Inhibitors of platelet aggregation

Anticoagulants

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Basic principles of blood coagulation

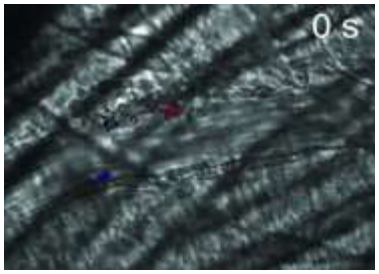
Normal (not damaged) vascular endothel - antithrombogenic

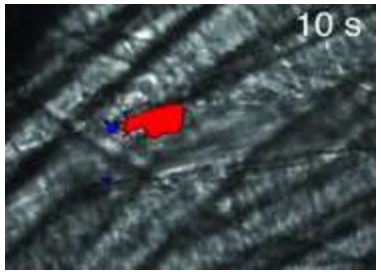
Vascular injury

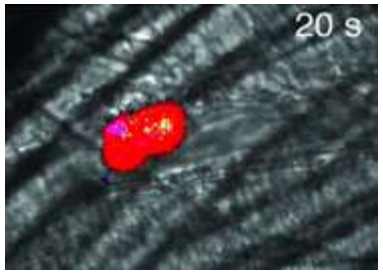
initiates events leading to

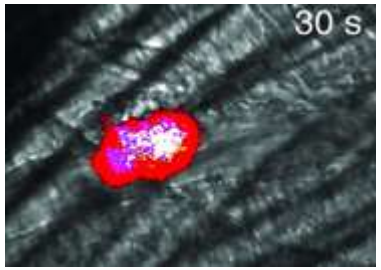
- **vasospasm**
- **platelet adhesion and aggregation - white thrombus**
- **blood coagulation - red thrombus**
- **fibrinolysis**
- **cell proliferation, repair mechanisms**

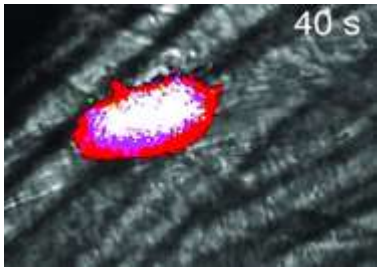
Birth of a thrombus

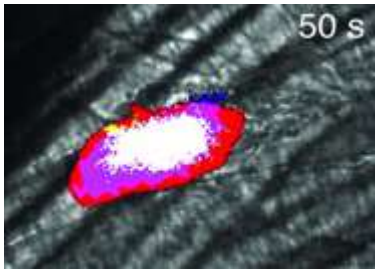


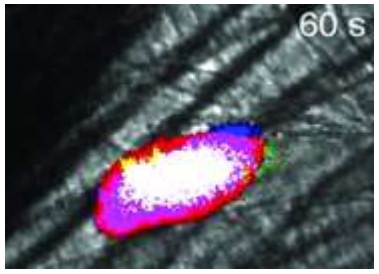


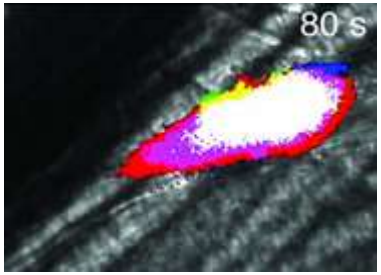


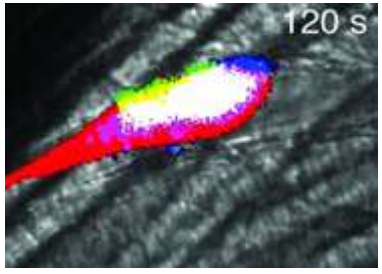


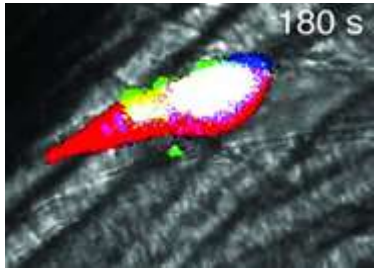












platelets

tissue factor

fibrin

platelets + tissue factor

platelets + fibrin

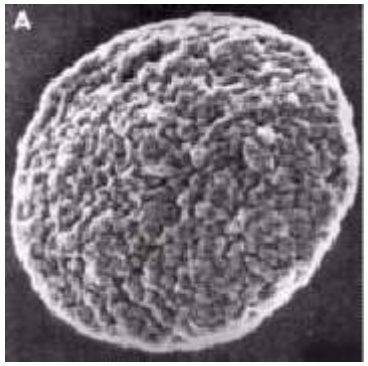
platelets + fibrin + tissue factor

Furie B, Furie BC. Thrombus formation in vivo. J Clin Invest. 2005

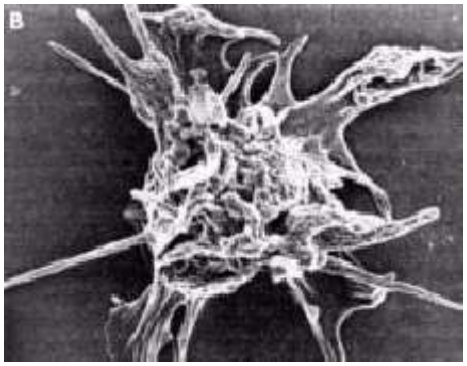
DRUGS USED IN THROMBOEMBOLIC DISORDERS

- **Antiplatelet drugs**
- **Anticoagulants**
- **Fibrinolytics**

Antiplatelet drugs



Thrombocytes I.

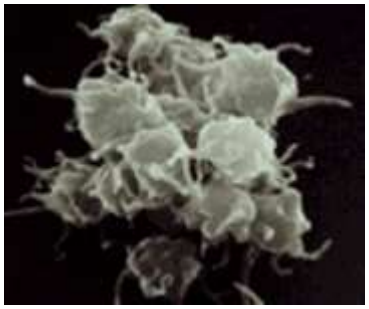


Circulating platelets at rest - small, discoid anuclear cells

Vascular injury - platelet adhesion, activation, aggregation

- **collagen-contact - adhesion of platelets to surface**
  - **glycoprotein Ib/IX - vWF, fibronectin, vitronectin, thrombospondin**
- **activation**
  - **release reaction (e.g. ADP, serotonin)**
  - **pseudopods (higher surface)**
  - **synthesis of thromboxan A_2**

Thrombocytes II.



