BLOOD COAGULATION

Blood coagulation is a dynamic and complex process that limits blood loss from the site of vascular injury. In the physiological setting, this process is finely regulated so as to avoid vessel occlusion. Thrombosis refers to the pathological condition where the perfusion of the vital organ(s) is attenuated due to the occlusion of vessel(s) by blood clots and, in most cases, poses a life-threatening emergency. Drugs that are used for the treatment and prophylaxis of thromboembolic diseases are the subjects of this lecture.

The first step of the physiological process eventually leading to clot formation on the site of vascular injury is platelet adherence and activation via the exposed sub-endothelial molecules such as collagen and von Willebrand factor (Figure 1). Vasoconstriction and platelet aggregation then follows, by the effects of thromboxane A_2 (TXA_2), ADP and 5HT that are released from the activated platelets. Platelet plug thus formed is then stabilized by the fibrin clot generated by the coagulation cascade. Platelet aggregation is central to normal hemostasis and thromboembolic disease, and is the target of several drugs known as antiaggregant (or antiplatelet) drugs.

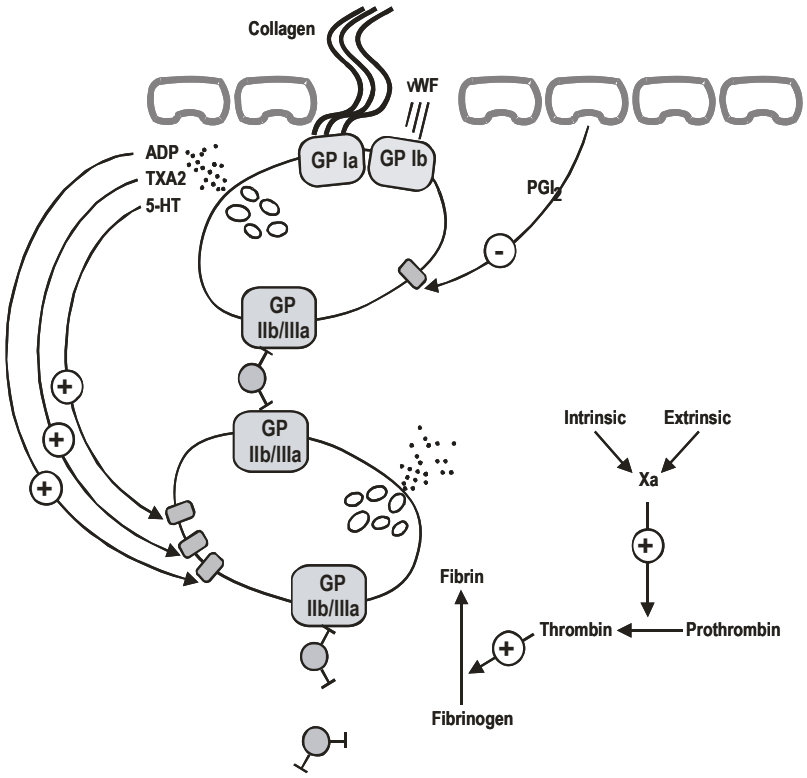

Figure 1. Platelet adherence, activation and aggregation at the site of vessel injury

Blood coagulates when thrombin converts soluble fibrinogen to insoluble fibrin, which in turn forms polymers that bind to and stabilize platelet plug. Thrombin (Factor IIa) is generated as the end product of a series of reactions known as coagulation cascade. The clotting factors (shown as roman numbers in Fig. 1) involved in this cascade are zymogens: In each step, a clotting factor is activated by limited proteolysis. Several of these factors are the targets of anticoagulant drugs.

