BLOOD COAGULATION

(1)

* Normal circumstances,
a delicate balance between coagulation and fibrinolysis prevents both thrombosis and Haemorrhages.
Any alterations in these balance → Thrombosis

Hemostasis

↓

Cessation of blood loss from a damaged vessel.

3 stages → 1. Platelet Aggregation
2. Clot or fibrin formation
3. Fibrinolysis

* **Platelet Aggregation** → platelet aggregation occur by adherence to macromolecules in the subendothelial regions of the injured blood vessel and get activated.
↓
to form an Haemostatic plug.

Platelets adhere via receptors
Specific Glycoprotein Ia/IIa → Platelet collagen receptor.

GP Ia/IIa & GP Ib → platelet receptors that bind to collagen & Von Willebrand factor

↓
Causing platelets to adhere to the subendothelium of a damaged Blood vessel.

↓
Causes platelet activation leading to release of preformed platelet granule constituents

including ADP → makes platelet stick together

↑
& denovo generation of mediators of coagulation ←

↓

Primary Hemostatic plug by losing their individual membranes → viscous mass

↓

promote the assembly of clotting factors

↓

amplify thrombin formation

PAR-1 & PAR-4

↓

Protease activated Receptors that respond to Thrombin ($\underline{II}a$)

& P_2Y_1 ; P_2Y_{12}

↓

Receptors of ADP

→ when stimulated by agonists

↓

activate Fibrinogen binds to Binding Protein (Integrin) ← GP $\underline{II}b/\underline{III}a$

& COX-1

↓

promote platelet aggregation and secretion.

Agonists
- Sheer stress
- collagen
- ADP
- Adrenaline
- Thrombin

Secrete TXA_2

↓

platelet activation

PGI_2 Prostacyclin syn. by endothelial cells.

circulating fibrinogen binds to an activated platelet receptor glycoprotein $\underline{II}b/\underline{III}a$ (integrin)

↓

converted to fibrin.

Coagulation :→

Circulating fibrinogen binds to an activated platelet receptor GP IIb/IIIa.

↓

procen of deposition of fibrin

↓

Coagulation.

↓

Initiated by intrinsic pathway

Initiated by Extrinsic pathway

↓

merge by activation of factor X → Xa.

Intrinsic pathway

Prekallikrein

XII → (kininogen) XIIa

↓

XI → XIa

↓

IX → (Ca2+) IXa.

↓

X → Xa

VIII, Ca2+, platelet factor.

Extrinsic pathway

II → IIa.

↓

I → Ia. (soluble)

↓ XIIIa ← XIII

FIBRIN. (insoluble)

Common pathway.

I Fibrinogen

II Prothrombin

III Thromboplastin

IV Ionic Calcium

V Proaccelerin, Hereditary labile factor

VI Accelerin

VII Proconvertin

VIII Anti Hemophilic factor

IX Plasma thromboplastin antecedent.

X Stuart power factor

XI Plasma thromboplastin antecedent

XII Hageman factor

XIII Fibrin stabilising factor.

XIV Prekallekrein

XV Kallekrein

XVI platelet factor.

Coagulation involves a series of Zymogen activation reactions.

Each reaction Zymogen (precursor protein)
↓ converted to
Active protease by cleavage of
One or more peptide bonds

The final protease generated is Thrombin.

Haemophilia → deficiency of
factor IX or factor VIII

Fibrinolysis

Fibrinolytic system dissolves intravascular fibrin through the action of plasmin.

Single Chain plasminogen —(Plasminogen activators)→ Two chain plasmin

By cleavage of specific peptide bond.

Plasminogen activators → Tissue plasminogen activator (t-PA)
→ Urokinase plasminogen activator (u-PA)

↓

Synthesized by Endothelial cells.

t-PA → dominates most conditions & drives intravascular fibrinolysis.

u-PA → in response to inflammatory stimuli promotes Extravascular fibrinolysis.

Fibrinolytic system is regulated such that unwanted fibrin thrombi removed, while fibrin in wounds is preserved to maintain Hemostasis.

t-PA → released from endothelial cells in response to many stimuli ~~that occurs~~ including stasis that occurs when thrombi occludes vessels.

↓

released t-PA is quickly cleared from blood or inhibited by plasminogen activator inhibitor (PAI-1) & PAI-2

↓

∴ little effect is seen in absence of fibrin.

The efficiency of t-PA increases when plasminogen levels increase over 300 fold. in presence of fibrin.

↓

promotes plasmin generation on its surface.