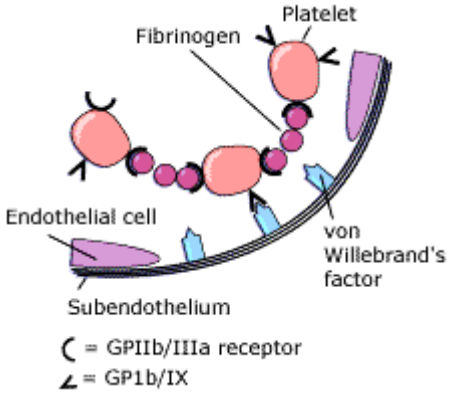
Activators of thrombocytes

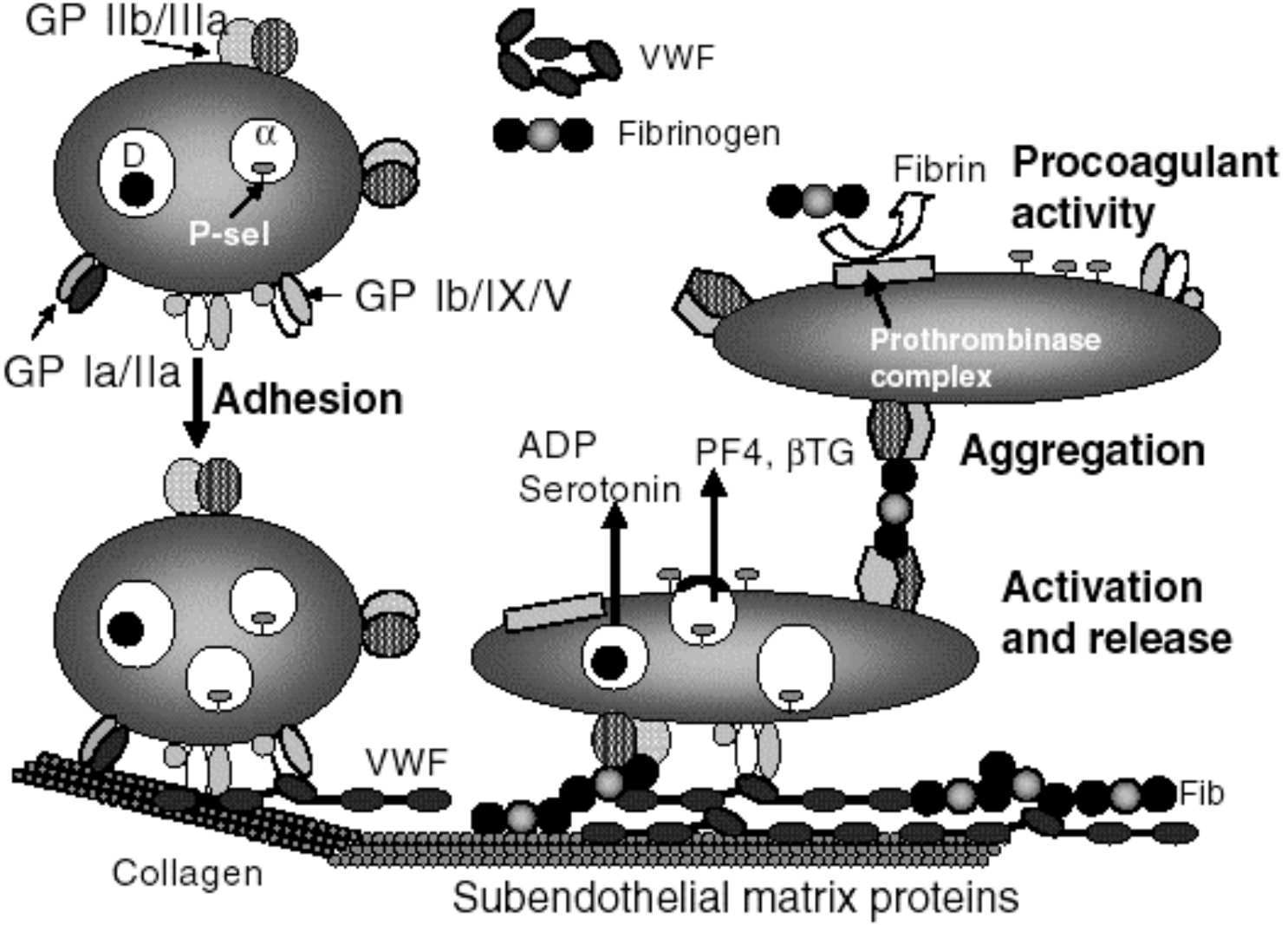
- collagen-contact
- agonists of the G-protein coupled surface receptors
  - thrombin (PAR-1 receptor)
  - ADP ($P2Y_1$ and $P2Y_{12}$ receptors)
  - serotonin ($5\text{-}HT_{2A}$ receptor)
  - epinephrine (α_2 receptor)

Role of eicosanoids: thromboxane $A_2 \leftrightarrow$ prostacycline

Aggregation of platelets

- GP IIb/IIIa receptor - binding to fibrinogen and other RGD proteins (-Arg-Gly-Asp e.g. vWF, vitronectin) - bridges between thrombocytes




GP IIb/IIIa
D
α
P-sel
GP Ib/IX/V
GP Ia/IIa
Adhesion
VWF
Fibrinogen
Fibrin
Procoagulant activity
Prothrombinase complex
Aggregation
ADP
Serotonin
PF4, βTG
Activation and release
VWF
Fib
Collagen
Subendothelial matrix proteins

Antiplatelet drugs

Mechanisms of antiplatelet action

- **Inhibition of thromboxane A_2 synthesis**

  acetylsalicylic acid

- **Antagonism of surface $P2Y_{12}$ receptors**

  clopidogrel, prasugrel, ticagrelor, cangrelor

- **Antagonism of PAR-1 receptors**

  vorapaxar

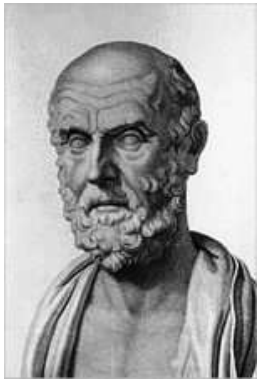
- **Antagonism of surface GP IIb/IIIa receptors**

  abciximab, eptifibatide, tirofiban

- **Phosphodiesterase inhibitors**

  dipyridamole, cilostazole

The antiplatelet drugs are effective only in arterial thromb. disorders



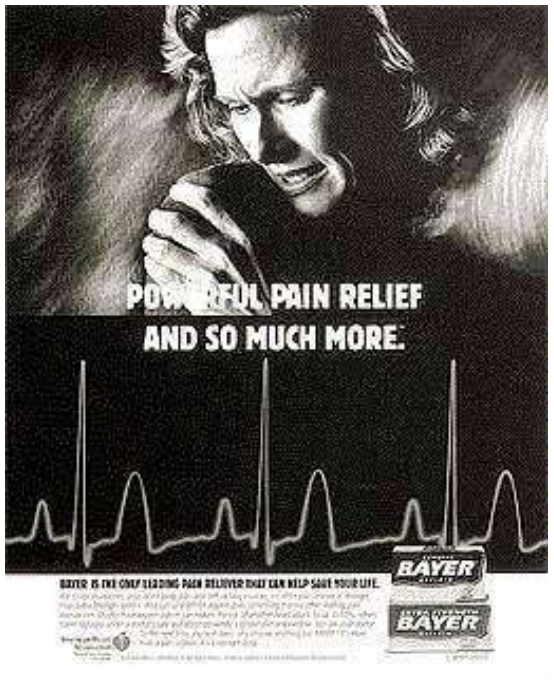
willow bark (Salix alba)

