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An investigation of the pharmacological response to vitamin K_1 in the rabbit

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1 The relationship between pharmacological response and disposition of a dose of vitamin K_1 ($10\,mg\,kg^{-1}$, i.v.) in normal rabbits and in rabbits treated with the coumarin anticoagulant brodifacoum, has been studied.

2 High performance liquid chromatography (h.p.l.c.) with electrochemical detection (EC) was used to determine concentrations of vitamin K_1 in plasma, whole liver homogenate, and liver microsomes.

3 After intravenous administration of vitamin K_1, plasma concentrations of the vitamin declined in a tri-exponential fashion. There were no differences between the two groups over the first 24 h of the experiment. However, between 24 h and the end of the study, plasma concentrations of vitamin K_1 in the presence of brodifacoum were significantly ($P \leqslant 0.05$) below those of vehicle-treated rabbits.

4 Seventy-two hours after administration of vitamin K_1, plasma concentrations of the vitamin were not different from normal.

5 Three hours after administration of vitamin K_1, the concentrations of the vitamin in whole liver were $46.6 \pm 4.3\,\mu g\,g^{-1}$ in the presence of brodifacoum, and $32.8 \pm 6.4\,\mu g\,g^{-1}$ in the absence of brodifacoum; and were significantly ($P \leqslant 0.05$) greater than normal ($127.7 \pm 44.3\,ng\,g^{-1}$). Likewise, microsomal concentrations of vitamin K_1 ($4.00 \pm 2.38\,\mu g\,mg^{-1}$ protein, and $2.65 \pm 1.01\,\mu g\,mg^{-1}$ protein, in the presence and absence of brodifacoum, respectively) were significantly ($P \leqslant 0.01$) greater than normal ($16.0 \pm 3.5\,ng\,mg^{-1}$ protein).

6 In conclusion, there appears to be no direct effect of coumarins on clearance of vitamin K_1 from either plasma or liver; the need for large doses of vitamin K_1 during coumarin poisoning is due to a greatly increased requirement for the vitamin.

Introduction

Vitamin K_1 is a cofactor for the postribosomal γ-carboxylation of clotting factor precursor proteins II, VII, IX and X, and also the endogenous anticoagulant proteins C and S (Jackson & Suttie, 1977; Friedman, 1984). During γ-carboxylation of these proteins, the vitamin is first reduced to vitamin K_1 hydroquinone, and the hydroquinone is then oxidised, forming the inactive species, vitamin K_1 epoxide (Willingham & Matschiner, 1974). γ-Carboxylation of clotting factor precursor proteins appears to be very closely linked to epoxidation of vitamin K_1 hydroquinone (see Bell, 1978). The cyclic inter-conversion of vitamin K_1 to its hydroquinone, followed by epoxidation, and the subsequent reduction of the epoxide back to the parent compound is known as the vitamin K-epoxide cycle (Bell, 1978). It is the active recycling of vitamin K_1 which explains the very low dietary requirements for the vitamin (approximately $1\,\mu g\,kg^{-1}$; Frick *et al.*, 1967; Barkhan & Shearer, 1977).

Coumarin anticoagulants are believed to block clotting factor synthesis by the inhibition of vitamin K_1 epoxide reductase (Bell & Matschiner, 1972). There is also evidence that coumarins block quinone reductase (Whitlon *et al.*, 1978). Inhibition of either, or both of these enzymes prevents the recycling of vitamin K_1 and leads to anticoagulation. Warfarin is the coumarin most commonly used for therapeutic purposes, but several other compounds share the

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same mechanism of action. For example, brodifacoum is more lipophilic than warfarin and has an extremely long duration of action (Hadler & Shadbolt, 1975; Barlow *et al.*, 1976). Thus, the latter drug has proved useful for experimental purposes (Park & Leck, 1982; Breckenridge *et al.*, 1985).

After coumarin poisoning, large doses of vitamin K_1 (1–10 mg) are given as an antidote (Lowenthal & MacFarlane, 1964; Van der Meer *et al.*, 1968). Such doses are far in excess of normal dietary intake. High concentrations of vitamin K_1 may be needed in order to displace coumarins from their sites of action in the liver. Yet *in vitro*, studies indicate that warfarin becomes bound irreversibly to epoxide and quinone reductases (Fasco & Principe, 1982). A second explanation for the interaction between vitamin K_1 and coumarins is that the disposition of the vitamin may be affected by the presence of these drugs. However, coumarin anticoagulants do not appear to affect the disposition of vitamin K_1 in plasma (Breckenridge *et al.*, 1985; Choonara *et al.*, 1985), but, these investigations have been limited by the sensitivity of the assays used.

There is now a highly sensitive and specific liquid chromatographic assay available which uses electrochemical detection to measure picogram concentrations of vitamin K_1 in plasma (Hart *et al.*, 1985). In the present studies we have used a development of this method to investigate the relationship between concentrations of vitamin K_1 in plasma, and also at the site of action of vitamin K_1, in the liver, and in liver microsomes, both in the absence and presence of brodifacoum. These experiments were performed to rationalize the requirements for vitamin K_1 during coumarin poisoning, and also to determine an *in vivo* indicator of the mode of action of anticoagulation produced by these drugs.

Methods

Male, New Zealand White rabbits (weights 2–3 kg: Buxted Rabbit Suppliers, Norfolk) were used. Rabbits were housed in the department for at least a week before the experiments were begun, and had free access to food and water throughout (Diet R14: Labsure Animal Foods, Poole, Dorset). The average dietary intake of vitamin K_1 from this diet was approximately 60 $\mu g\,kg^{-1}$ per day.

