The Chloro Analog of Vitamin K: Antagonism of Vitamin K Action in Normal and Warfarin-Resistant Rats (37411)

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The mechanism by which the coumarin anticoagulants block the action of vitamin K has not been clearly established. Although it is often assumed (1) that they function as direct antagonists of the vitamin at its metabolically active site, there is little experimental evidence to support this view. Bell and Matschiner (2) have suggested that coumarins act by increasing the liver concentration of the 2,3-epoxide of vitamin K which then antagonizes the action of vitamin K. A third theory has been proposed by Lowenthal, who has suggested (3) that warfarin functions as an anticoagulant by blocking some specific cellular transport route for vitamin K. The theory is based on the nature of the antagonism observed between vitamin K and the vitamin K antagonist, 2-chloro-3-phytyl-1,4-naphthoquinone (chloro-K) (4), in vitamin K-deficient, and warfarin-treated rats. Lowenthal's observations were based on the response of plasma factor VII (proconvertin) to the various treatments utilized. The activity of this clotting factor increases faster than that of prothrombin, and may respond to levels of the vitamin which show little effect on prothrombin concentrations. We have therefore studied the effect of chloro-K on prothrombin production, and have extended these observations to the warfarin-resistant rat. These animals, which have hereditary resistance to warfarin (5), provide an additional system for investigating the mechanism of action of warfarin, and these studies provide additional information regarding this strain of rats.

Methods. Normal Holtzman strain rats, and rats homozygous for the warfarin-resistant trait (6, 7) were used. The rats (165–170 g) were housed in coprophagy preventing cages (8) and fed a diet low in vitamin K (9) for 6 to 7 days (normal) or 2 to 3 days (warfarin resistant). After this time, their plasma prothrombin concentrations were usually less than 15 units/ml plasma (normal values are 210–240 units).

Blood samples were obtained under light ether anesthesia by cardiac puncture. Blood (0.9 ml) was drawn in a syringe containing 0.1 *M* potassium oxalate (0.1 ml) and centrifuged at 2000*g* for 20 min in refrigerated centrifuge. Prothrombin concentration was measured by the two-stage method of Ware and Seegers as modified by Shapiro and Waugh (10) and expressed as Iowa units per milliliter of plasma. The chloro analog of vitamin K_1 was emulsified with Tween 80 before use, and a commercial emulsion of vitamin K_1 (Aquamephyton) was used. The emulsified vitamin K and chloro-K were diluted with physiological saline just prior to each experiment and were administered intravenously via the exposed jugular or tail vein.

Results. The response to vitamin K administration in both normal and warfarin-resistant vitamin K-deficient, hypoprothrombinemic rats is shown in Fig. 1. Both strains of rats showed a characteristically rapid increase in prothrombin concentration 1 hr following vitamin K administration, and a small additional increase by 2 hr. The maximum response in normal rats was reached with about 0.75 μg vitamin K/100 g. The data also indicate that the warfarin-resistant rats had about a 20-fold higher requirement of vitamin K_1 than did the normal rats. At each dose of vitamin K_1 administered, male warfarin-resistant rats showed lower prothrombin response than female warfarin-resistant rats (Fig. 1). Vitamin K-deficient normal female rats (data not shown) showed a similar re-

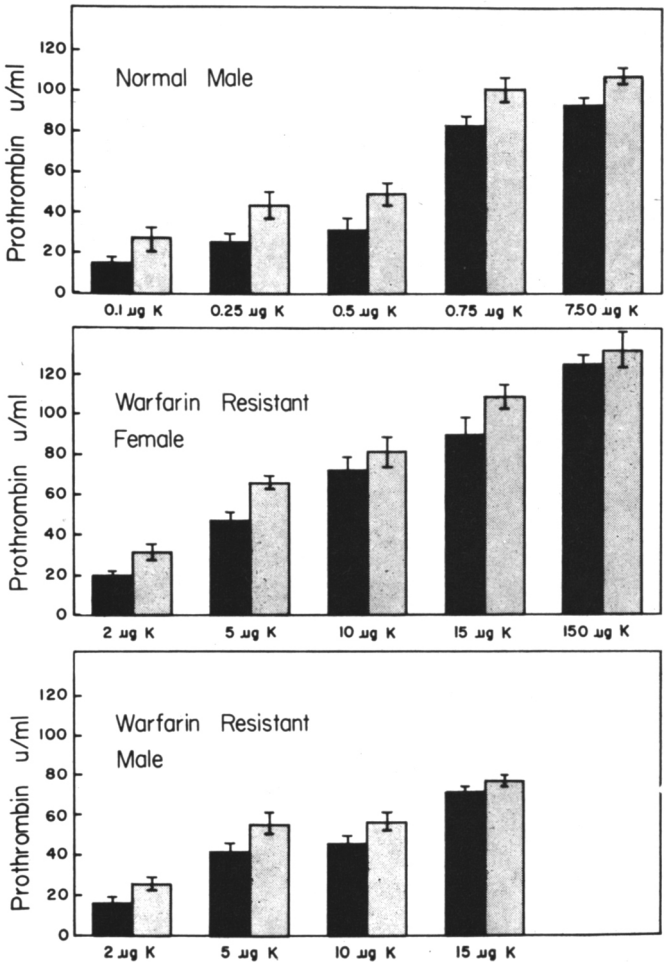

FIG. 1. Increase in plasma prothrombin concentration at 1 hr (dark bar) and 2 hr (light bar) following the intravenous administration of vitamin K to vitamin K-deficient hypoprothrombinemic rats. The amount of vitamin K administered per 100 g of body weight is shown. The vertical bars indicate standard error of the mean for 5–7 rats/group.

sponse to that obtained with male rats at each dose of vitamin K_1. The normal female rats did however require a slightly longer period in coprophagy preventing cages (10–11 days) than the male rats (6–7 days) to produce the same degree of hypoprothrombinemia.