↓

plasmin then degrades fibrin.

plasminogen and plasmin bind to lysine residues on fibrin. ⟶ fibrin degradation.

Natural anticoagulant mechanism

*) NO, PGI_2 ⟶ syn. by endothelial cells

↓

Induce vasodilation

⊗ platelet activation & aggregation

*) ANTITHROMBIN III ⟶ plasma protein that ⊗ coagulation enzymes of intrinsic & common pathways.

Heparan SO4 proteoglycans syn. by endothelial cells.

↑ activity of Antithrombin III by 1000 fold.

*) PROTEIN C & PROTEIN S ⟶ associated with activation of Thrombin as well as factor X

*) TISSUE FACTOR PATHWAY INHIBITOR (TFPI)

↓

natural anticoagulant found in lipoprotein fraction of plasma

TFPI → binds to factor Xa → (X) Factor VIIa

*) Natural flow / Rapid flow of Blood.

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* Rapid flow of Blood
* Anti thrombin III
* Removal of fibrinolysis of traces of fibrin ~~removal~~ formed in circulation

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* Stasis within Venous system
* Injury / disease of the vessel wall
* Hypercoagulable state of Blood.

} * coagulation in Blood ↓?

ANTI COAGULANTS

CLASSIFICATION

I) **INVIVO ANTI COAGULANTS**

a) FAST ACTING : Heparin
Bivalirudin
Lepirudin
Desirudin, Argatroban
Drotrecogin Alfa
Anti Thrombin

b) SLOW ACTING :

(i) COUMARIN Derivatives

Eg: Bishydroxy coumarin
Ethyl bis coumacetate
Warfarin Sodium

(ii) INDANDIONE Derivatives

Eg: Phenindione

Other Eg: Dabigatran Etexilate, Rivaroxaban

II) **INVITRO ANTICOAGULANTS**

Eg: Citrates, oxalates, EDTA

INR :→ International Normalized Ratio. It is a laboratory measurement of how long it takes blood to form a clot.

$$INR = \frac{\text{Prothrombin (Test)}}{\text{Prothrombin control}} \times ISI$$

ISI → International sensitivity index.

Healthy people 1.1 or below.

INR Range 2 to 3 → in patients

*) Strongest organic acid in the body.

HEPARIN :→ *) Glycosaminoglycan → found in secretory granules of Mast cells.

(2)

↓

*) Synthesized from UDP-sugar precursors as a polymer of alternating D-glucouronic acid and N-acetyl D-glucosamine residues

*) 10-15 glycosaminoglycan chains with 200-300 saccharide units with a mass of 750-1000 KDa.

↓

undergo modifications like N-acetylation & N-sulfation & epimerisation etc

↓

Heparin.

Source Extracted from porcine intestinal mucosa

↓

rich in mast cells.

History discovered in 1916 by McLean, a medical student → looking for a coagulant in liver.

found in Mast cells which are abundant in liver & lungs.

When released, these are degraded by Macrophages & hence heparin cannot be detected in normal plasma.

Purified Heparin (Unfractionated Heparin)

↓

from different animals. → different activities.

Bioassay of Heparin

↓

Capacity of heparin to prevent clotting of sheep or cattle plasma under standardized conditions.

↓

compared with activity of standard Heparin powder 1 mg of dry material from cattle lung = 150 USP units.

→ The anticoagulant activity is attributed to its strong electronegative charge.

→ It is used as sodium salt.

Heparin derivatives

These include Low Molecular weight Heparins (LMWHs) & FONDAPARINUX

	Heparin	LMWHs	Fondaparinux
Source	Biological	Biological	Synthetic.
weight	15,000 Da	5000 Da	1500 Da
affects	Xa & IIa	Xa & IIa	Xa
$t_{1/2}$	1 hr	4 hr	17 hrs
Renal excretion	No	Yes	Yes-
Thrombocytopenia	<5%	<1%	<1%

Mechanism of Action

Anticoagulant effect both invitro & invivo. & act on all 3 stages.

*) Bind to Antithrombin III. → accelerate the rate at which it inhibits various coagulation proces.

↓

which then inactivates factor IXa, Xa and Thrombin.

*) ↑ the formation of Thrombin – Antithrombin Complex

*) Has action on Both intrinsic & Extrinsic pathway & NO action on thrombin bound to fibrin.

Heparin, LMWHs, fondaparinux act in a catalytic fashion. After binding to antithrombin and promoting the formation of covalent complexes b/w antithrombin & target proteases, it dissociates & then catalyse other antithrombin molecules.

