Blood Hemostasis

Antiplatelets
Anticoagulants

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- An estimated 300,000 VTE-related deaths occur in the US each year
- VTE causes over 500,000 deaths in Europe every year
- Deaths attributable to VTE are estimated to exceed the total combined number of deaths from breast cancer, prostate cancer, AIDS, and traffic accidents annually
- An estimated 2.2 million people in the US and 4.5 million in the EU have atrial fibrillation

Blood has been considered sacred since ancient times.

The Chinese character/ hieroglyphical of blood

A diagonal stroke over the symbol of the plate (SYMBOL) signifies
"the sacrifice placed on the plate"
or
"a sacrifice placed in a vessel"

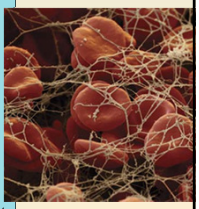
The ability to transform blood components from liquid to solid form represents a network of enzymatic activation and inhibition.

- The modern concept of coagulation was presented in 1964 as the Waterfall/Cascade model, which might overwhelm many nonhematologists with its complexity

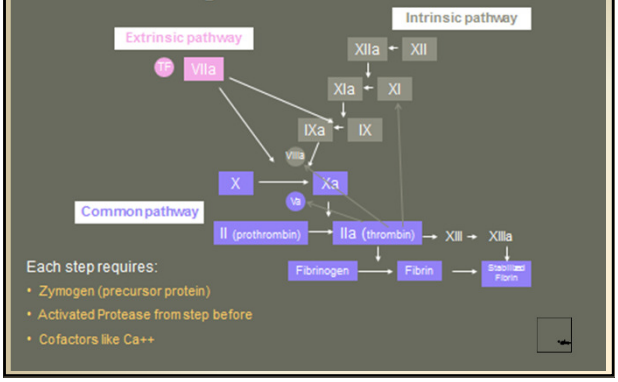
Tamakar K A et al. Anesth Analg 2009;108:1433-1446, ©2009 by Lippincott Williams & Wilkins

Hemostasis

- Is part of an important host defense mechanism termed **hemostasis** (the cessation of blood loss from a damaged vessel).
- Upon vessel injury, platelets adhere to macromolecules in the subendothelial tissues and then aggregate to form the primary hemostatic plug.
- The platelets stimulate local activation of plasma coagulation factors, leading to generation of a fibrin clot that reinforces the platelet aggregate.



Coagulation Cascade



Each step requires:

- Zymogen (precursor protein)
- Activated Protease from step before
- Cofactors like Ca++

Control of homeostasis

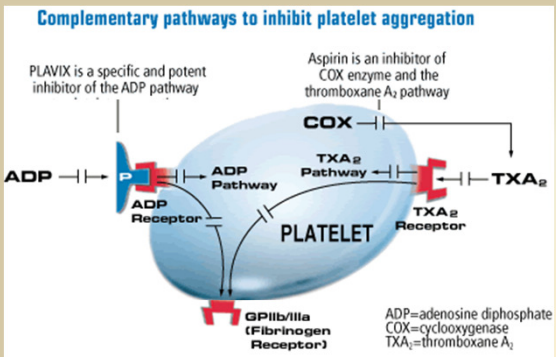
- **Antiplatelets**
- **Anticoagulants**

Platelet Inhibitors

- Inhibit the aggregation of platelets
- Indicated in progressing MI, TIA/CVA
- Side Effects: uncontrolled bleeding
- No effect on existing thrombi

Aspirin, PLAVIX (clopidogrel bisulfate)

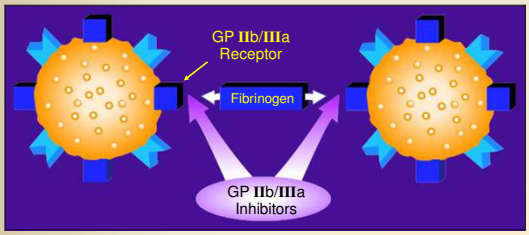
- Aspirin inhibits the cyclo-oxygenase enzyme - COX, thereby inhibiting TXA-2 production and subsequent activation of the GPIIb/IIIa binding site on the platelet, which allows fibrinogen to bind.
- PLAVIX inhibits adenosine diphosphate (ADP)-induced platelet aggregation



- Results from the CAPRIE (*Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events*) trial demonstrated that PLAVIX reduced the rate of the combined end points of first occurrence of MI, stroke, or other vascular death in patients with recent MI, recent stroke, or established PAD.

