PHTX 441
Drugs that affect Coagulation and Clot Integrity
Steve Sawyer
Sept 28, 2004

**Anticoagulants and Thrombolytic Agents**

**Learning Objectives:**
1. Coagulation cascade- learn in vivo pathway and key steps in blood coagulation and platelet reaction in both hemostasis and thrombosis.
2. Injectable anticoagulants- mechanism of heparin and hirudin action
3. Oral anticoagulants- warfarin and related compounds-action in Vitamin K-dependent reactions
4. Vitamin K mechanism of action
5. Agents that accelerate and suppress Fibrinolysis- TPA, streptokinase, tranexamic acid
6. Agents that promote clotting- Vitamin K, Clotting factors for replacement, Desmopressin
Hemostasis

- Hemostasis is the arrest of blood loss from damaged vessels and is essential for survival.

- The main phenomena are:
  1) platelet activation,
  2) blood coagulation and
  3) vascular contraction.

- The lecture is primarily focused on blood coagulation.

Blood Clot formed by Fibrin

Fibrin forms framework of clot: fibrin acts to trap blood cells that form bulk of clot

Thrombosis

Thrombosis is the pathological condition of (unnecessary) clotting

1. Venous thrombosis due to slow circulation without significant platelet activation. Thrombus may break away from vessel wall forming an embolus. Emboli clogging vessels in the heart, lungs and brain results in tissues deprived of circulation (oxygen) and are a major cause of death.

2. Arterial thrombosis usually associated with arteriosclerosis and inappropriate platelet activation. Heart Attack and Stroke result primarily from arterial thrombosis in either heart or brain.

FIGURE 1. Simplified version of coagulation that results in formation or thrombus from either vascular damage or atherosclerotic plaque

HEMOSTASIS AND THROMBOSIS

1. Hemostasis is the arrest of blood loss from damaged vessels and is essential for survival. The main phenomena are i) platelet adhesion and activation and ii) blood coagulation (fibrin formation), and iii) vascular contraction.

2. Thrombosis is a pathological condition. Venous thrombosis usually results from slow blood flow and coagulation without significant initial platelet activation. Arterial thrombosis usually is associated with arteriosclerosis and platelet activation. Thrombus may break away from vessel wall becoming an embolus. Emboli clogging vessels in the heart, lungs and brain results in tissues deprived of circulation (oxygen) and are a major cause of death.
Much of knowledge of the molecular / biochemical basis of Blood Coagulation comes from the study of genetic diseases that affect coagulation. An example is as classic hemophilia which results from a mutation of Factor VIII on the X chromosome.

In Vivo Pathway of Blood Coagulation

i. Tissue factor exposed by vessel damage-the binding site of VIIa located at the wound site, additional binding sites (acidic phospholipids) provided by activated platelets.

ii. Common pathway after factor X. Factor Xa cleaves factor II (prothrombin) to IIa (thrombin) in the presence of factor Va bound to membranes.

iii. Thrombin, IIa, is central in control of coagulation-acts to cleave factor I, fibrinogen, to insoluble fibrin. Fibrin meshwork traps blood cells to form the clot. Also converts XIII to XIIIa to stabilize the fibrin meshwork, activates factors V, VII, VIII, & XI, activates platelets, and acts on endothelial cells.

THE IN VIVO OR EXTRINSIC PATHWAY

THE CONTACT SYSTEM OR INTRINSIC PATHWAY

1. Clotting system is a cascade of enzymes and cofactors-factors 1 through XIII.
2. Inactive precursors are activated in series, each giving rise to the next.
3. The last enzyme, thrombin, derived from prothrombin, converts soluble fibrinogen (factor I) into insoluble meshwork of fibrin in which blood cells are trapped, forming the clot.
4. There are two pathways in the cascade: the extrinsic or in vivo pathway while the intrinsic is triggered in the test tube.
5. Both pathways activate Factor X, which converts prothrombin to thrombin.
6. Calcium and negatively charged phospholipids are required in three enzymatic cleavage steps: IX on X, XI on X, and X on II.
7. Negative phospholipids are provided by activated platelets that have adhered to the site of injury. This localizes the site of clot formation.
8. Binding proteins as well as enzymes are used, i.e. factor V in X cleaving II.

A. Hemostasis-physiological arrest of blood loss

1. Platelet Reactions
   a. Adhesion, activation, and aggregation of platelets
   b. Exposure of acidic (negatively charged) phospholipids that are binding sites for coagulation factors
   c. Release of factors: [ADP, TXA2, PAF]
   d. Further Aggregation of Platelets
Vitamin K
Discovered in Denmark and named the “Koagulation” Vitamin
Necessary for efficient blood coagulation

Vitamin K Function
& Inhibition of Vitamin K re-use by Warfarin

Necessary for efficient blood coagulation

The activation of prothrombin (Factor II) by factor Xa

II. Agents that increase coagulation.

A. Vitamin K - Phytonadione (Aqua Mephyton, Konakion), Menadiol Sodium (Synkavite)

1. Action - required for post-translational modifications of glutamic acid residues on clotting factors that allow these proteins to bind to the membranes (negatively charged phospholipids) of damaged cells in the vessel wall.

