MILESTONE 2

Warfarin: from rat poison to clinical use

The anticoagulant drug warfarin is widely used to prevent and treat deep-vein thrombosis and pulmonary embolism, and to prevent stroke in patients who have atrial fibrillation, valvular heart disease, or a prosthetic heart valve.

The discovery of warfarin originated in the 1920s on the prairies of Canada and North America. Previously healthy cattle began dying from internal bleeding with no obvious cause. The cattle and sheep had grazed on sweet clover hay (*Melilotus alba* and *Melilotus officinalis*); the haemorrhaging occurred most commonly when the climate was damp and the hay had become infected with mould. The spoiled hay would normally have been discarded, but financial hardship in the 1920s meant that farmers could not afford replacement fodder. The haemorrhagic disease became known as 'sweet clover disease'.

Frank W. Schofield and Lee M. Roderick, two local veterinary surgeons, showed that sweet clover disease could be reversed by removing the mouldy hay or by transfusing

fresh blood into the bleeding animal. Roderick referred to the acquired coagulation disorder as a 'plasma prothrombin defect'.

Despite recommendations not to feed mouldy hay to their cattle, many farmers did not follow the advice, and sweet clover disease remained prevalent a decade later. In desperation, Ed Carlson, a Wisconsin farmer, drove a dead cow 200 miles to an agricultural experimental station, where he presented biochemist Karl Link with a milk can of unclotted blood. Link and colleagues set about identifying and isolating the active compound that caused the haemorrhagic disease. They adopted a new *in vitro* clotting assay using plasma from rabbits to guide chemical fractionation of compounds found in the hay.

By 1940, after 6 years' work, Link and colleagues established that a natural substance called coumarin was oxidized in mouldy hay to produce 3,3'-methylene-bis(4-hydroxycoumarin), which would become better known as dicoumarol. The work was funded by the Wisconsin

“ Warfarin transitioned into clinical use ... and was approved for use in humans in 1954 ”

Alumni Research Foundation (WARF), who were awarded the patent for dicoumarol in 1941.

In 1945, Link considered using a coumarin derivative as a rodenticide. Dicoumarol acted too slowly to be a practical poison. Link and colleagues worked through a list of 150 variations of coumarin, and number 42 was found to be particularly potent. The compound was named 'warfarin' after the funding agency, and was successfully marketed in 1948 as a rodenticide.

In 1951, a US Army inductee attempted suicide with multiple doses of warfarin in rodenticide, but fully recovered after being treated with vitamin K in hospital. Studies then began on the use of warfarin as a therapeutic anticoagulant. Clinical anticoagulants were available at this time, but heparin required parenteral administration, and dicoumarol had a long lag period before onset of a therapeutic effect. The main advantages of warfarin were high oral bioavailability and high water solubility; it was more potent than dicoumarol, but its effect could still be reversed by vitamin K. Therefore, warfarin transitioned into clinical use under the trade name Coumadin, and was approved for use in humans in 1954.

An early recipient of warfarin was US president Dwight D. Eisenhower, who was prescribed the drug after a myocardial infarction in 1955. Despite its widespread use, the mechanism of action of warfarin was not discovered until 1978, when John W. Suttie and colleagues demonstrated that warfarin disrupts vitamin K metabolism by inhibiting the enzyme epoxide reductase.

Gregory B. Lim,
Chief Editor, *Nature Reviews Cardiology*

ORIGINAL ARTICLES Roderick, L. M. The pathology of sweet clover disease in cattle. *J. Am. Vet. Med. Assoc.* **74**, 314–325 (1929) | Roderick, L. M. A problem in the coagulation of blood: 'sweet clover disease of cattle'. *Am. J. Physiol.* **96**, 413–425 (1931) | Campbell, H. A. et al. Studies on the hemorrhagic sweet clover disease: I. The preparation of hemorrhagic concentrates. *J. Biol. Chem.* **136**, 47–55 (1940) | Campbell, H. A. et al. Studies on the hemorrhagic sweet clover disease: II. The bioassay of hemorrhagic concentrates by following the prothrombin level in the plasma of rabbit blood. *J. Biol. Chem.* **138**, 1–20 (1941) | Campbell, H. A. & Link, K. P. Studies on the hemorrhagic sweet clover disease: IV. The isolation and crystallization of the hemorrhagic agent. *J. Biol. Chem.* **138**, 21–33 (1941) | Stahmann, M. A. et al. Studies on the hemorrhagic sweet clover disease: V. Identification and synthesis of the hemorrhagic agent. *J. Biol. Chem.* **138**, 513–527 (1941) | Whitton, D. S. et al. Mechanism of coumarin action: significance of vitamin K epoxide reductase inhibition. *Biochemistry* **17**, 1371–1377 (1978)
FURTHER READING Wardrop, D. & Keeling, D. The story of the discovery of heparin and warfarin. *Br. J. Haematol.* **141**, 757–763 (2008)