- Powerful data from a subsequent study, the CURE (*Clopidogrel in Unstable angina to prevent Recurrent Events*) trial, demonstrated that PLAVIX added to aspirin achieves risk reduction in thrombotic events (MI, stroke, or cardiovascular [CV] death) in patients with acute coronary syndrome (unstable angina/non–Q-wave MI), including patients who undergo percutaneous coronary intervention (with or without stent) or CABG.

GP IIB/IIIA Inhibitors



GP IIB/IIIA Inhibitors

- abciximab (ReoPro®)
- eptifibitide (Integrilin®)
- tirofiban (Aggrastat®)
  - Adjunct to heparin & aspirin therapy for prevention of CI complications in pt's undergoing coronary interventions
  - Short-term MI risk reduction in pt's with unstable angina not responding to conventional Rx

Anticoagulants

- Interrupt clotting cascade at various points
  - No effect on platelets
- Unfractionated heparin
- Low Molecular Weight Heparin
- Factor Xa inhibitors
- Warfarin (Coumadin®)

Unfractionated Heparin

- Endogenous - Released from mast cells/basophils
- It is derived from porcine intestinal mucosa, standardized for anticoagulant activity
- Binds with *antithrombin III*
- Antithrombin III binds with and inactivates excess thrombin to regionalize clotting activity.
  - Most thrombin (80-95%) captured in fibrin mesh.
- Antithrombin-heparin complex 1000X as effective as antithrombin III alone
- Also binds to serine centres of IXa, Xa, XIa and XIIa and inactivates factor II

Heparin

- It slows the process of thromboplastin synthesis, decelerates the conversion of prothrombin to thrombin, and inhibits the effects of thrombin on fibrinogen, blocking its conversion to fibrin
- The agent also causes an increase in the number of negatively charged ions in the vascular wall, which helps prevent the formation of intravascular clots.

Heparin…

- Cannot be taken orally – amino acid
- MW 5000 – 30000 Dalton
- Indications: MI, PE, DVT, ischemic CVA
- Antidote for heparin OD: protamine SO_4

  (Protamine was originally isolated from the sperm of salmon and other species of fish but is now produced primarily through recombinant biotechnology.)

  – MOA: heparin is strongly negatively charged. Protamine is strongly positively charged.

  – 1:1 dosing i.e. 1mg / 100U heparin

Administration.

- Continuous infusion with monitoring of the APTT (activated partial thromboplastin time).
  – Intermittent IV administration with more doses of heparin and increased risk of bleeding.
- Subcutaneous. Prophylaxis before surgery and during pregnancy

Low molecular weight heparins (LMWH).

- Enoxaparin and dalteparin.
- MW 3500 – 5000.
- More action against Xa than against IIa.
- Enoxaparin follows first order kinetics with a half-life of 60 min.
- More predictable anticoagulant effect.
- Does not need monitoring.

