Warfarin therapy

Rat poison and the prevention of thrombosis

Thrombosis, which has been defined as blood clotting in the wrong place, is among the principal causes of death in the Western world. One of the drugs that is most frequently used to prevent arterial thrombosis is the oral anti-coagulant, warfarin, which was first marketed as rat poison in the 1940s. In 1999, warfarin was the eleventh most prescribed drug in the USA, with annual sales of approximately US$500 m.

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The discovery of warfarin[1]

In the early part of the 20th Century, cattle farmers in the northern prairie states of Canada and the USA began planting sweet clover plants (*Melilotus alba* and *M. officinalis*) that were imported from Europe (see Figure 1). Although the sweet clover proved to be nutritious when used as fresh fodder or as hay, it also brought a fatal disease in which the cattle developed severe, spontaneous bleeding. In 1922, Schofield first attributed the bleeding disease to the consumption of improperly cured sweet clover hay (unspoiled fodder had no effect) and in 1929, Roderick observed that the affected cattle were deficient in a blood clotting factor, prothrombin. At the same time, Dam was investigating a severe bleeding condition with similar depletion of prothrombin in hens that were maintained on sterol-depleted diets. This work led to the discovery of vitamin K, for which Doisy and Dam received the Nobel Prize for Medicine in 1943 (see Figure 2). In 1940, Link and co-workers published the purification and synthesis of dicumarol (3,3′-methylenebis-9 [4-hydroxycoumarin]), the active component in the spoiled sweet clover (see Figure 2). Dicumarol is derived from oxidation of coumarin (the plant product responsible for the sweet smell of *Melilotus*) by the action of fungi in the mouldy hay. Clinical investigators began immediately to experiment with the therapeutic use of dicumarol, but renewed impetus to the development of oral anticoagulants came in 1946, again from Link, who had turned his interest to the development of rat poison. Dicumarol proved to be relatively ineffective as a rodenticide and the most potent compound Link and co-workers discovered was 3-phenylacetyl ethyl 4-hydroxycoumarin (see Figure 2). Link assigned patent rights of this compound to the Wisconsin Alumni Research Foundation, from which the name warfarin was derived. Warfarin was launched as the ideal rat poison in 1948. Although it was thought at first to be too toxic for human use, in 1951 the failed attempted suicide of a navy recruit who had taken a large dose of rat poison led clinicians to discard dicumarol in favour of warfarin. The first clinical study with warfarin was reported in 1955. In the same year, President Eisenhower was treated with warfarin following a heart attack.

Figure 1. Sweet clover. Photo credit: copyright Br. Alfred Brousseau, St Mary's College of California

Mechanism of action

In 1978, two groups independently reported that warfarin acts by inhibiting vitamin K epoxide reductase, which catalyses an essential reaction in the vitamin K-dependent pathway by which clusters of glutamyl residues in certain proteins are γ-carboxylated (γ-carboxyglutamic acid). The post-translational modification of what

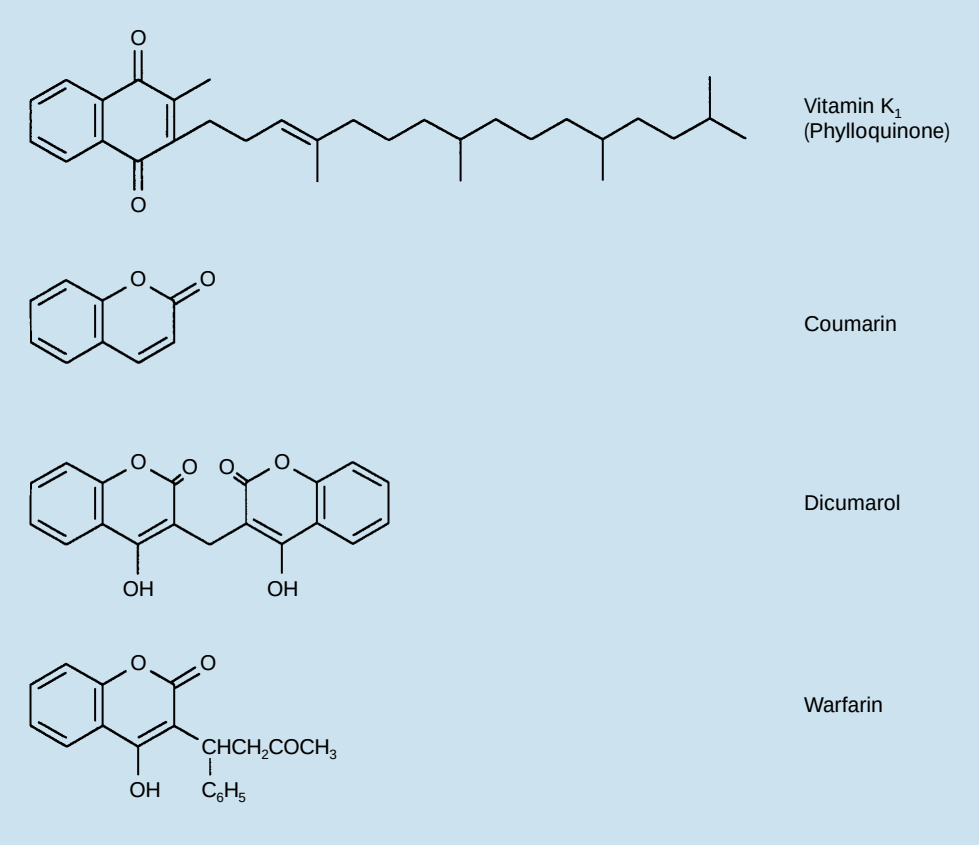

Figure 2.

are known collectively as the vitamin K-dependent proteins is carried out by a carboxylase, an integral membrane glycoprotein that binds both the propeptide sequence of the protein undergoing modification and the reduced form of vitamin K (hydroquinon)[2]. As yet, the mechanism by which vitamin K acts as a cofactor for the carboxylase is not fully understood. One hypothesis is that an oxygenated species of vitamin K is generated that extracts a proton from the γ-carbon of glutamic acid, thereby permitting the addition of CO_2. The activated vitamin K subsequently "collapses" to vitamin K epoxide. The regeneration of the reduced form of vitamin K from the epoxide, and also from the naturally occurring quinone form of vitamin K, is due to vitamin K epoxide reductase, a membrane-bound, multiprotein enzyme complex associated with the endoplasmic reticulum.