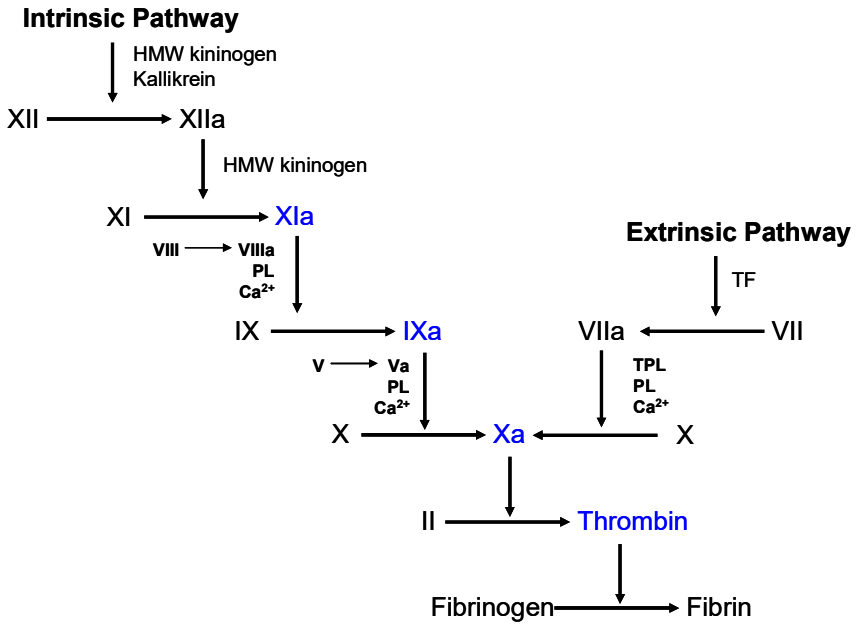

Figure 2. Coagulation cascade

Clot formation is normally limited to the site of injury by the so-called natural anti-coagulant mechanisms such as inactivation of active coagulation factors by antithrombin, protein C and protein S. Plasmin, generated from its circulating inactive precursor plasminogen by tissue plasminogen activator (t-PA), limits the extension of thrombus by proteolytic digestion of fibrin. Plasminogen to plasmin conversion is the target of fibrinolytic drugs.

ANTICOAGULANT DRUGS

Drugs that exert antithrombotic effect by interfering with the coagulation system are called anticoagulants. Based on the mechanism of action, they can be grouped as follows:

- Indirect thrombin inhibitors
- Warfarin and other coumarin anticoagulants
- Direct Factor Xa inhibitors
- Direct thrombin inhibitors

I. Indirect Thrombin Inhibitors

Unfractionated heparin (UFH), low molecular weight heparin (LMWH) and synthetic pentasaccharide Fondaparinux exert anticoagulant effect by enhancing antithrombin activity. Thus, their anticoagulant effect is indirect. Antithrombin inhibits activated clotting factors (shown as blue in Fig. 2), especially thrombin and factor Xa, by forming stable complexes with them. In the absence of heparin, these reactions are slow; when heparin binds to antithrombin, they are accelerated 1000 fold. All three drugs enhance inactivation of factor Xa. UFH, and to a lesser extend LMWH also enhance inactivation of thrombin.

Commercial UFH is a heterogeneous mixture of sulfated mucopolysaccharides with different lengths: Only about a third of the molecules in these preparations have an enhancing effect since the remainder lacks the minimum pentasaccharide structure (Fig. 3) required for the high affinity binding to antithrombin. LMWH is obtained by fractionation of UFH.

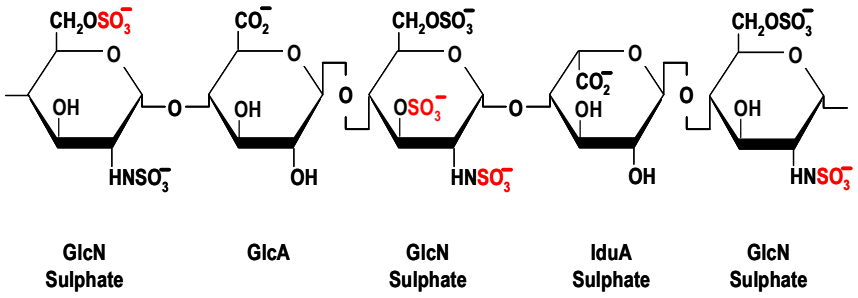

Figure 2. Minimum pentasaccharide structure of heparin

Properties of indirect thrombin inhibitors are summarized in Table 1.

