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

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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.

![Blood Coagulation](image-url)

*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-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:
2. Enzyme: activated factor X (Xa).
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-thrombodulin, 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.

![Coagulation Cascade and Natural Anticoagulant Systems](image)

**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.
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. **a2 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.

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

![Fibrinolytic System](image)

**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 cofactor.

**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)