Filipendula (spiraea) ulmaria - bridewort,meadowsweet

Charles Frederic Gerhardt 1853 - synthesized acetylsalicylic acid

Felix Hoffmann 1897 - rediscovered acetylsalicylic acid
(for his fathers' rheumatism)
and it was marketed as Aspirin

(the name comes from the '**A**" in acetyl chloride, the "**spir**" in spiraea ulmaria (the plant they derived the salicylic acid from) and the '**in**' was a familiar name ending for medicines)



1980's - The FDA approves aspirin for reducing the risk of recurrent myocardial infarction (MI) and preventing first MI in patients with unstable angina.

The FDA also approved the use of aspirin for the prevention of recurrent transient-ischemic attacks and made aspirin standard therapy for previous strokes.

Acetylsalicylic acid

Classical indications:
pain, fever, inflammation

Irreversible inhibitor of the COX enzyme

→ inhibits the synthesis of both thromboxane A_2 and prostacycline

their balance is still shifted to an increased prostacycline/thromboxane A_2 ratio
→ inhibition of the platelet aggregation

- **thromboxane A_2 is from thrombocytes - anuclear cells, do not sythetize new enzimes - inhibitory effect is cumulative; prostacycline is from endothelial cells - continuously synthetize new enzimes**
- **low dose oral aspirin (100 mg/day) in sustained release preparations provides relative high concentrations continuously in the portal vein, but low concentrations (metabolism in the liver to the reversible inhibitor salicylic acid) in the systemic circulation at the endothelial cells**
- **low dose aspirin affects more the the thromboxane A_2 synthesis (COX-1), than the prostacycline (COX-1 and COX-2),**

Acetylsalicylic acid (Aspirin)

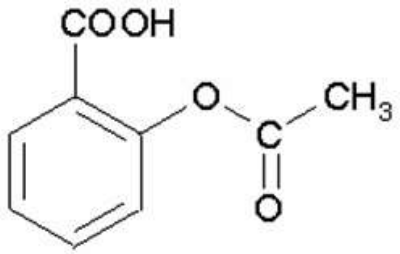
Pharmacokinetics

good oral absorption

first pass metabolism → salicylic acid

high plasma protein binding

metabolism in the liver



Indications related to coagulation

secondary or primary profilaxis (treatment) of arterial thromboembolic diseases

– in acute cases higher loading dose (250-500mg), then ~100 mg/day

e.g. unstable angina pectoris, myocardial infarction, coronary angioplasty, cerebrovascular circulatory problems (stroke) etc.

Adverse effects

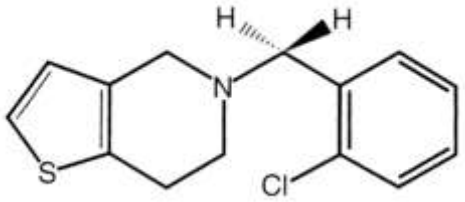
bleeding, peptic ulcer etc.

$P2Y_{12}$ receptor antagonists

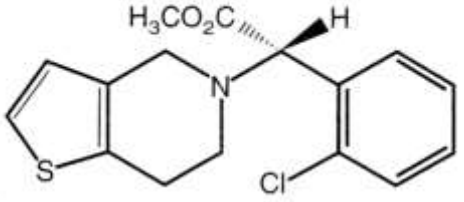
Thienopyridines: ticlopidine, clopidogrel and prasugrel

- **non-competitive antagonists at the platelet ADP receptor**
- **prodrugs, activated in the liver**
- **good absorption, high protein binding**
- **additive synergistic action with other antiplatelet drugs**
- **indications: cardio- and cerebrovascular circulatory problems (e.g. TIA, stroke)**

Ticlopidine



Clopidogrel



$P2Y_{12}$ receptor antagonists

Thienopyridines: ticlopidine, clopidogrel and prasugrel

- **adverse effects: gastrointestinal problems, minor bleedings, rarely leuko- and thrombocytopenia (esp. in case of ticlopidine → regular blood tests in the first 3 months)**
  - **clopidogrel and prasugrel causes less side effects, esp. less hematologic problems → blood control is not necessary**
- **interactions: CYP2C19 inhibitors (omeprazol, fluoxetin or fluconazol) inhibit the activation of clopidogrel**

Ticagrelor (oral), cangrelor (i.v.)

- **more rapid onset (not prodrugs, activation is not needed)**
- **combination with aspirin:**
  cangrelor – i.v. percutaneous coronary interventions,
  ticagrelor – oral, prevention of arterial thromboembolic disorders
- **adverse effect: bleeding**

PAR-1 antagonists

Vorapaxar

- orally active
- antagonist of the thrombin receptor
- indication: myocardial infarction (sec. prohylaxis), peripheral art. thromb. (combination with aspirin)
- adverse effects: bleeding
- contraindications: bleeding, TIA, stroke
- metabolismus: liver (CYP3A – interactions)
- withrawn – no longer authorized in EU (2017)

GP IIb/IIIa receptor antagonists

Abciximab

- monoclonal antibody against the IIb/IIIa complex, binds with high affinity, irreversible antagonist; binds to the endothelial cells and vitronectin receptors as well
- must be given i.v., has a short metabolic half-life (30 min), but its biologic half-life (duration of action) is 18-24 hours
- indications: percutaneous coronary interventions in coronary syndromes
- adverse effects: bleeding (major bleeding - 4%), thrombocytopenia (0,5-2%), hypotension, bradycardia, nausea, vomiting; severe bleeding may require thrombocyte transfusion (long duration of action)

Synthetic, competitive antagonists

- active only i.v., shorter duration of action (2-4 hours), more selective action on the thrombocytes
- eptifibatide: cyclic heptapeptide, analogue of the fibrinogen carboxy terminal
- tirofiban: smaller molecule, non-peptide