Advantages of LMWH over UH

- Decreased "heparin resistance"
  - pharmacokinetics of UH are influenced by its bindings to plasma protein, endothelial cell surfaces, macrophages, and other acute phase reactants
  - LMWH has decreased binding to nonanticoagulant-related plasma proteins

Advantages of LMWH over UH

- No need for laboratory monitoring
  - when given on a weight-adjusted basis, the LMWH anticoagulant response is predictable and reproducible
- Higher bioavailability - 90% vs 30%
- Longer plasma half-life
  - 4 to 6 hours vs 0.5 to 1 hour
  - renal (slower) vs hepatic clearance

Advantages of LMWH over UH

- Less inhibition of platelet function
  - potentially less bleeding risk, but not shown in clinical use
- Lower incidence of thrombocytopenia and thrombosis (HIT syndrome)
  - less interaction with platelet factor 4
  - fewer heparin-dependent IgG antibodies

Monitoring of LMWH

- Unnecessary in majority of patients
- May be useful in specific instances
  - renal insufficiency (creatinine >2.0 mg/dl)
  - obese patients with altered drug pK
  - major bleeding risk factors
- aPTT not useful - low anti-IIa activity
- anti-factor Xa assay is more appropriate, but not widely available

HEPARIN Side-effects

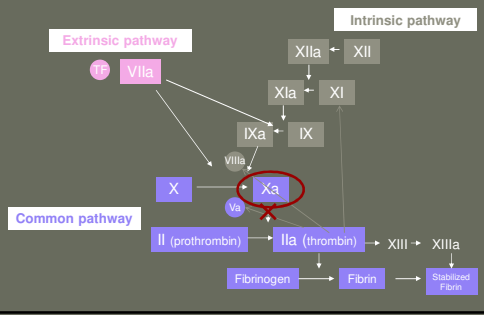
- Bleeding
- Heparin Induced Thrombocytopenia (HIT)
- Cardiovascular/Respiratory - allergic vasospastic reaction can cause chest pain and possible ischemia
- Effects on the Liver - Elevation of liver enzymes
- Local Injection Site Reactions – erythema, pain, hematoma and ulceration
- Allergic Type Reaction

Heparin-induced thrombocytopenia

Gangrene of the legs following arterial thrombosis

Direct Thrombin Inhibitors
Factor Xa Inhibitors

- Parenteral or Oral
- Direct or Indirect mech. of inhibition



Direct Xa Inhibitors
Parenteral

- The formation of a blood clot is the end product of a highly regulated enzymatic cascade process called the coagulation cascade.
- Factor Xa is the critical link in the coagulation cascade, where the extrinsic and intrinsic pathways meet to form the common pathway.
- Its formation enables the conversion of prothrombin to thrombin, which catalyses the formation of fibrin from fibrinogen.

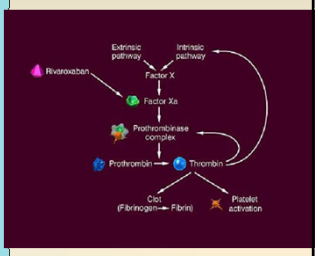
Factor Xa Inhibitors

Fondaparinux

- Parenteral anticoagulant
- first selective factor Xa inhibitor approved 55% better than enoxaparin (LMWH) at reducing risk of VTE
- synthetic pentasaccharide: "represents the oligosaccharide consensus sequence of heparin"
- Indirect inhibition: binds to antithrombin and increases antithrombin's affinity for factor Xa by 300-fold

Direct Xa Inhibitors… Oral

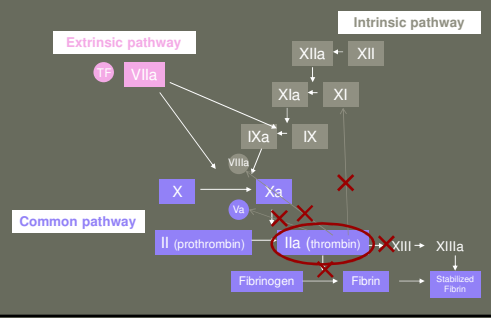
- Rivaroxaban is a direct Factor Xa inhibitor that can be administered orally.
- A once-daily dose produces 24-hour inhibition of Factor Xa and thrombin generation levels



Direct Xa Inhibitors…

- Data from the rivaroxaban clinical trials programme suggest the time is fast approaching when a once-daily, oral anticoagulant that has a low propensity to interact with other medicines and doesn't require regular monitoring becomes a clinical reality. I

