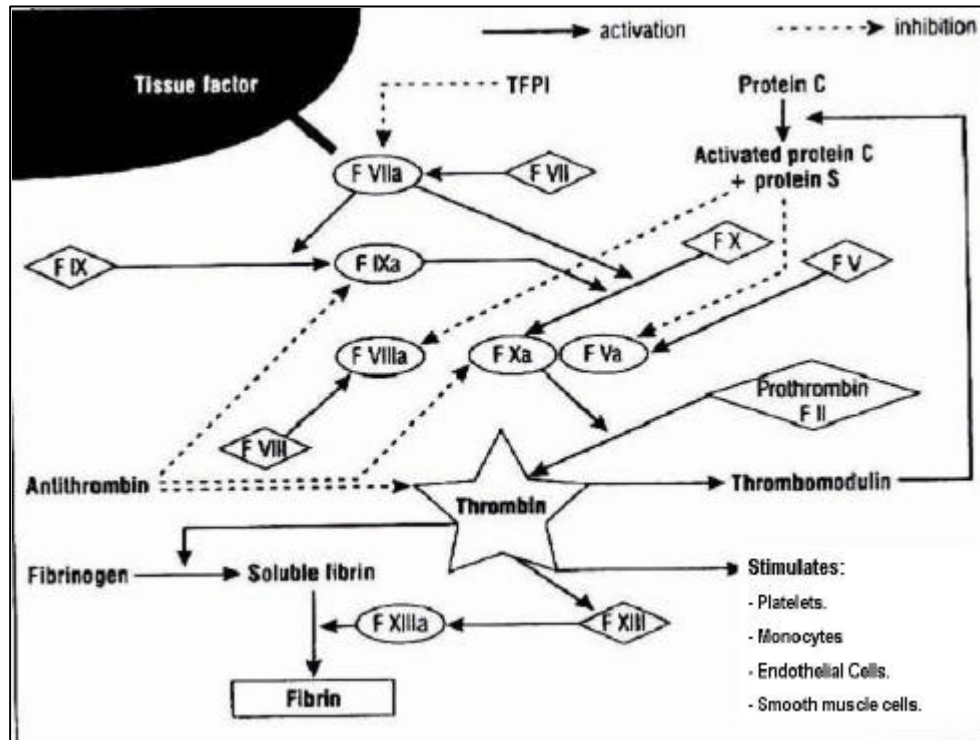


Figure (16-2): Coagulation Cascade and Natural Anticoagulant Systems

Source: Oldgren and Wallentin in Handbook of acute coronary syndromes-2004.

- **Protein C- thrombomodulin, protein S mechanism** (Figure 16-3)
 - Protein C is a zymogen, when activated it breaks down and inactivates coagulation co factors Va and VIIIa.
 - Thrombomodulin (TM) is an endothelial cell receptor.
 - Binding thrombin to TM induces a change in thrombin which makes it able to activate protein C, while loosing its ability to breakdown fibrinogen or activate platelets.
 - Activation of protein C by thrombin TM complex is enhanced by protein S.

Tissue Factor Pathway Inhibitor (TFPI)

- TFPI is synthesized by vascular endothelium and circulates in plasma.
- TFPI inhibits:
 - Factor X activation directly.
 - TF/VIIa complex, thereby impairing the extrinsic pathway.

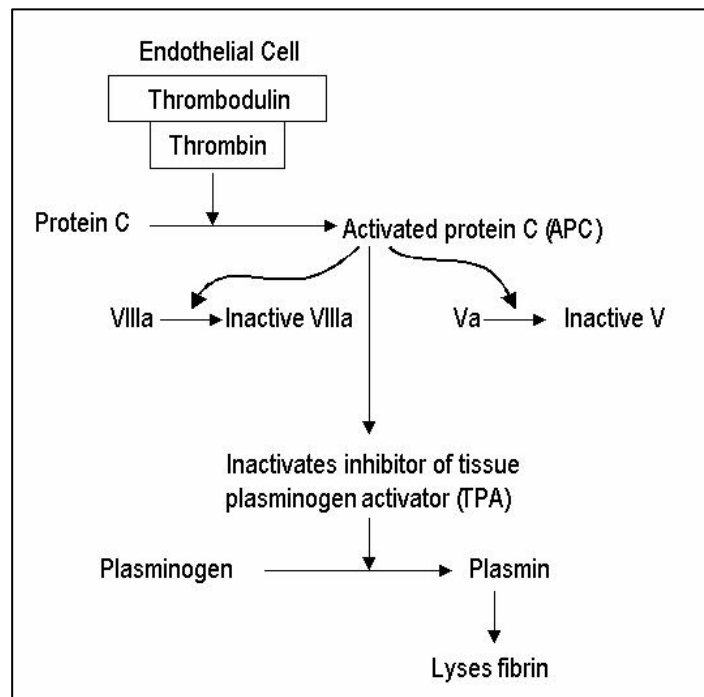


Figure (16-3): The fibrinolytic system and its regulation by protein C.