	Heparin	LMWH	Fondaparinux
Source	Biological	Biological	Synthetic
Mol. Weight	15000	5000	1500
Target	Xa and IIa	Xa and IIa	Xa
Bioavailibility	30%	90%	100%
$T_{1/2}$(h)	1	4	17
Renal Elimination	No	Yes	Yes
Antidote	Complete	Partial	No
Thrombocytopenia	>5%	>1%	>1%

Pharmacokinetics

None of the indirect thrombin inhibitors is orally bioavailable: They have to be given parenterally. When given by intravenous route, anticoagulant effect starts rapidly. Thus, they are the drugs of choice in the emergency cases such as pulmonary thromboembolism. Heparin has the lowest and the most unpredictable bioavailability when given subcutaneously. LMWH preparations such as enoxaparin, dalteparin, and tinzaparin have mostly replaced UFH, as they have equal efficacy, increased bioavailability from the subcutaneous site of injection, and less frequent dosing requirements. However, heparin is the drug of choice when the anticoagulant effect has to be terminated abruptly, as in the case of coronary artery by-pass surgery (compare the elimination half lives in Table 1).

Doses of heparin are specified in international standard units (IS), those of enoxaparin and fondaparinux in milligrams, and those of Dalteparin and tinzaparin in anti-factor Xa units.

Monitoring of the Heparin Effect

Patients receiving UFH must be monitored by activated partial thromboplastin time (aPTT) throughout the therapy. LMW heparin levels can be determined by anti-Xa units. Therapy with LMWH requires less frequent monitoring except in the setting of renal insufficiency, obesity, and pregnancy.

Toxicity

The major adverse effect of heparin and its derivatives is bleeding. Close monitoring of the patients receiving these drugs is necessary for reducing the risk. In the case of excess anticoagulant activity the drugs must be discontinued. If bleeding occurs, heparin action can be neutralized by administration of protamine sulfate, a highly basic peptide that forms stable complexes with heparin. Neutralization of LMWH by protamine is only partial. Fondaparinux can not be neutralized by protamine.

Another serious adverse effect of heparin is heparin-induced thrombocytopenia (HIT). HIT is a hypercoagulable state that leads to thrombotic events in the course of heparin treatment. Patients receiving heparin should be closely monitored by frequently performing platelet counts and thrombocytopenia should be considered suspicious for HIT. Any new thrombus occurring during the heparin therapy should also raise the suspicion of HIT. In the case of HIT, heparin is discontinued and patient is treated with the direct thrombin inhibitor argatroban.

Heparin is contraindicated in patients with:

- HIT
- hypersensitivity to the drug
- active bleeding

- hemophilia
- significant thrombocytopenia
- purpura
- severe hypertension
- intracranial hemorrhage
- infective endocarditis
- active tuberculosis
- ulcerative lesions of the gastrointestinal tract
- threatened abortion
- visceral carcinoma
- advanced hepatic or renal disease

Heparin should be avoided in patients who have recently had surgery of the brain, spinal cord, or eye; and in patients who are undergoing lumbar puncture or regional anesthetic block. Despite the apparent lack of placental transfer, heparin should be used in pregnant women only when clearly indicated.

II. Warfarin and Other Coumarin Anticoagulants

Warfarin is a synthetic derivative of a natural anticoagulant bishydroxycoumarin and, among the substances known as coumarin anticoagulants, the only antithrombotic agent used in humans.

Warfarin (and other coumarin anticoagulants) inhibits γ-carboxylation of several glutamate residues in factors II, VII, IX, and X as well as the endogenous anticoagulant proteins C and S. γ-carboxylation is essential for coagulant activity of these factors. Thus, in the presence of warfarin, biologically inactive coagulation factors are produced by the liver. Inhibition of γ-carboxylation is due to the competitive inhibition of the enzyme vitamin K epoxide reductase (VKORC1) and thus blockade of reduction of oxidized vitamin K, the by-product of the γ-carboxylation reaction (Fig. 3).