Direct Thrombin Inhibitors



Direct Thrombin Inhibitors

Ximelagatran

- First target-specific oral anticoagulant in trials
- Ximelagatran is the oral prodrug of Melagatran
- Hepatatoxicity
  - Did not receive FDA approval in 2004
  - On the market in Europe but pulled in 2006
- 'proof of principle'
  - "efficacious" as warfarin
  - Wider therapeutic index
  - Little dosage adjustment/ no monitoring

Direct Thrombin Inhibitors

Dabigatran etexilate

- Prodrug converted to Dabigatran (471 mw) by esterases in blood after absorbed in stomach in microenvironment from tartaric acid capsule
- Peak 2hr; Half life 14 – 17 hr
- Not metabolized by cytochrome system = no anticipated drug interactions
- Farthest along in trials
- History:
  - early 1990s: German scientists at Boehringer-Ingleheim began research
  - 1996 Synthesized novel drug
  - 1999 Phase I trials
  - 2001-3 Phase II trials
  - 2004-present Phase III trials
  - 2008 entering European as Pradaxa®
  - RE-LY trial results in 2009
- Promising; improvement in liver safety

Vitamin K antagonists

History of Warfarin

- 1930s: cows hemorrhaging after eating spoiled sweet clover silage
- 1939: bishydroxycoumarin (dicoumarol) identified
- 1948: potent form as rodenticide
  - Called *Warfarin* (**W**isconsin **A**lumni **R**esearch **F**oundation)

Anticoagulant in humans? No, too toxic!?

- 1951: Army inductee's failed attempt at suicide with high dose of warfarin rodenticide
- Clinical use for over 60 years

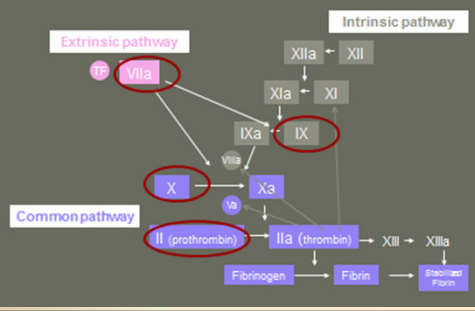
Warfarin

- Factors II, VII, IX and X all vitamin K dependent factors
- Warfarin competes with vitamin K in the synthesis of these enzymes → False factors
- Cannot bind to Calcium and thus to phospholipids
- Depletes the reserves of clotting factors.

Vitamin K antagonists

Effect on Coagulation

- Vitamin K dependent clotting factors: Factors II, VII, IX, and X



Warfarin…

- Initial effect only on factor VII (short half-life).
- It takes 3-5 days to be effective:

- **II → 42-72 hours**
- **VII → 4-6 hours**
- **IX→ 21-30 hours**
- **X → 27-48 hours**

→ Overlap with heparin therapy

INR: International Normalized Ratio

- **A mathematical "correction" (of the PT ratio) for differences in the sensitivity of thromboplastin reagents**
- **Relies upon "reference" thromboplastins with known sensitivity to antithrombotic effects of oral anticoagulants**
- **INR is the PT ratio one would have obtained if the "reference" thromboplastin had been used**
- **Allows for comparison of results between labs and standardizes reporting of the prothrombin time**

INR Equation

$$\text{INR} = \left(\frac{\text{Patient's PT in Seconds}}{\text{Mean Normal PT in Seconds}}\right)^{\text{ISI}}$$

INR = International Normalized Ratio
ISI = International Sensitivity Index

The International Normalized Ratio (INR) is used to monitor the effectiveness of warfarin

The test result for PT depends on the method used, with results measured in seconds and compared to the average value in healthy people.

Most laboratories report PT results that have been adjusted to the International Normalized Ratio (INR) for patients on anti-coagulant drugs.

These patients should have an **INR of 2.0 to 3.0** for basic "blood-thinning" needs. For some patients who have a high risk of clot formation, the INR needs to be higher - about 2.5 to 3.5.

