Discovery of warfarin
- 1920: appearance of new disease in N. American cattle
  o hemorrhage following minor surgery
- 1921-24: “Sweet Clover Disease” – duplicated in rabbits
- 1929: LM Roderick – affected animals lacked the blood clotting factor prothrombin
- 1933-39: KP Link – anticoagulant dicoumarol
  1. Oxidation of coumarin by fungi during spoilage to 4-hydroxycoumarin
  2. Coupling of two 4-hydroxycoumarin molecules with naturally occurring formaldehyde produces dicoumarol
- 1948: Warfarin (Wisconsin Alumni Research Foundation)
  o Rodenticide: odorless, tasteless & slow acting
- 1951: Warfarin OD reversed by Vitamin K
- 1954: Warfarin approved for use in humans (Coumadin)
- 1955: Eisenhower myocardial infarct
- 1978: MOA: Warfarin inhibits coagulation that is dependent on Vitamin K

Chemistry of Warfarin
- Water-insoluble lactones
- Weakly acidic
  o Warfarin sodium salt
- Warfarin cyclic hemiketal may be the active form in vitro

Coagulation: prevent blood loss from damaged blood vessels by producing a platelet/fibrin plug

Dysfunction
- Pathological reduction: hemorrhage
- Pathological increase: thrombosis

3 major clotting pathways
- Tissue Factor Pathway
- Contact Activation Pathway
- Platelet Activation

Vitamin K – “Koagulation”: fat-soluble organic compounds required for modification & activation of protein cascades required for blood coagulation and metabolism in various tissues (bones)

- Naturally occurring K₁ (phyloquinone, phyomenadione, phytonadione)
  o Plant-derived, primary dietary source from green leafy vegetables
  o Used as is or converted to K₂ for use in blood coagulation
- Naturally occurring K₂ (menaquinones), MK-n (isoprenoids) – humans & other mammals
  o MK-4 (arteries, pancreas, testes)
  o MK-7-11 (colonic bacteria)
- Synthetic K₃, K₄, K₅

Vitamin K: cofactor for activation of enzymes by carboxylation of glutamate residues

Factors II, VII, IX, X

Inactive protein precursor (Glu)

Prozymogen

Active γ-carboxylated protein (Gla)

Active enzyme

Vitamin K Epoxide

Reduces

Vitamin K Epoxide Reductase (VKOR)

Vitamin K

Reductive

pathway for VKOR reduction

Dietary Sources

Activated Factors II, VII, IX, X

Vitamin K Hydroquinone

Reduced

Vitamin K Quinone Reductase

NAD+ NADH

Vitamin K

SH SH SH SH

Vitamin K Epoxide

Oxidized

Vitamin K Epoxide Reductase (VKOR)

Vitamin K-dependent gamma-glutamyl carboxylase

Ca²⁺ needs another CO₂

IVA can't help but Ca²⁺
Determination of warfarin dosing: prothrombin time (12-13 sec) = time for plasma to clot after addition of TF
INR (international normalized ratio) = (PT test / PT normal)$^{16}$ ISI = international sensitivity index of
manufacturers tissue factor

- 0.8 – 1.2 = basal
- 2.0 – 3.0 = warfarin
- 2.5 – 3.5 = extreme

Above range: risk of bleeding increases
Below range: risk of thromboembolic events increases

Drug interactions: inhibition of CYP2C9 $\rightarrow$ ↑ risk of bleeding

Food interactions: need to keep consistent dietary intake of Vit K

Warfarin MOA: binds to and inhibits VKOR(C1)

Warfarin binds to site close to redox active site of VKOR = steric competition

- Vitamin K Hydroquinone (reduced) is reduced
  - Can’t further activate the 4 tissue factor enzymes dependent on Vitamin K

Warfarin metabolism

- Vitamin K Epoxyde
- Vitamin K Hydroquinone
- 7-OH-WARFARIN
- 6-OH-WARFARIN
- 8-OH-WARFARIN

Limitations of warfarin therapy

- Main cause of ER admissions for severe bleeding
- Main cause of bleeding as ADR
- 15% minor bleeding; 1% die
- Causes 8% of cerebral bleeds
- Adverse drug interactions
- Narrow therapeutic index
- Requires regular coagulation monitoring

Warfarin indications

- Oral anticoagulant used in prevention of:
  - Thrombosis: formation of blood clots in blood vessels
  - Thromboembolism: migration of blood clots
- Atrial fibrillation
  - Increased risk of blood clots (inefficient pumping)
  - Clots can migrate to & block arteries in brain $\rightarrow$ STROKE (4-5 x higher incidence)
- Mechanical heart valves
  - Constant anticoagulant therapy due to high shear stress, stagnation, and flow separation
  - Risk of mechanical hemolytic anemia, RBC hemolysis
- Prevention of secondary myocardial infarct
  - Irregular pumping common $\rightarrow$ increased risk of clots
- Conditions that promote increased incidence of blood clots
  - Hypertension / CVD
  - Altered expression or activity of clotting pathway enzymes
  - Hip/knee replacement surgery, coronary stents

Varied response to warfarin

- Increased dose indicated: 65/100
  - ↑ risk of blood clot at intermediate dose
- Intermediate dose: 32/100
- Lower dose indicated: 3/100
  - ↑ risk for excessive bleeding at intermediate dose

Age: large decrease in CYP2C9 activity

- Mean warfarin daily dose requirements fall by 0.5 – 0.7 mg per decade between 20 – 90 years old

Drug interactions: inhibition of CYP2C9 $\rightarrow$ ↑ risk of bleeding

Food interactions: need to keep consistent dietary intake of Vit K

Warfarin metabolism

- Vitamin K Hydroquinone
- Vitamin K Epoxyde
- 7-OH-WARFARIN
- 6-OH-WARFARIN
- 8-OH-WARFARIN

Elimination via kidney and bile
**Warfarin variation: pharmacogenomics** – mutations account for 40-63% of variability in warfarin dosing

<table>
<thead>
<tr>
<th>VKOR activity &amp; warfarin dosing</th>
<th>CYP2C9 activity &amp; warfarin dosing</th>
</tr>
</thead>
<tbody>
<tr>
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**VKORC1 and CYP2C9 Polymorphisms**

| VKORC1 variant – decreased expression = decreased activity |
| CYP2C9 variant 2 – decreased activity |
| CYP2C9 variant 3 – no activity |

**NOTE:** there are mutations that increase VKOR activity → contribute to warfarin resistance or hypersensitivity (increased risk of hemorrhage)

### Anticoagulant alternatives to warfarin

- Dabigatran, lepirudin: inhibits thrombin
- Heparin: activates anti-thrombin III
- Rivaroxaban, apixaban: inhibits Xa

**Heparin**

- Activates anti-thrombin III, inhibits unbound thrombin
- SC or IV dosing – high negative charge & size precludes gut absorption
- Unfractioned heparin: $t_{1/2}$: 1-2 hours
- LMW heparin: $t_{1/2}$: 4-5 h
- Heparin-induced thrombocytopenia: platelets targeted by immune response
- Protamine sulfate – binds heparin
- Not appropriate for long-term anticoagulant treatment
  - o Start of heparin while warfarin takes effect = “bridging”

**Newer warfarin alternatives**

- Dabigatran, Lepirudin: direct thrombin inhibitor, can bind free & fibrin-bound
- Rivaroxaban, Apixaban: direct factor Xa inhibitor
  - → Fast acting (4 hours)
  - → Doesn’t require frequent blood tests for INR monitoring
  - → No specific way to reverse during major bleeding event