→ Prolongs clotting time 2 to 2½ times the control and activated partial thromboplastin time. to 1.5 to 2 times the control.

Pharmacological actions :→

→ Blood coagulation (X)

→ Heparin and lipoprotein lipase : → Abolishes the cloudiness of the Hyperlipemic plasma within minutes after its administration.

Invivo → in doses too small than anticoagulant effect.

→ ⊗ Aldosterone secretion

↓

Causes Hyperkalemia

→ ⊗ growth of many cells like vascular muscle, endothelium etc

→ Some anti-inflammatory action.

ADME :→

A :→ Not effective orally, ∴ given parenterally.

SC or IV.

↓ ↓

every 8–12 hrs. infusion every 4–6 hrs.

↓ ↓

onset of action delayed by 1–2 hrs. Immediate onset of action

D :→ Taken up by mast cells in their meta chromatic granules.

↓

act as storage depot for exogenously admn. Heparin.

also taken up by Endothelial cells.

M :→ metabolized in liver by the enzyme Heparinase.

$t_{1/2}$ depends on dose of Heparin admn.

100, 400, 800 units/kg of Heparin.

with $t_{1/2}$ 1, 2.5 & 5 hrs respectively.

E :→ Reticulo endothelial system
undegraded Heparin appears in Urine.

LMWH's, Fondaparinux → $t_{1/2}$ more than Heparin (4-6 hrs) (17 hrs)
there are eliminated through kidneys.
∴ drugs can accumulate in Renal impairement
↓
Lead to Bleeding.

∴ Contraindicated in patients with Clearance < 30 ml/min.

Fondaparinux CI in patients with hip fracture, Knee replacement, Hip replacement or Abdominal surgery.

Adverse effects :→

*) Allergic and anaphylactoid reactions.
asthma, urticaria, rhinitis, fever.
∴ A trial dose of 1000 units of Heparin given before

*) Bleeding → Primary unfoward effect
1-5% of patients with Venous Thrombosis

Excessive use may produce bleeding from ulcer, kidneys, Hemorrhoids or wound Hematoma
May bleed with normal aPTT or prolonged aPTT.

*) Thrombocytopenia → mild Thrombocytopenia in 25% of cases.

LMWH's → prepared by fractionation of native Heparin.

Mol. wt b/w 4000 – 6500 Da.

Given SC once / twice daily

II^{r} antithrombotic activity as native Heparin.

*) absorption is more uniform than Heparin

*) Longer duration of action $t_{1/2}$ – 4 hrs.

*) Selectively inactivate Factor $\overline{X}a$

$\overline{X}a : \overline{II}a :: 2:1 : 4:1$

*) Predictable anticoagulant effect

*) less interaction with platelets

*) less Antigenic

Eg: ENOXAPARIN
DALTEPARIN Na.
TINZAPARIN
PAMAPARIN
REVIPARIN

FONDAPARINUX :→ Synthetic pentasaccharide unit of Heparin that binds to Antithrombin and enhances inactivation of Factor $\overline{X}a$

$t_{1/2}$ → 17hrs.

Slow Acting Anticoagulants / ORAL ANTICOAGULANTS

COUMARIN DERIVATIVES :→

Bishydroxy coumarin / Dicoumarol → first coumarin compound isolated from spoiled sweet clover in 1943-44 & was proved to be causative factor in cattle disease termed "sweet clover disease"

↓

Characterised by severe haemorrhagic tendency.

→ Most commonly used drug is Warfarin sodium.