Dipyridamole

- vasodilator and inhibitor of the platelet function
- possible mechanism of action
  - phosphodiesterase inhibition
  - adenosine uptake inhibition
- by itself no (or little) beneficial effect
- in combination with warfarin can be used for the primary profilaxis of thromboemboli in patients with prosthetic heart valves
- in high i.v. dose (avoid it !) - risk of coronary steal effect

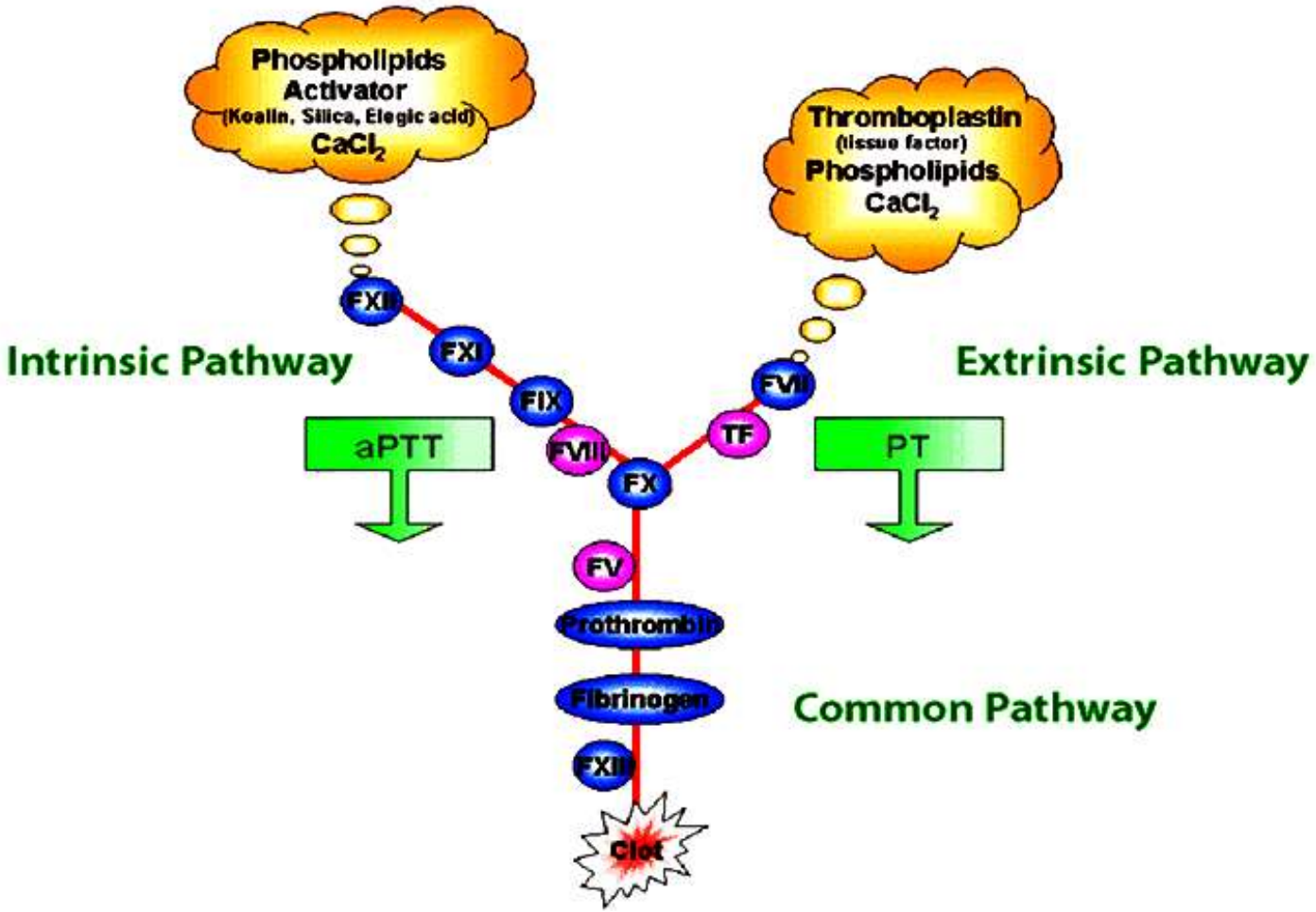
Newer phosphodiesterase inhibitor: cilostazole - for treatment of intermittent claudication