The inhibition of the reductase by warfarin results in insufficient vitamin K hydroquinone to support full carboxylation of the vitamin K-dependent proteins involved in blood coagulation.

Vitamin K-dependent proteins

A number of γ-carboxyglutamic acid-containing proteins have been identified (Table 1). The consensus site within the propeptide that identifies a protein for carboxylation is defined by a Zaa-Phe-Zaa-Xaa-Xaa-Xaa-Xaa-Ala motif, where Zaa is an aliphatic hydrophobic amino acid and Xaa is any amino acid. The vitamin K-dependent blood coagulation factors contain 10–12 closely associated γ-carboxyglutamic acid residues within the first 40 residues of the N-terminus of the mature protein (known as the Gla domain).

Function of the Gla domain in blood coagulation

The ability of the body to control the flow of blood following injury is paramount to survival. Blood coagulation occurs by the proteolytic cleavage of fibrinogen by thrombin, which generates fibrin monomers that rapidly polymerize to form a blood-retaining mesh. Proteolytic cleavage is also responsible for the generation of thrombin from its inactive precursor prothrombin, through the action of factor Xa. The cleavage by factor Xa is very slow when the enzymes are mixed in free solution. In addition to Ca^{2+}, two factors are required to accelerate the reaction; factor Va and a cell surface that expresses relatively high levels of phosphatidylserine on the outer leaflet (as is provided by the surface of activated platelets). Factor Xa and prothrombin are, coincidentally, bound on to the cell surface by the Gla domain, thereby reducing the apparent K_m for the reaction.

In the absence of Ca^{2+}, γ-carboxyglutamic acid residues of factor Xa and prothrombin are exposed to solvent and the Gla domain has no affinity for the cell surface. After the binding of seven calcium ions, the γ-carboxyglutamic acid residues are internalized and hydrophobic residues within the Gla domain become exposed. The affinity of the Ca^{2+}-loaded Gla domain for the cell surface is thought to be caused by the penetration of these hydrophobic residues into the phospholipid membrane to a depth of 7 Å[3]. This penetration probably permits further interactions between the protein and the negatively charged phospholipid to achieve full binding.

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Therapeutic use of warfarin

Three coumarin-derived drugs are currently used therapeutically as anti-coagulants. These do not differ in their effect on vitamin K metabolism, but are different in terms of pharmacokinetics. The elimination half-life of warfarin is 40–70 h, whereas those of acenocoumarol and phenprocoumon are 3-10 h and 90–140 h respectively. These differences have implications in patient management: for example phenprocoumon takes over 2 weeks to reach steady-state, whereas acenocoumarol has to be administered on a twice daily basis[4].

Steady-state levels of the drugs are set so that only approximately 20% of the normal blood level of the vitamin K-dependent coagulation factors contain γ-carboxyglutamic acid residues. This reduced level is associated with a decrease in the functional levels of each coagulation factor (rather than the antigenic levels of each protein). Oral anticoagulant therapy is controlled by measurement of the prothrombin time (the clotting time of plasma in the presence of tissue factor). The clotting time is expressed as the ratio of the observed clotting time to the clotting time of normal plasma (the International Normalized Ratio). Patients need to be monitored regularly, since there are a multiplicity of drugs that interfere with oral anti-coagulant therapy[5]. As well as ensuring the effectiveness of the anti-coagulant therapy, monitoring also reduces the risk of bleeding, which is a distinct risk in patients undergoing this treatment. Fatal bleeding is much rarer and it has been calculated that oral anti-coagulation in stroke patients prevents 20 strokes for every bleeding episode that it causes.

In addition to their importance in blood clotting, recent work suggests a role for the Gla proteins in vascular calcification. Vascular calcification, including coronary and aortic calcification, is very common and clinically significant in atherosclerosis and heart failure. What has stirred interest in the Gla proteins was the observation of extensive vascular calcification (and early death) in matrix Gla protein (MGP) knockout mice. MPG, which was initially isolated from bone, acts as an inhibitor of bone morphogenetic protein-2. The significance of this new insight to warfarin treatment waits to be investigated[6].

Warfarin resistance, which has been the subject of much research in rats, is also occasionally seen in warfarinized patients, especially where the infusion of large doses of vitamin K has been used to reverse anti-coagulant therapy. The mechanism responsible for the resistance in rats has been found to vary from strain to strain. There are reports of accelerated clearance of the poison or lower affinity of warfarin for the reductase complex.

Table 1.

Known vitamin K-dependent proteins

Blood clotting-associated	*Bone proteins*	*Other proteins*
	Osteocalcin	Gas6
Prothrombin	Matrix Gla protein	$PRGP_1$
Factor VII		$PRGP_2$
Factor IX		
Factor X	*Conopeptides*	
Protein C		
Protein S	Conantokin G	
Protein Z	Conantokin T	

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