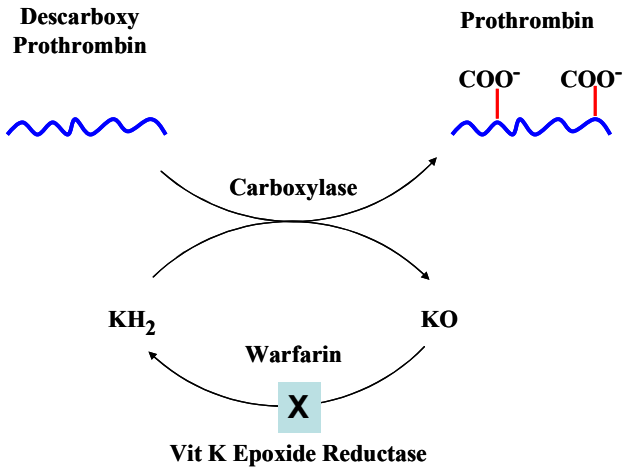

Figure 3. Action mechanism of Warfarin

Anticoagulant effect of warfarin starts after a lag period of at least 8-12 hours which reflects the time required for the replacement of the vitamin K-dependent clotting factors in the systemic circulation with their newly synthesized biologically inactive counterparts (Fig.4). Hence, warfarin can not be used in acute cases: Therapy in active thromboembolic states should always be initiated with UFH or LMWH to achieve immediate anticoagulation until adequate warfarin-induced depletion of the procoagulant clotting factors is achieved. The duration of this overlapping therapy is generally 5–7 days, after which warfarin can be administered alone.

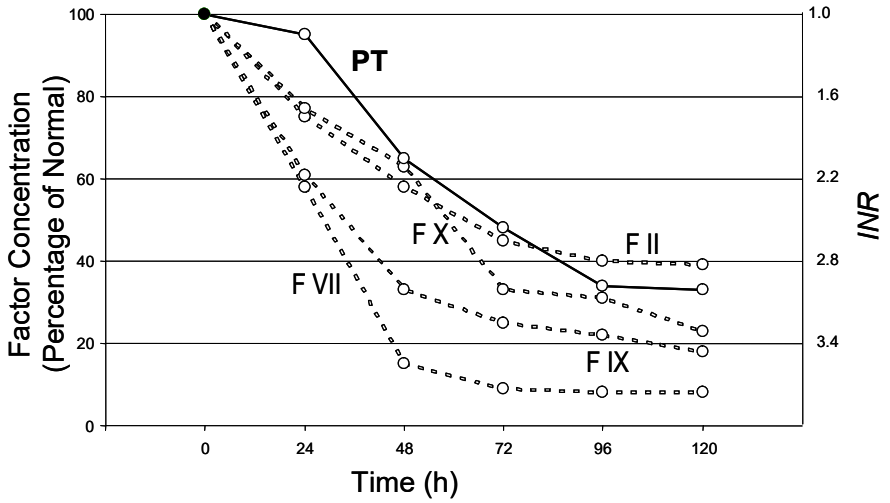

Figure 4. Time course of Warfarin-induced depletion of active coagulation factors and increase in PT (in INR)

Pharmacokinetics

Warfarin is also known as the oral anticoagulant; its oral bioavailability is 100%. Over 99% of warfarin is bound to plasma albumin. Commercial warfarin preparation is a racemic mixture composed of equal amounts of two enantiomorphs. The levorotatory S-warfarin is four times more potent than the dextrorotatory R-warfarin. Warfarin is metabolized in the liver by cytochrome P450 CYP2C9 enzyme.

Monitoring of the Warfarin Effect

Patients receiving warfarin should be monitored regularly by protrombin time (PT) determinations. The therapeutic range for oral anticoagulant therapy is defined in terms of an international normalized ratio (INR). The INR is the prothrombin time ratio (patient prothrombin time/mean of normal prothrombin time for lab)^{ISI}, where the ISI exponent refers to the International Sensitivity Index and is dependent on the specific reagents and instruments used for the determination. The recommended INR for prophylaxis and treatment of thrombotic disease is 2–3.

Toxicity

The major adverse effect of warfarin is bleeding. Close monitoring of the patients receiving warfarin is necessary for reducing the risk. Excessive anticoagulant effect and bleeding from warfarin can be reversed by stopping the drug and administering oral or parenteral vitamin K1, fresh-frozen plasma, prothrombin complex concentrates, and recombinant factor VIIa (rFVIIa). Disappearance of the excessive effect is not correlated with the plasma concentration but with the reestablishment of the normal activity of the clotting factors.