International Normalized Ratio Blood Test-INR

Normal INR Values: 1 to 2

The INR is used to make sure the results from a PT test is the same at one lab as it is at another lab.

Common drugs influencing INR values :	
INR ↑ / PT decreases	**INR ↓ /PT increases**
amoxicillin	rifampin
kinolons	antihistamin
cephalosporins	barbiturat
makrolid antibiotics	carbamazepin
paracetamol	digoxin
salicylate	diuretics
amiodaron	pills
allopurinol	coffein
omeprasol	pentoxiphyllin
heparine	Vitamin K
NSAID & COX2 inh.	
tricyclic antidepressants	

Warfarin…

- Can add aspirin.
- Atrial fibrillation: warfarin.
- INR control: weekly and then 2 monthly.
- Should be taken between 17h00 and 19h00.
- INR measured between 9h00 and 11h00.

Warfarin
Complications

- Haemorrhage.
- A warfarin induced skin necrosis.
- Teratogenecity: saddle nose and microcephalus, brain and eye defects.
- Haemorrhage during labour.
- Preparation before surgery:Add vit K 1-2 mg daily until INR is reduced with 50%.

Warfarin
Complications – Skin Necrosis

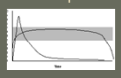
Warfarin
Complications – Gum Bleeding

Problems with Warfarin

- Food and drug interactions

- Genetic variation in metabolism

- narrow therapeutic window



- slow onset of action

dosage adjustments & freq. monitor with INR

overlap with parenteral drugs

Warfarin: Drug interactions
Potentiation

- Phenylbutazone.
- Aspirin
- Inhibition of biotransformation via liver enzymes
- Anabolic steroids
- Quinidine and d-thyroxine.
- Tetracyclines effect on Vit K.

Warfarin: Drug interactions
Inhibition

- Cholestyramine binds to warfarin.
- Induction of liver enzymes.
- Oestrogens increase synthesis

Table 4. Summary of Antithrombotic Drugs

Drug	Heparin	Warfarin	LMWH	Fondaparinux	Rivaroxaban[1]
Mechanism of Action	Inhibit thrombin/fXa	Reduce vitamin K factors	Inhibit fXa > thrombin	Inhibit fXa	Inhibit fXa
Indication	PCI, CPB, thrombosis	Stroke/DVT prophylaxis	DVT/PE prophylaxis	DVT/PE prophylaxis	DVT/PE prophylaxis
Route	IV, SQ	Oral	SQ	SQ	Oral
Half life	1–2.5 h	36–42 h	3–5 h	17 h	5–10 h
Elimination	Hepatic	Hepatic	Renal	Renal	Renal
Monitoring	aPTT/ACT	PT/INR	Anti-Xa	Anti-Xa	Anti-Xa
Comments	May cause HIT/HITT	↓Protein C/protein S	↓Dose in reduced kidney fx	↓Dose in reduced kidney fx	

IV = intravenous; SQ = subcutaneous; LMWH = low molecular weight heparin; APC = activated protein C (drotrecogin alpha); sTM = recombinant human soluble thrombomodulin; PCI = percutaneous coronary intervention; DVT = deep venous thrombosis; PE = pulmonary embolism; DIC = disseminated intravascular coagulation; PT/INR = prothrombin time/international normalized ratio; aPTT = activated partial thromboplastin time; ACT = activated clotting time; kidney fx = kidney function; HITT = heparin-induced thrombocytopenia with thrombosis; HIT = heparin-induced thrombocytopenia; EPCR = endothelial protein C receptor; CPB = cardiopulmonary bypass.

[1] Under clinical development.

[2] Argatroban blocks catalytic site of thrombin.

[3] Lepirudin and bivalirudin block catalytic site and Exosite I of thrombin.

[4] Available in Japan for DIC/United States Phase II trial for DVT/PE.

[5] Antibody may increase anticoagulant effect, but allergic reaction is rare.

The End