Mechanisms of action of the anticoagulants warfarin, 2-chloro-3-phytylnaphthoquinone (Cl-K), acenocoumarol, brodifacoum and difenacoum in the rabbit

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Vitamin K_1 is essential for normal blood coagulation as a co-factor in the postribosomal synthesis of clotting factors II (prothrombin), VII, IX, and X. The vitamin K_1 dependent step in clotting factor synthesis involves the γ-carboxylation of glutamic acid residues in clotting factor 'precursors' during which vitamin K_1 is converted to vitamin K_1 epoxide by vitamin K_1 epoxidase. For the continued synthesis of clotting factors vitamin K_1 must be regenerated from the biologically inactive epoxide by vitamin K_1 epoxide reductase (Esnouf, 1977). Apart from studies of warfarin in man (Shearer, McBurney, Breckenridge & Barkhan, 1977), previous studies on the mechanism of anticoagulants which act by interfering with the vitamin K_1-epoxide cycle have been carried out in the rat (Willingham, Laliberte, Bell & Matschiner, 1976). We have studied the relationship between vitamin K_1 metabolism and prothrombin complex activity (PCA) in the rabbit, thus allowing their temporal relationship to be studied in the same individual animal.

After a single dose of anticoagulant the metabolism of [^{3}H]-vitamin K_1 was studied by reversed-phase chromatographic analysis of blood samples taken hourly for 6 h after a 10 μCi dose. PCA was measured at 4 hourly intervals using a one-stage prothrombin time method.

Warfarin and Cl-K are thought to act by inhibiting the vitamin K_1-epoxide cycle at the reductase and epoxidase steps respectively (Willingham *et al.*, 1976). Warfarin did not affect the plasma elimination of [^{3}H]-vitamin K_1 but did cause an accumulation of [^{3}H]-vitamin K_1 epoxide resulting in a significant increase in the maximum [^{3}H]-vitamin K_1 epoxide: [^{3}H]-vitamin K_1 ratio in plasma to 1.29 ± 0.21 compared with controls, 0.44 ± 0.09. In contrast Cl-K decreased the maximum ratio of [^{3}H]-vitamin K_1 epoxide: [^{3}H]-vitamin K_1 in plasma to 0.13 ± 0.04, in keeping with the hypothesis that it is a direct antagonist of vitamin K_1 epoxidase (Willingham *et al.*, 1976). To test further the relationship between the epoxidase and the reductase in the vitamin K_1-epoxide cycle warfarin and Cl-K were administered together. A dose of Cl-K which reduced PCA to $< 5\%$, inhibited the accumulation of vitamin K_1 epoxide normally produced by warfarin.

In our system, we have studied the effects of acenocoumarol and two novel anticoagulants, brodifacoum and difenacoum which are more potent than warfarin and are effective in warfarin-resistant rats (Hadler & Shadbolt, 1975). With all 3 drugs the plasma half-life of disappearance of [^{3}H]-vitamin K_1 from plasma was similar to that seen with control animals while the mean half-lives of degradation of PCA were similar to the mean half-life obtained with warfarin. All three drugs caused a significant ($P < 0.01$) increase in the plasma [^{3}H]-vitamin K_1 epoxide: [^{3}H]-vitamin K_1 ratio indicating that they block clotting factor synthesis by inhibiting vitamin K_1 epoxide reductase.

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