Anticoagulants

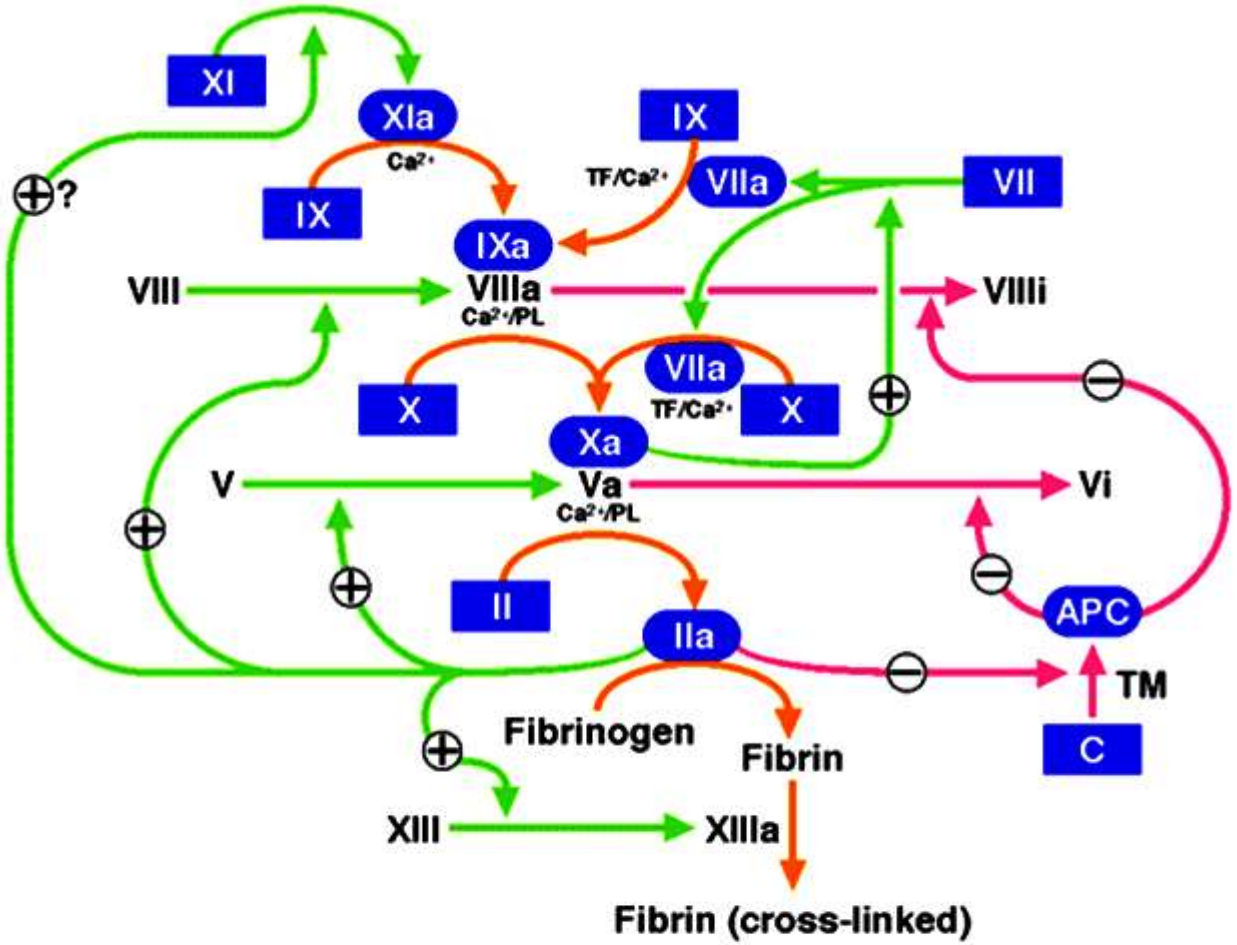
The coagulation cascade

Transformation of soluble fibrinogen into insoluble fibrin

Cascading series of limited proteolytic reactions by serin-proteases



The coagulation cascade



TF = tissue factor
PL = platelets
APC = activated protein C
Vi = degraded Va
VIIIi = degraded VIIIa

⊕ Procoagulant
⊖ Anticoagulant

Anticoagulants

Inhibitors of the coagulation cascade

Mechanism of action

1. Binding to the coagulation factors and their inactivation (only parenterally active)

- **either as a part of an inactivation complex**

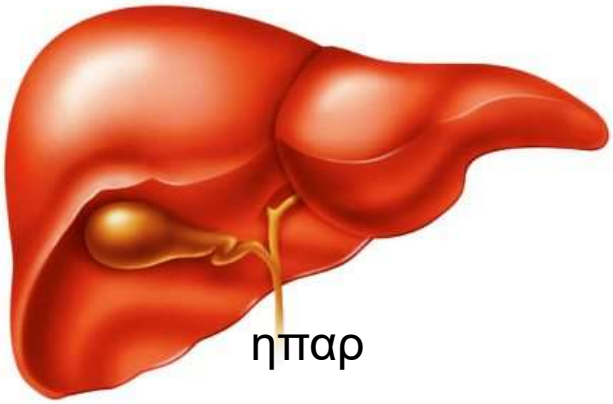
  heparin, LMWH, danaparoid, fondaparinux

- **or directly**

  hirudin, bivalirudin, argatroban, dabigatran, rivaroxaban

2. Inhibition of the the coagulation factor synthesis leading to the synthesis of functionally inactive factors

warfarin and related compounds (oral anticoagulants)

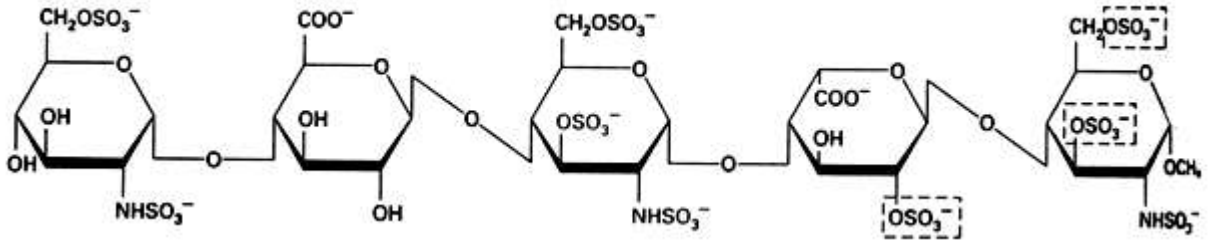


Jay McLean, 1915-1916

William Howell, 1918

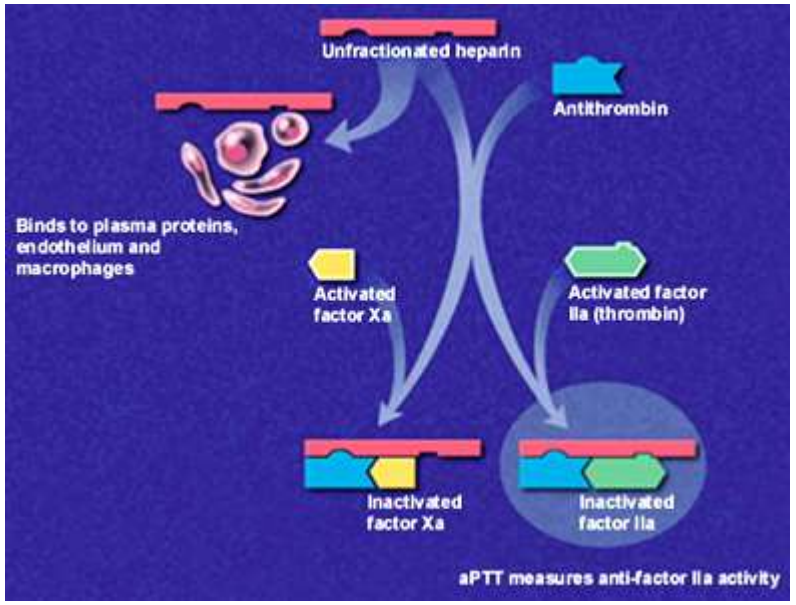
Heparin

- **Heparin is a heterogeneous mixture of sulfated mucopolysaccharides, a negatively charged glucosaminoglycane (MW: 5-30 kD)**
- **Low molecular weight heparin (LMWH) preparations (MW: 1-10 kD) can be isolated from the natural heparin**
    - **Heparin is produced by mast cells, but in vivo plays a little role - a heparine-like compound secreted by endothel cells is heparan sulfate**
- **Functional unit of heparin is a pentasaccharid, which binds to antithrombin III, and accelerates its activity 1000fold.**