The data in Fig. 2 illustrate that the administration of increasing amounts of cloro-K along with a constant amount of vitamin K to vitamin K-deficient hypoprothrombinemic rats results in an inhibition of the normal response. In the normal rat, 10-fold more chloro-K (7.5 μg/100 g) than vitamin K is without effect while 10 and 20 μg/100 g produce partial inhibition, and 30 μg/100 g almost completely inhibit the response to vitamin K_1. When vitamin K-deficient, warfarin-resistant female or male rats of nearly the same nutritional status are used similar results are obtained (Fig. 2 middle, bottom). Even though a larger dose of vitamin K_1 (20-fold) is required to produce a comparable rise in the plasma level of prothrombin in vitamin K-deficient, warfarin-resistant rats, the doses of the chloro analog required for partial or almost complete inhibition of the rise in prothrombin concentration in vitamin K-deficient warfarin-resistant rats is only about 1.5 times that re-

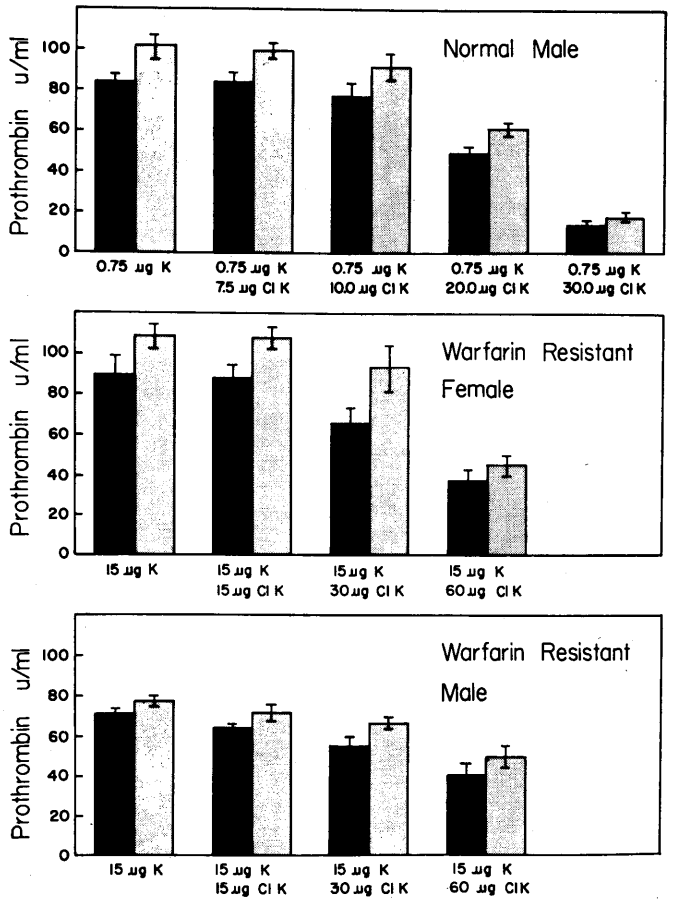

FIG. 2. Increase in plasma prothrombin concentration at 1 hr (dark bar) and 2 hr (light bar) following the intravenous administration of varying ratios of vitamin K and chloro K to vitamin K-deficient, hypoprothrombinemic rats. The amount of vitamin K and chloro K administered per 100 g of body weight is shown. The vertical bars indicate the standard error of the mean for 5–7 rats/group.

quired in normal rats. The ratio of chloro-K to vitamin K which is needed to prevent the vitamin K-induced response is therefore considerably less in the warfarin-resistant rat.

Another difference in the behavior of the two different strains of rats was shown when they were both given increasing amounts of a constant ratio of chloro-K to vitamin K which was shown in Fig. 2 to be ineffective in blocking the prothrombin response. In normal vitamin K-deficient rats there was no inhibition of the rise in prothrombin concentration when 0.75 μg/100 g of vitamin K_1 and 7.5 μg/100 g of chloro analog were given together (Fig. 3, top). But when the doses of each were increased 4-fold and the ratio of vitamin K_1 to the chloro analog was kept constant, partial inhibition was observed. A further 2-fold increase of the doses (vitamin K_1, 6.0 μg/100 g; chloro analog 60 μg/100 g) produced marked inhibition. In contrast to this observation, when similar experiments were carried out in vitamin K-deficient, warfarin-resistant female and male rats, the higher doses were without any effect (Fig. 3). The degree of inhibition in the rise of plasma prothrombin remained constant when the dose was increased 2-, 4-, or 8-fold. Even at a ratio of vitamin K_1:chloro-K of 1:4 (data not shown) which produced some inhibition at the lower level, the in-