2. Clinical use of Vitamin K
   i. bleeding due to excess oral anticoagulants
   ii. hemorrhagic disease of newborns
   iii. vitamin K deficiencies - i.e. adsorption problems
Agents that increase coagulation.
Replacement of missing or defective Plasma Clotting Factors - treatment of genetic diseases of coagulation

a. Factor VIII, lacking in classic hemophilia or Hemophilia A (Hemofil M, Monoclate P, Koate HP, Profilate OSD),
b. Factor IX lacking in Hemophilia B (Konyne 80, Proplex T, Humate-P, Profilnine)
c. Desmopressin (DDAVP, STIMATE, RHINYLE): posterior pituitary hormone used to maintain hemostasis in hemophilia and Von Willebrand’s disease by promoting release of Factor VIII from stores in platelets and/or liver, Intranasal or IV route

Vitamin K Antagonist
Warfarin - both rat poison and oral anticoagulant for humans

Vitamin K Function
& Inhibition of Vitamin K re-use by Warfarin

Agents that inhibit coagulation

A. Oral anticoagulants - Warfarin (Coumarin) and Bisphodyxoumarin (Dicumarol)
- These drugs act as competitive inhibitors in the reduction of oxidized vitamin K that regenerates active vitamin K from the inactive vitamin K.
- Vitamin K loses hydrogen atoms to the reaction (is oxidized) to put COOH groups onto coagulation factors that allow them to bind to phospholipids.
- An enzyme normally puts these hydrogen atoms back onto vitamin K (reducing Vitamin K). The oral anticoagulants bind to this enzyme and prevent it from restoring oxidized vitamin K to a functional form. Thus even though coagulation factors are synthesized, they do not function due to the lack of functional vitamin K necessary to carry out the final step in production.
Side effects of Oral anticoagulants

Side effects: Oral anticoagulants have delayed effects and interaction with other drugs is often a problem causing:
1) Birth defects,
2) Interactions with other drugs,
3) Risk of bleeding.

The effects of these agents can be reversed with Vitamin K but the Vitamin K acts slowly since new clotting factors must be synthesized. Typical therapy for reversal of oral anticoagulants is transfusion of plasma and/or whole blood to supply new clotting factors.

B. Injectable anticoagulants

i. Cofactor for Antithrombin III (ATIII)- Heparin

a. Heparin- family of sulfated glycosaminoglycans or mucopolysaccharides of 3,000 to 40,000 molecular weight. Heparin binds to ATIII, an inhibitor of coagulation factors, and accelerates the binding of ATIII to factors Xa and thrombin, primarily, but others as well. The extracellular matrix of cells lining the vessel walls, the endothelium, have heparin-like molecules that probably act to reduce coagulation in undamaged vessels.

Heparin- an injectable, fast-acting anticoagulant that acts in concert with antithrombin III
Heparin injected IV- (Lipohepin, Liquaemin)

- Side effects: 1) excessive bleeding, 2) allergic reactions since heparin is an animal product, 3) thrombocytopenia (reduced platelets in the circulation)

Injecting protamine sulfate that forms an inactive complex with heparin can reverse action of heparin.

b. Low molecular weight heparin (Enoxaparin, Dalteparin, Ardeparin, Danparoid)- smaller heparin that accelerate the binding of ATIII to Xa but not thrombin. This is longer acting and more bioavailable than heparin when given by subcutaneous injection. Used to prevent deep vein thrombosis in patients undergoing abdominal surgery and hip or knee replacement.

ii. Antithrombin III-independent anticoagulants

a. Hirudin- anticoagulant protein from leeches is the most potent inhibitor of thrombin known. Either leeches are applied to patient on area of desired effect or this protein is made by recombinant DNA technology and injected intravenously.

b. Hirugen*- small peptide derived from Hirudin

VI. Fibrinolysis or Thrombolysis- Physiological Pathway by which Clots are dissolved

A. Plasmin (fibrinolysin)- proteinase that degrades the fibrin meshwork of the clot

- Plasmin is formed by the action of plasminogen activator on an inactive precursor molecule- Plasminogen. [Plasmin is a trypsin-like enzyme that cleaves Arg-Lys bond in not only fibrin, but fibrinogen, factors II, V, and VIII and many other proteins.]* Its action is confined to the clot by many plasmin inhibitors in the circulation.