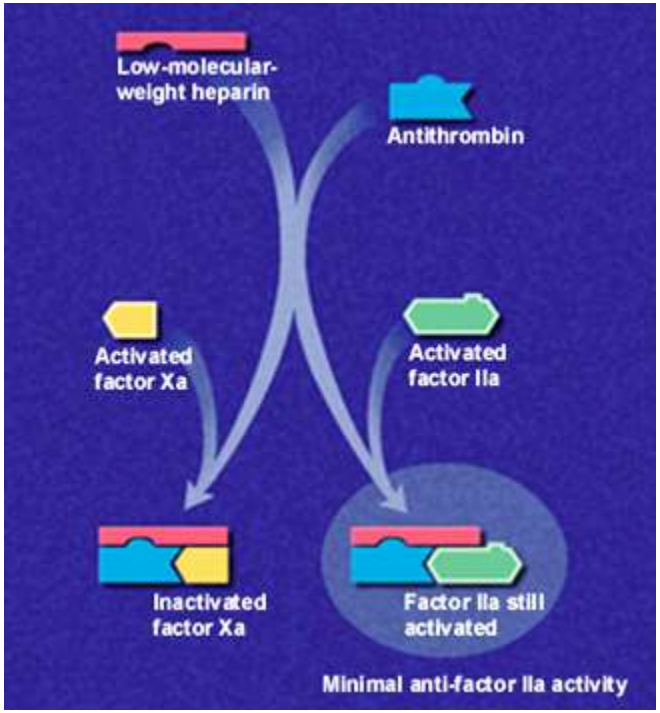


- **Antithrombin III is a serpine (serin protease inhibitor), inactivating thrombin and factor Xa (and IXa)**

Heparin



• **Heparin chains longer than 18 monosaccharid units (UFH) inactivate both thrombin and factor Xa (ratio 1:1)**



• **Heparin chains shorter than 18 monosaccharid units (LMWH) inactivate preferentially factor Xa (compared with thrombin the ratio is 2-4:1)**

Heparin

Pharmacokinetics

- bad absorption - only parenteral use (i.v. or s.c.)
- after s.c. adiministration
  - UFH about 30%, but uncertain bioavailability
  - LMWH stable and high (>90%) bioavailability
- distribution
  - UFH - binding to endothel, macrophages, plasma proteins → at the beginning of the treatment these binding sites must be saturated first, it also complicates its elimination
  - LMWH - limited binding to these binding sites - more predictible dose-effect relation and elimination
  - UFH and LMWHs do not cross the placenta
- elimination
  - UFH - 60-90 min half-life
  - LMWH - longer (2-4 h) half-life

Heparin

Clinical use

UFH

- **treatment of deep venous thrombosis, acute pulmonary emboli, arterial embolies**
- **prophylaxis of postoperative venous thromboses and recurrent thromboembolism**
- **myocardial infarction (after or without thrombolysis), unstable angina**
- **anticoagulant therapy during pregnancy**
- **extracorporal circulation**

dosage

- **prophylactic use - usually 2-3 x 5000-7500 IU s.c or 5-7 IU/kg/h i.v. inf.**
- **acute therapy - usually 5000 IU starting bolus i.v., followed by 1000-1500 IU/h i.v. inf.**

uncertain pharmacokinetics $\rightarrow$ effect must be monitored by measuring aPTT (1,5-2,5 times prolonged compared with control value)

Heparin

Clinical use

LMWH

- **enoxaparin, certoparin, dalteparin, ardeparin, nadroparin, reviparin, tinzaparin**
- **different LMWH preparations are not the same (composition, pharmacokinetics etc.)**
- **similar indications as by natural heparin, in massive embolism natural heparin is used**

dosage

- **prophylactic use - usually 2500-5000 IU once daily**
- **acute treatment - usually 175-200 IU/kg s.c. 1x or 2x daily**

aPTT is not prolonged by LMWHs, their effect can not be monitored by aPTT, but usually it is not needed due to the more predictible pharmacokinetics (except renal failure - anti-Xa assay)

Heparin

Adverse effects

- **bleeding (major bleeding - 3-6%, lethal <1%)**

  i.v. therapy is associated with higher risk of bleeding

- **heparin induced thrombocytopenia (HIT)**

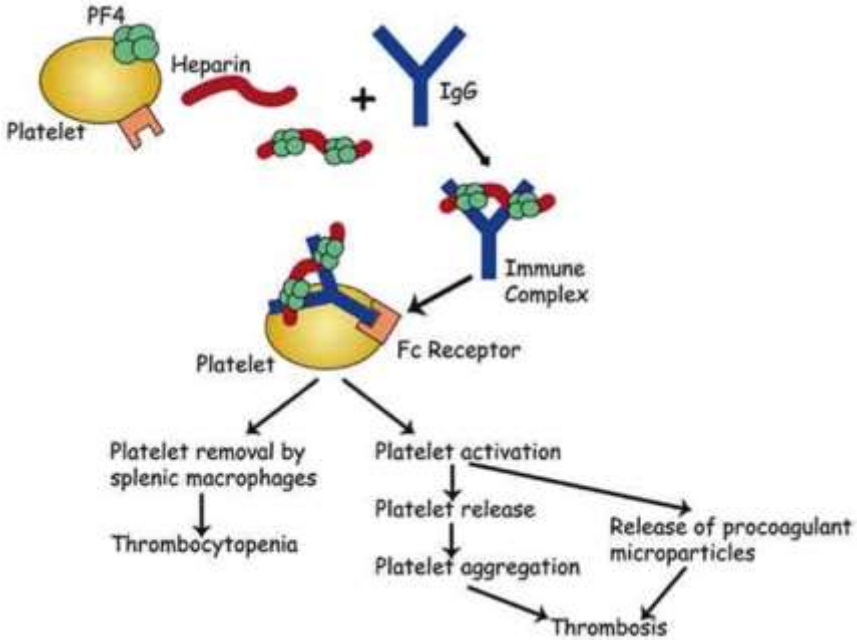
  type I - 5-10% - reversible, transient (usually in the first 4 days)

  type II - 0,5-3% - very dangerous (20-30% lethal), antibody-mediated thrombocyte aggregation → paradoxically thromboembolic complications

- **rarely: hair loss, allergic reactions, mild transaminase elevation, high doses - impaired aldosterone synthesis, osteoporosis may be associated with long-term (3-6 months) therapy**

- **most side effects are less frequent when LMWH is used**

Mechanism of HIT type II



Reversal of heparin action

- **protamine sulfate**
- **derived from salmon sperm**

- **highly basic (positively charged) protein, neutralizes heparin in blood (LMWH only partially)**