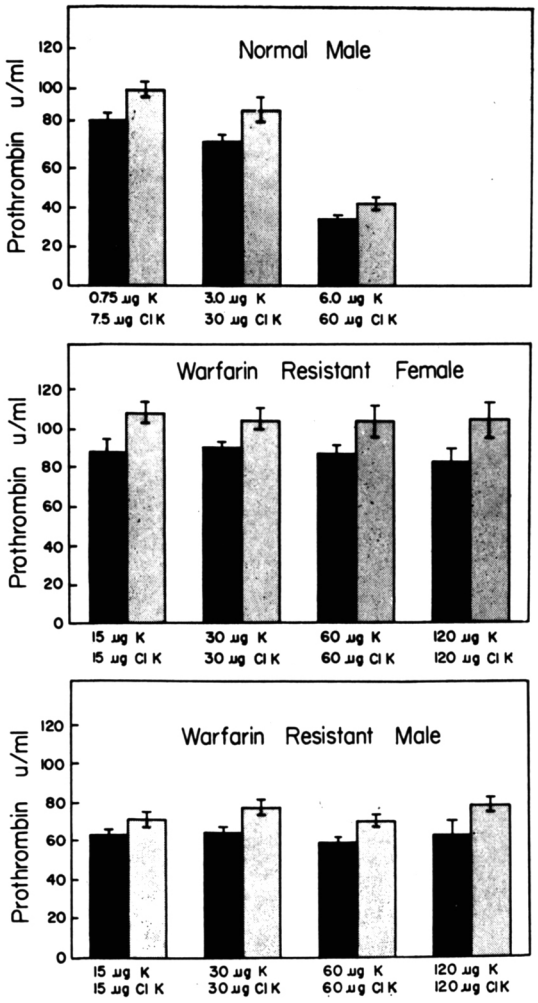

FIG. 3. Increase in plasma prothrombin concentrations at 1 hr (dark bar) and 2 hr (light bar) following the intravenous administration of varying amounts of a constant ratio of vitamin K and chloro K to vitamin K-deficient hypoprothrombinemic rats. The amount of vitamin K and chloro K administered per 100 g of body weight is shown. The vertical bars indicate the standard error of the mean for 5–7 rats/group.

hibition was not increased by higher doses.

Discussion. The data in Fig. 1, which show about a 20-fold difference in the amount of vitamin K needed to produce the equivalent prothrombin response to warfarin-resistant as in normal hypoprothrombinemic rats confirm the original observations of Hermodson, Suttie and Link (11) that these rats have a higher vitamin K requirement than normal rats. The effect of chloro-K on the vitamin K-induced increase in prothrombin was the same as Lowenthal had observed (12, 13) for a factor VII response. At a ratio of about 30 times as much chloro-K as vitamin K, a significant inhibition of prothrombin formation occurred. Lowenthal has previously observed that a noninhibitory ratio of chloro-K to vitamin K becomes inhibitory if the total dose is increased, while the ratio of the two compounds is kept constant. These data would confirm that observation.

The behavior of the warfarin-resistant rats toward chloro-K administration was different. A lower ratio of chloro-K to vitamin K was effective in inhibiting a prothrombin response in hypoprothrombinemic warfarin-resistant

rats. This is consistent with the observation (14) that plasma prothrombin concentrations in these rats are decreased by lower doses of chloro-K than are needed for normal rats. As the amount of a noninhibiting ratio of the two compounds was increased, there was no indication that the treatment was having any greater inhibitory effect on prothrombin synthesis in warfarin-resistant rats. These results are similar to what Lowenthal has observed when chloro-K was administered to rats made hypoprothrombinemic by prior treatment with warfarin. He has interpreted his data by assuming that warfarin blocks a normal physiological transport route for vitamin K, and both it and the chloro analog must reach a vitamin K receptor protein via an alternate route which is functional only at higher doses of the vitamin. The chloro analog cannot use the normal route, so in vitamin K-deficient animals it acts as a noncompetitive inhibitor of the vitamin. Increasing the dose of an ineffective ratio of the two compounds will eventually produce an inhibition as sufficient chloro-K will get through the alternate route to block vitamin K action. In the warfarin-treated animal both compounds must travel the alternate route, and the antagonism is competitive. This model is hypothetical in the sense that there is no direct demonstration of more than one route of vitamin K transport, nor any indication what cellular pools of the vitamin are involved. If the model is assumed, however, the defect in the warfarin-resistant rat could be explained as a loss of the normal vitamin K transport route so that all of the vitamin is transported via the alternate, nonwarfarin-sensitive route. The increased vitamin K requirement of the warfarin-resistant rat would be consistent with this type of metabolic defect. However, there may be a number of biochemical alterations in the warfarin-resistant rat (7, 15–17) and the basis for its resistance to warfarin, or the action of warfarin in normal rats, has not been satisfactorily explained at this time.

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