Warfarin crosses the placenta and may cause bleeding as well as bone-related birth defects in the fetus. Hence, warfarin should never be used in pregnant women. Cutaneous necrosis, and infarction of the breast, fatty tissues, intestine, and extremities may occur and are probably due to the warfarin-induced depletion of protein C.

Drug Interactions

Coumarin anticoagulants interact with several drugs and disease states, some of which are given in Table 2

Drugs and disease states that increase the anticoagulant effect of Warfarin		Drugs and disease states that decrease the anticoagulant effect of Warfarin	
Farmacokinetic	Farmacodynamic	Farmacokinetic	Farmacodynamic
Amiodarone Cimetidine Disülfiram Metronidazole Fluconazole Phenylbutazone Sulfinpyrazone Trimethoprim-sulfamethoxazole	Aspirin Cephalosporins Heparin, argatroban, dabigatran, apixaban, rivaroxaban Liver disease Hyperthyrodism	Barbiturates Cholestyramine Rifampin	Diuretics Vitamin K Genetic resistance Hypothyrodism

III. Direct Factor Xa Inhibitors

Factor Xa inhibitors, including rivaroxaban, apixaban, and edoxaban represent a new class of anticoagulant drugs that can be given in fixed doses and do not require monitoring. Along with oral direct thrombin inhibitors this new class of direct oral anticoagulant drugs is having a major impact on antithrombotic pharmacotherapy.

Andexanet alfa which is a recombinant analogue of factor Xa acts as a decoy for oral factor Xa inhibitors and has been shown to be useful for reversal of anticoagulant effect of direct factor Xa Inhibitors.

IV. Direct Thrombin Inhibitors

The direct thrombin inhibitors bind to the active site of thrombin and inhibit its downstream effects. Bivalirüdin and argotraban are administered by parenteral route whereas dabigatran etexilate mesylate is orally active.

The direct oral anticoagulant drugs have consistently shown equivalent antithrombotic efficacy and lower bleeding rates when compared with traditional warfarin therapy. In addition, these drugs offer the advantages of rapid therapeutic effect, no monitoring requirement, and fewer drug interactions in comparison with warfarin, which has a narrow therapeutic window, is affected by diet and many drugs, and requires monitoring for dosage optimization.

Therapeutic uses of anticoagulant drugs are given in Table 3

Drugs	Therapeutic Uses
Unfractionated Heparin	
Heparin	• Prophylaxis/treatment of venous thromboembolism • Acute coronary syndrome • Percutaneous coronary intervention • Cardiopulmonary bypass surgery • Disseminated intravascular coagulation
Low-Molecular-Weight Heparin	
Enoxaparin Dalteparin Tinzaparin	• Prophylaxis against venous thrombosis • Initial treatment of venous thromboembolism •Maintenance treatment in cancer-associated thromboembolism • Acute coronary syndrome
Fondaparinux	
Fondaparinux	• Prophylaxis against venous thromboembolism • Initial treatment of venous thromboembolism • Heparin-induced thrombocytopenia • Acute coronary syndrome
Vitamin K Antagonist	
Warfarin	• Treatment of venous thromboembolism in tandem with parenteral anticoagulation • Secondary prevention of venous thromboembolism • Prevention of stroke in atrial fibrillation • Prevention of stroke in patient with mechanical heart valves or ventricular assist devices
Direct Parenteral Thrombin Inhibitors	
Desirudin	• Thromboprophylaxis after hip arthroplasty
Bivalirudin	• Percutaneous coronary intervention • Heparin-induced thrombocytopenia
Argatroban	• Heparin-induced thrombocytopenia