Fondaparinux

- **synthetic pentasaccharid, analogue of the antithrombin III binding subunits of heparin**
- **the „lowest molecular weight heparin”**
- **inactivates only factor Xa**
- **only parenteral use, s.c. - 100% bioavailability, half-life 15-17h**
- **deep venous thrombosis can be its major indication, does not cause HIT type II., but may cause bleeding and can not be reversed by protamin**
- **longer acting analogue: idraparinux - one s.c. inj./week**

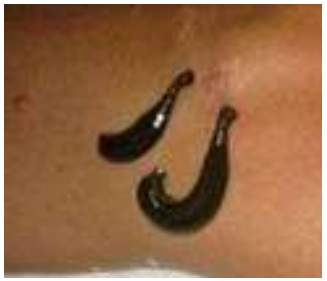
Heparinoids

Danaparoid

- **mixture of heparan sulfate (84%), dermatan sulfate (12%) and chondroitin sulfates**
- **inactivates mainly factor Xa (by accelerating antithrombin III)**
- **only parenteral use, s.c. - 100% bioavailability, half-life 25h**
- **used in case of HIT type II**
- **major toxicity is bleeding; can not be antagonized by protamine**

Direct thrombin inhibitors

Hirudin



- derived from medicinal leeches (Hirudo medicinalis)
- recombinat analogue: desirudin
- binds directly to thrombin and irreversibly inactivates it
- parenteral use only (s.c. - 100% bioavailability)
- eliminated by the kidneys, half-life 1-1,5h (renal insuff. → much longer)
- indication: anticoagulant action in case of HIT type II.
- monitoring its action: aPTT (it should be 1,5-3x higher than the control)
- adverse effects: bleeding
- no antidote

Direct thrombin or Xa inhibitors

Bivalirudin

- synthetic hirudin-like compound (direct thrombin inhibitor)
- more rapid onset and shorter duration of action than that of hirudin
- only i.v. use - percutaneous coronary angioplasty
- elimination is mostly independent from kidney

Argatroban

- synthetic thrombin inhibitor
- short half-life, elimination is independent from kidney, influenced by liver diseases
- used i.v. in case of HIT type II.

Direct oral antikoagulants (DOACs) novel oral antikoagulants (NOACs)

Dabigatran etexilate

- **orally active prodrug**
- **after absorption – conversion to dabigatran, direct thrombin inhibition, 1-2x/d**
- **antidote: idarucizumab (antibody)**

Rivaroxaban, Apixaban, Edoxaban, Betrixaban

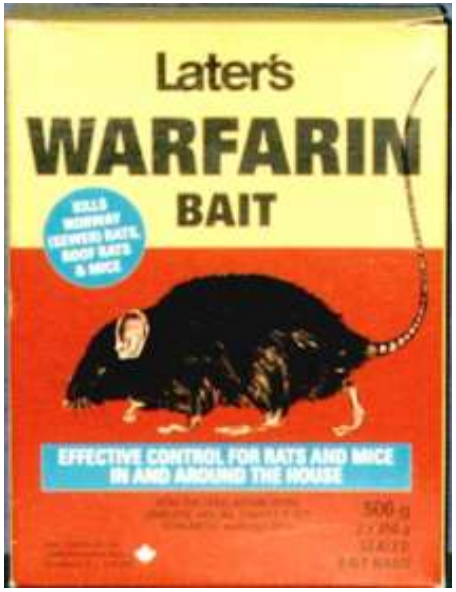
- **orally active**
- **direct factor Xa-inhibitors, 2x/d**
- **Indications: prophylaxis of deep vein thrombosis and pulmonary embolism in patients undergoing knee or hip replacement surgery; reduce the risk of stroke and systemic embolism in patients with nonvalvular atrial fibrillation; acute and chronic treatment of deep venous thrombosis and pulmonary embolism**
- **potential antidotes:**
  adexanet-alfa (recombinant analogue of factor Xa) – only Xa-inhibitors
  ciraparantag – Xa-inhibitors, dabigatran, heparin

Anticoagulants inhibiting clotting factor synthesis: cumarins

Anticoagulants inhibiting clotting factor synthesis: cumarins

History

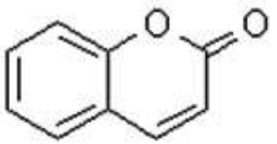
- at the end of the XIXth century - sweet clover was planted in North-USA and Canada
- 1924 - reports on the hemorrhagic disorders of cattles resulted from the ingestion of spoiled sweet clover silage
- 1939 - haemorrhagic agent was identified: dicoumarol
- use of cumarins as rodenticides
- 1948 - warfarin (name: Wisconsin Alumni Research Foundation was the patent holder + cumarin) was introduced as a rodenticide
- since 1951 - use of warfarin as an oral anticoagulant



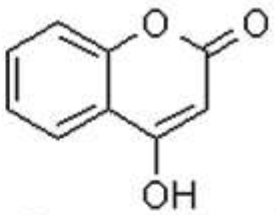
Cumarins

Chemistry

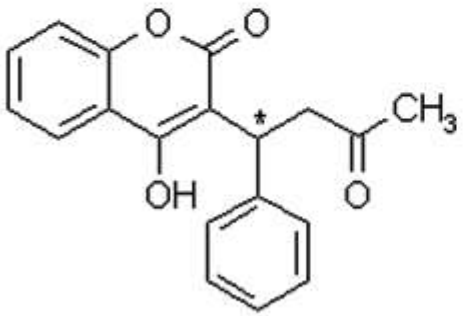
- **4-hydroxy-cumarin derivatives - differences only in potency and duration of action**