B. Plasminogen activator- Proteinase that diffuses into the clot and cleaves plasminogen to plasmin

1. Tissue-type plasminogen activator (TPA)
Main plasminogen activator in fibrinolysis-derived from endothelium of small vessels and phagocytic cells.
2. Urokinase- enzyme used for tissue remodeling
THROMBUS

PLASMINOGEN

PLASMIN

FIBRIN FIBRIN DEGRADATION PRODUCTS

FIBRINOGEN

Natural PLASMINOGEN ACTIVATORS-

ANTIFIBRINOLYTIC DRUGS-

TRANSEXAMIC ACID

TPA-Alteplase

STREPTOKINASE

UROKINASE

Blood Vessel

FIBRINOLYTIC AGENTS

Blood Clot formed by Fibrin and degraded by Plasmin

Fibrin framework of clot is degraded by activation of plasmin from plasminogen

Thrombin

FIBRINOGEN

FIBRIN

ANTIFIBRINOLYTIC DRUGS-

TRANSEXAMIC ACID

PLASMINOGEN

PLASMIN

Natural PLASMINOGEN ACTIVATORS-

TPA-Alteplase

STREPTOKINASE

UROKINASE

Blood Vessel

FIBRINOLYTIC AGENTS

Therapy for Heart Attack: TPA Breaks Up Thrombus/Clot in Coronary Artery

Figure A is X-ray image showing blockage of coronary artery in a patient suffering a heart attack. After injection and continuous infusion of the fibrinolytic agent, TPA, for 3 hours, the X-ray in figure B shows that the reappearance of the white (X-ray reflective) marker of blood circulation reveals that the blockage has been removed.

VII. Fibrinolytic and Antifibrinolytic Agents

A. Injected plasminogen activators that directly cleave plasminogen to plasmin in vivo. Used to dissolve unwanted thrombi in heart attack and stroke. A critical factor is rapid use (within 2 hours preferably and useless after 3 to 6 hours since tissue will become necrotic without blood flow).

1. Alteplase or TPA (Activase) - recombinant human tissue-type plasminogen activator - nonantigenic, high clot selectivity, short half-life, high cost

2. Reteplase (Retavase) - alternative human tissue plasminogen activator similar to TPA, longer half-life than Alteplase

3. Urokinase or u-PA (Abbokinase Open-Cath) - plasminogen activator isolated from cultures of human embryonic kidney cells (used primarily for clearance of catheters)

B. Proteins that act like plasminogen activators

Streptokinase (Streptase) - protein from beta-hemolytic streptococci that has no enzymatic activity. Streptokinase binds to plasminogen and causes a change in conformation that converts the normally inactive plasminogen into an active fibrin-cleaving enzyme. It is proven to reduce deaths from myocardial infarction when infused intravenously for more than 1 hour. However, it is antigenic such that repeated use or past streptococci infection may result in anaphylactic reactions. Much cheaper than TPA to use but poor selectivity of clots.
Special Rules in using Fibrinolytic drugs for treatment of Stroke

- Treatment of Stroke victims limited to TPA but not streptokinase
- Stroke victim must be first evaluated for cranial bleeding because 20% of strokes are hemorrhagic in nature; i.e. the thrombus/clot blocks circulation, blood pressure rises and blood vessel bursts.
- Stroke Victim must have been awake when stroke symptoms first appeared so that exact time of stroke initiation is known. TPA must be given as soon as possible and therapy after three hours is more dangerous than helpful.

C. Antifibrinolytic agents

1. Tranexamic acid-Used reduce GI bleeding because it promotes clot formation by slowing the destruction of naturally formed clots by plasmin. It blocks conversion of Plasminogen into plasmin by blocking plasminogen activator activity.
2. Aprotinin
3. PCC
4. APCC

Blood Clot formed by Fibrin and degraded by Plasmin

Fibrin framework of clot is degraded by activation of plasmin from plasminogen

Blood Vessel

Coagulation Issues in Dentistry

A. Cause of Excessive and Prolonged Bleeding from procedures that induce gum trauma
   - Genetic defects
     - Hemophilia A or B
     - Other rare mutations in coagulation
   - Anti-platelet drugs (aspirin etc)
   - Oral anticoagulants (warfarin)
   - Injectable Anticoagulants (heparin and LMW heparins)
   - Antifibrinolytic drugs
Coagulation Issues in Dentistry

B. Cause of Minor but prolonged bleeding

1. Genetic defects in coagulation
   • Heterozygous genetic defects (2% of population as mild von Willibrand disease)
   • Factor XI defects (very rare)
2. Antiplatelet drugs
3. Oral anticoagulants

Coagulation Issues in Dentistry

What can the dentist do to reduce bleeding problems with patients?

1. Ask if patient or family has history of coagulation problems with before starting procedure that will trigger moderate hemorrhage.
2. Inquire if patient has taken prescription and non-prescription drugs that affect blood coagulation and consult with patient’s physician if dose of oral anticoagulant, antiplatelet drug, etc can be stopped or reduced before initiating procedure that will trigger hemorrhage.
3. Patients with genetic disorders require factor replacement therapy or Desmopressin therapy to increase concentration of clotting factors. Consult with patient’s Hematologist!