Direct Oral Thrombin Inhibitor	
Dabigatran etexilate	• Treatment of acute venous thromboembolism after at least 5 days of parenteral anticoagulation • Secondary prevention of venous thromboembolism • Prevention of stroke in atrial fibrillation • Thromboprophylaxis after hip or knee arthroplasty
Direct Oral Factor Xa Inhibitors	
Rivaroxaban	• Treatment of acute venous thromboembolism • Secondary prevention of venous thromboembolism • Prevention of stroke in atrial fibrillation • Thromboprophylaxis after hip or knee arthroplasty • Prevention of recurrent ischemia in stabilized acute coronary syndrome patients
Apixaban	• Treatment of acute venous thromboembolism • Secondary prevention of venous thromboembolism • Prevention of stroke in atrial fibrillation • Thromboprophylaxis after hip or knee arthroplasty
Edoxaban	• Treatment of acute venous thromboembolism after at least 5 days of parenteral anticoagulation • Secondary prevention of venous thromboembolism • Prevention of stroke in atrial fibrillation

FIBRINOLYTIC DRUGS

Streptokinase, urokinase and recombinant human tissue plasminogen activators (t-PAs; alteplase, reteplase and tenecteplase) are currently used fibrinolytic drugs that lyse thrombi by catalyzing the formation of plasmin from its precursor, plasminogen. Streptokinase is a protein synthesized by streptococci and acts by binding to proactivator plasminogen. Urokinase and t-PAs directly convert plasminogen to plasmin. All fibrinolytic drugs are administered by intra venous route.

Indications for fibrinolytic drugs are:

- pulmonary embolism with hemodynamic instability
- severe deep venous thrombosis such as the superior vena caval syndrome
- ascending thrombophlebitis
- Acute myocardial infarction

Fibrinolytic drugs create a generalized lytic state and break down both protective hemostatic thrombi and target thromboemboli. Hence, they can cause bleeding as a major adverse effect. Use of fibrinolytic drugs is contraindicated in patients with:

- Intracranial hemorrhage
- Structural lesions in cerebral blood vessels
- Malignant intracranial tumors

- Ischemic attack in the last three months
- Suspected aortic dissection
- Active bleeding or bleeding diathesis
- Closed cranial or facial trauma in the last three months

Fibrinolytic therapy should also be avoided in patients with:

- Uncontrolled hypertension
- Major surgical operation in the last three weeks
- Internal bleeding in the last two weeks
- Known streptokinase hypersensitivity (for streptokinase)
- Pregnancy
- Peptic ulcer
- Warfarin use (INR>1.7)

ANTIAGGREGANT DRUGS

Among the substances that regulate platelet aggregation (see Fig. 1), several are targets for antiaggregant drugs. Currently used antiaggregant drugs exert their effect by:

- Inhibition of prostaglandin (thromboxan A_2) synthesis (aspirin)
- Inhibition of ADP-induced platelet aggregation (clopidogrel, prasugrel, ticlopidine)
- Blockade of glycoprotein IIb/IIIa (GP IIb/IIIa) receptors on platelets (abciximab, tirofiban, and eptifibatide)

Aspirin inhibits the synthesis of thromboxane A_2 by irreversible acetylation of the enzyme cyclooxygenase. It is used for the secondary prophylaxis of vascular events.

Clopidogrel, prasugrel and ticlopidine are irreversible antagonists of ADP $P2Y_{12}$ receptor on platelets. Ticlopidine is approved for prevention of stroke in patients with a history of a transient ischemic attack (TIA) or thrombotic stroke, and in combination with aspirin for prevention of coronary stent thrombosis. Clopidogrel is approved for patients with unstable angina or non-ST-elevation acute myocardial infarction in combination with aspirin; for patients with ST-elevation myocardial infarction; or recent myocardial infarction, stroke, or established peripheral arterial disease. Prasugrel is approved for patients with acute coronary syndromes. Cangrelor and and ticagrelor are newer reversible $P2Y_{12}$ receptor antagonists.

Activation of the platelet GP IIb/IIIa (integrin $\alpha IIb\beta 3$) receptor complex is the final common pathway for platelet aggregation. The GP IIb/IIIa antagonists are used in patients with acute coronary syndromes. Abciximab is a chimeric monoclonal antibody directed against the IIb/IIIa complex and has been approved for use in percutaneous coronary intervention and in acute coronary syndromes. Eptifibatide is a cyclic peptide derived from rattlesnake venom. Tirofiban is a peptidomimetic inhibitor. Because of their short half-lives, the GP IIb/IIIa antagonists must be given by continuous infusion.