FIBRINOLYTIC SYSTEM

- Hemorrhage or thrombosis depend on a delicate balance between the procoagulant system and the fibrinolytic system.
- Components:
 1. *Plasminogen.*
 2. *Plasmin.*
 3. *TPA: tissue plasminogen activator.*
 4. *PAI: plasminogen activator inhibitor.*
 5. *α 2 antiplasmin.*
- Proenzyme (zymogen) plasminogen is converted via many pathways into the active enzyme plasmin (figure 16-4).
- Plasmin is a serine protease with a broad spectrum of activity and a large number of substrates.
- Plasmin hydrolyzes both fibrinogen and fibrin into degradation products (FDPs).
- One of the major FDPs is D-dimer, which consists of two D domains from adjacent fibrin monomers that have been cross linked by activated factor XIII.
- Plasmin degrades factors V, VIII, IX and XI, insulin and other proteins.
- Physiologic activation of the fibrinolytic system involves the endothelial cell-derived plasminogen activator (TPA) which converts plasminogen to plasmin directly.
- Plasminogen binds fibrin and TPA. This complex leads to the conversion of the proenzyme plasminogen to active proteolytic plasmin.

- Presence of FDPs in the circulation can compromise hemostasis by interference with fibrin monomer polymerization and platelet function.
- Plasmin activity is regulated by vascular endothelial cells that secrete both plasminogen activators (TPA and urokinase-type) and plasminogen activator inhibitors (PAI-1 and PAI-2).
- TPA circulates in plasma as a complex with its natural inhibitor PAI-1 and is rapidly cleared by the liver.
- Urokinase is the major activator of fibrinolysis in the extravascular compartment.
- PAI-1 is synthesized by endothelial cells and platelets.
- PAI-2 is synthesized by white blood cells and the placenta.
- Alpha-2 antiplasmin is a rapid inhibitor of plasmin activity. It is secreted by the liver and is present within platelets.
- Plasmin released into the circulation is rapidly inactivated by alpha-2 antiplasmin.

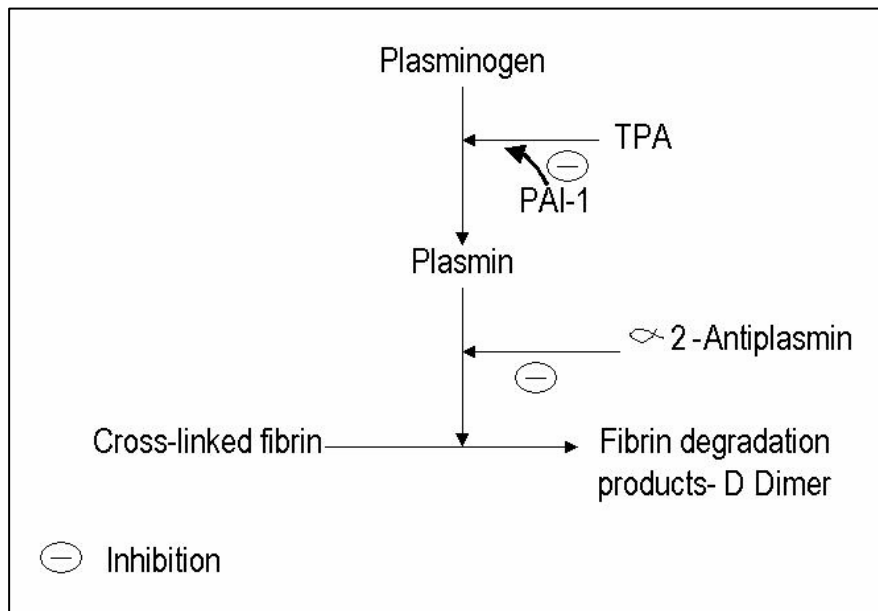


Figure (16-4): Fibrinolytic System

Thrombin-activatable Fibrinolysis inhibitor (TAFI)

- TAFI is a newly recognized physiologic substrate for the thrombin-thrombomodulin complex.
- Activated TAFI function as a fibrinolysis inhibitor. It diminishes the activation of plasminogen, leading to delayed clot lysis.

Some Components of Coagulation Cascade

Coagulation Factor VII

- A vitamin k-dependent proteolytic enzyme (serine protease).

- Plays a central role in initiating the extrinsic coagulation pathway.
- When combined with TF it activates factors IX and X.

Coagulation Factor VIII

- It circulates bound to von Willebrand factor.
- Activated factor VIII (VIIIa) forms a complex with factor Xa on platelet surface. It acts as a co factor.

Factors IX, XI and XII

- Make up the intrinsic coagulation pathway and activate each other reciprocally.
- Activated factor XII activates factor XI, which in turn upon activation activates factor IX.

Prothrombin Fragment 1+2

- Activated factor X splits prothrombin into thrombin and prothrombin fragment 1+2.
- Plasma level of prothrombin fragment 1+2 reflect thrombin generation.

D-dimer

- After formation of fibrin clot, the fibrinolytic system degrades it to produce fibrin degradation products such as D-dimer.
- Levels of D-dimer reflect the extent of fibrin turnover.

Classification of Antithrombotic Agents

I. Indirect Thrombin Inhibitors

Antithrombin dependent: Heparins

II. Direct Thrombin Inhibitors

Antithrombin independent

- a. Parenteral:
 1. Hirudin
 2. Bivalirudin
 3. Argatroban
- b. Oral: Ximelagatran

III. Thrombin Generation Inhibitors

- a. Factor Xa inhibitors.
 1. LMWHs.
 2. Pentasaccharide.
 3. TAP: Tick anticoagulant peptide.
- b. Tissue factor pathway inhibitor (TFPI)