↓

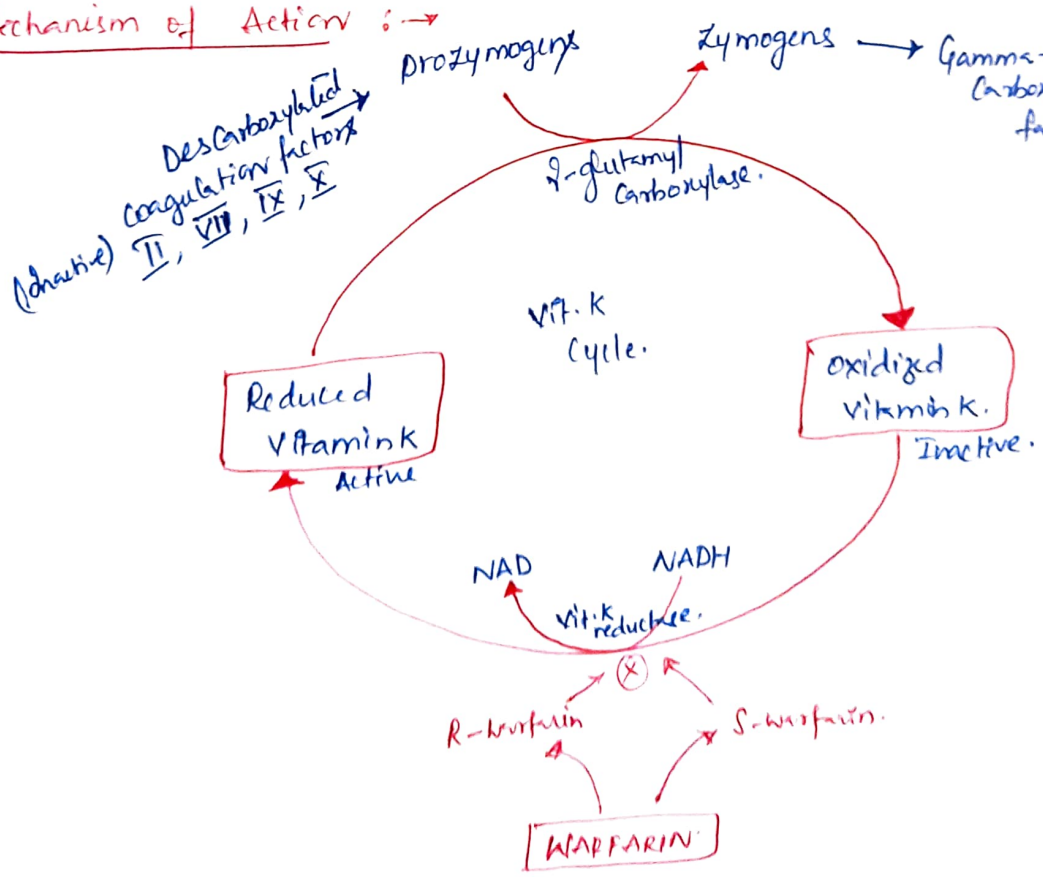
Name derived from the patent holder WARF (Wisconsin Alumni Research Foundation)

↓

Not used therapeutically till 1951. In 1951, an army inductee survived an attempted suicide with rodenticide (Warfarin). Since, then used for prevention of thromboembolic disease.

→ Used as Rodenticide

Mechanism of Action :→



1. Oral Anticoagulants are antagonists of Vitamin K
2. Coagulation factors II, VII, IX & X & anticoagulant proteins C & S are synthesized mainly in the liver & are biologically inactive till the amino-terminal glutamate residues are carboxylated to form Ca^{2+} binding Gla residues.
3. This reaction of decarboxy precursor protein requires CO_2, O_2 and reduced Vit.K which is catalysed by γ-glutamyl Carboxylase.
4. Carboxylation reaction is directly coupled to oxidation of Vit.K to its corresponding epoxide.
5. For sustained carboxylation & synthesis of competent proteins, Reduced Vit.K must be regenerated from epoxide. This is again catalysed by Vit.K epoxide reductase (VKOR) which is inhibited by Warfarin.
6. Vit.K also can be converted to corresponding Hydroquinone by another second reductase DT-diaphorase. This enzyme requires high Conc^n of Vit.K & is less sensitive to ~~Her~~ Coumarin drugs.
7. Large doses of Vit.K can counteract even large doses of Oral Anticoagulants.
8. In contrast to Heparin, there is a considerable lag b/w the time of peak plasma level of Coumarins and the therapeutic responses. This is because they prevent the formation of active essential clotting factors by the liver but do not destroy the circulating ones.
9. It takes 3-7 dys for prothrombin time to return to normal after cessation of therapy. As Coumarins have no direct action on coagulation factors, they are not effective in vitro.
10. Coumarin therapy is controlled by estimating prothrombin time which is expressed as INR (International Normalized Ratio). Bleeding time is unaffected.

$$INR = \frac{PT_{PT}}{PT_{Ref.}}$$

ADME

A → Slow & incomplete orally

D → Extensively Bound to plasma proteins. Cross placental Barrier & also secreted in milk.
96% distributed

M → Metabolised in liver & varies in metabolism from drug to drug.
CYP2C9

Adverse

- Bleeding
- Fetal toxicity
- Cutaneous gangrene.
- rarely Urticaria, Anorexia, Vomiting, Diarrhoea.