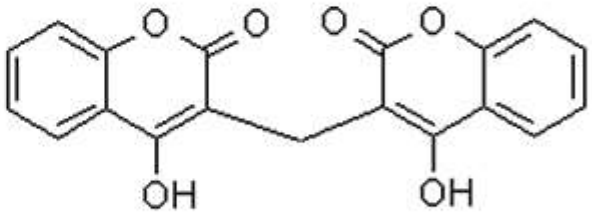
coumarin



4-hydroxycoumarin



warfarin
* chiral center

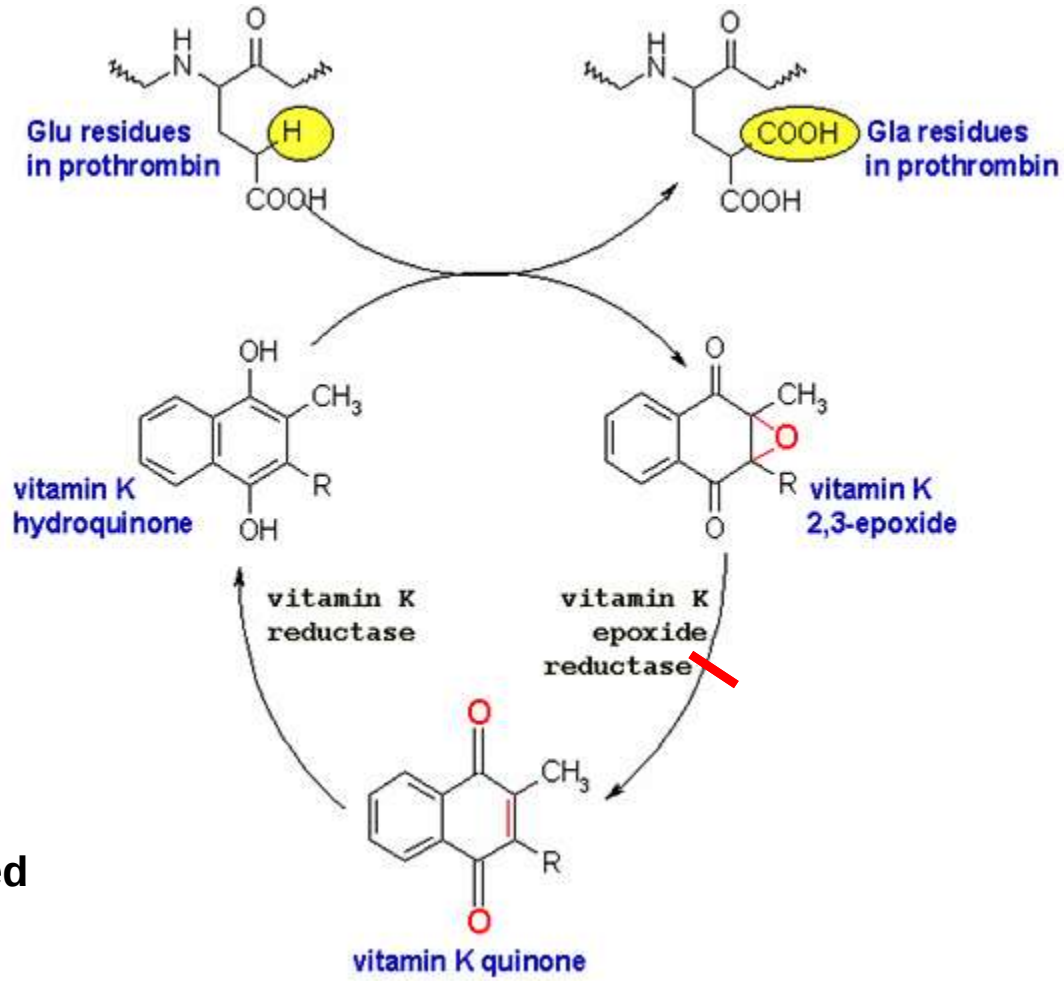


bishydroxycoumarin
(dicoumarol)

Cumarins

Mechanism of action

- complete synthesis of several clotting factors (factors II, VII, IX, X and protein C) in the liver includes a final γ-carboxylation on glutamate residues (necessary to Ca^{2+} binding and so binding to phospholipids)
- γ-carboxylation is coupled with the oxidation of reduced vitamin K (hydroquinone form) to epoxide form
- cumarins block the the vitamin K epoxide reductase and so the transformation of the vitamin back to its hydroquinone form → functionally inactive clotting factors are synthetized



- delay (8-12 hours) in the anticoagulant effect
- their action can be antagonized by vitamin K (with several hours delay)

Cumarins

Pharmacokinetics

- good absorption (almost 100%) → oral anticoagulants
- 90-99% albumin binding in plasma
- crossing the placenta, with the exception of warfarin presence in breast milk
- metabolism in liver (major step is glucuronid conjugation)
- excretion mainly with the urine, in part with the bile
- half-life - individual variations

  acenocoumarol 9-24h

  warfarin 25-60h (usually around 40h)

  phenprocoumon 130-160h

Cumarins

Clinical use

- **continuation of heparin therapy, prophilactic use to prevent thromboembolism**
- **dosage**

  warfarin 2-10 mg

  acenocumarol 1-12 mg

  phenprocoumon 0,75-6 mg

    in the first few days 2x the above doses
- **monitoring the effect, dosage adjustment based on the results**

  INR (international normalizes ration) = $(PT_{patient}/PT_{reference})^{ISI}$

  (ISI = international sensitivity index of the thromboplastin reagent)

  therapeutic goal: INR=1,5-3 (prophylactic use - sometimes only 1,2)

Cumarins

Toxicity

- bleeding (minor bleeding 10-20%, major bleeding <5%, lethal <1%), dramatically increased risk if INR>4
- malformations, death of the fetus (pregnancy is contraindication!)
- necrosis of the subcutaneous tissue (and the skin) - rare

  might affect the breast, fatty tissue, intestine, extremities

  possible reason - inhibition of protein C will be prominent at first - in the first week potentially increased risk of local thromboembolism
- additional rare adverse effects

  allergic reactions, gastrointestinal symptoms, alopecia

  purple toe syndrome
- reversal of the action - vitamin K_1 (slow onset of action), in more severe cases fresh-frozen plasma, factor concentrates

Cumarins

Interactions I.

- K-vitamin concentration in the blood - dependent on diet, intestinal bacterial flora
- pharmacokinetic interactions
  - at the absorption - inhibition by antacids, cholestyramin
  - at the albumin binding - several drugs (e.g. NSAIDs) - clinical importance is limited
  - at the metabolism
    - enzyme inhibitors: phenylbutazone, sulfinpyrazone, metronidazole, fluconazole, sulfonamides, amiodaron, disulfiram, cimetidine
    - enzyme inducers: barbiturates, rifampin, carbamazepine, phenytoin, griseofulvin

Cumarins

Interactions II.

- pharmacodynamic interactions
  - increased anticoagulation: heparin, aspirin, liver disease, hyperthyreoidism, certain cephalosporins with N-methyl-thiotetrazol substitution (e.g. cefamandol, cefoperazone)
  - decreased coagulation: vitamin K, hypothyreoidism, strong diuretic therapy, corticosteroids

Resistance to cumarin

- rare, due to mutation of vitamin K epoxid reductase

Cumarin sensitivity

- genetic polymorphism of the cumarin metabolizing enzyme (CYP2C9) - decreased activity
  - ~ 10% (caucasians 10-20%, afro-americans, asians <5%)

Contraindications: pregnancy, nursing women, active bleeding, incerased risk of dangerous bleeding