Determination of the endogenous plasma concentrations of vitamin K_1

Blood samples (5 ml) were collected from the right marginal ear vein of a group of rabbits ($n = 8$), at the same time for 4 successive days. Blood was collected into heparinized blood tubes. Plasma was obtained by centrifugation (2000 *g*; 10 min) and stored frozen (−20°C) until use.

Determination of plasma concentrations of vitamin K_1 in the presence and absence of brodifacoum

Twenty four hours before the administration of vitamin K_1, two groups of rabbits ($n = 6$ in each group) received either brodifacoum dissolved in polyethylene glycol 200, (10 $mg\,kg^{-1}$; 0.5 $ml\,kg^{-1}$), or polyethylene glycol 200 alone (0.5 $ml\,kg^{-1}$) via the left marginal ear vein. Vitamin K_1 (10 $mg\,kg^{-1}$; 1 $ml\,kg^{-1}$, i.v.) was administered 24 h later to all rabbits via the left marginal ear vein. Samples were collected from the right marginal ear vein into heparinized blood tubes at t = 0, 0.5, 1, 2, 4, 6, 10, 16, 24, 34, 48, 58, 72, 82, 96, 144, 168, 216 and 264 h after administration of vitamin K_1 in animals given vehicle only, and up to 77 h in the rabbits pretreated with brodifacoum. Plasma was obtained as described above, and was frozen for the subsequent determination of vitamin K_1. Samples taken between 30 min and 16 h were analysed by h.p.l.c. with ultraviolet detection (Wilson & Park, 1983); the remainder of the samples were analysed by h.p.l.c. with electrochemical detection (Hart *et al.*, 1985, and see below).

Determination of vitamin K_1 in plasma by h.p.l.c. with electrochemical detection

An aliquot of MK6 (internal standard) was added to the plasma (2–5 ml). After equilibration (15 min) at room temperature, absolute ethanol (5 ml) was added to precipitate plasma protein; this was followed by the addition of 5 ml hexane. Extraction was facilitated by gentle mechanical tumbling (30 r.p.m.; 20 min). The extraction mixture was then centrifuged (2000 *g*; 2–5 min), and the hexane layer carefully removed, transferred to a clean, silated test-tube, and evaporated *in vacuo* at 40°C. The residue was redissolved in hexane (1.5 ml) and transferred to a Sep-Pak silica cartridge (Waters Associates, Massachusetts, U.S.A.) with a silated glass syringe. The silica was washed with hexane (2 × 10 ml), and the fraction containing vitamin K_1 was eluted with 3% diethyl ether in hexane (10 ml). This fraction was then evaporated to dryness *in vacuo* at 50°C, and the residue dissolved in normal phase eluent (60 μl) for preparative h.p.l.c. Preparative h.p.l.c. was carried out by elution through a silica column (Partisil 10 ODS 25 cm × 4.5 mm i.d.), collecting the fraction containing both vitamin K_1 and MK-6. Solvent composition was 0.34% acetonitrile in hexane, at a flow rate of 2 $ml\,min^{-1}$.

The fraction containing vitamin K_1 and MK6 was evaporated to dryness *in vacuo* at 40°C, and the residue redissolved in methanol (200 μl) for reversed-

phase analytical h.p.l.c. The reversed phase analytical column was packed with Hypersil C_8 (250 × 4.5 mm i.d.). Mobile phase was 97% methanol with 3% 0.05 M sodium acetate buffer (pH 3); the addition of 5 mM EDTA (20 ml) enhanced the sensitivity of the system. Flow rate was 1 ml min^{-1}.

Detection of vitamin K_1 was carried out by dual cell electrochemical detection in the redox mode of operation (Coulochem, model 5100A). The upstream electrode (−1.30 V) reduced vitamin K_1 to the hydroquinone, which was then detected at the downstream electrode (+0.15 V) by re-oxidation to vitamin K_1. The range of concentrations measured by this assay were 0.76 to 2460 ng ml^{-1}; minimum sensitivity was 0.5 ng ml^{-1}.

Reversed-phase h.p.l.c. produced clear separation of vitamin K_1 and MK-6. The retention times were approximately 9 min, and 11.8 min for vitamin K_1 and MK-6, respectively (Figure 1). Plasma vitamin K_1 was determined from the ratio of vitamin K_1 peak height to the peak height of MK-6. The linear regression for the standard curve produced for this determination was $0.919 \times + 0.327$, $r = 0.999$. Intra-assay variation was calculated by repeated chromatography ($n = 6$) of a single rabbit plasma sample; the coefficient of variation was 8.2%.

Determination of prothrombin complex activity (PCA)

Further blood samples (0.9 ml) were taken at 0, 2, 6, 10, 24, 48, 72, and 77 h from the group of rabbits pretreated with brodifacoum (see above) for the immediate determination of PCA as described previously (Park *et al.*, 1979). Prothrombin times were measured by the method of Quick (1957) and converted to PCA by use of a standard curve produced by determining prothrombin times of normal rabbit plasma diluted in plasma obtained from rabbits treated with brodifacoum, at concentrations of 1–100%. Anticoagulated plasma had a prothrombin time ⩾ 500 s, indicating the absence of active vitamin K_1-dependent clotting factors. PCA of each experimental animal was expressed as a percentage of its own control by relating it to the standard curve (above). PCA of each animal at the beginning of the experiment was assumed to be 100%.

Determination of the concentrations of vitamin K_1 in liver homogenate

Four groups of rabbits were used. Two groups were given vitamin K_1 alone (10 mg kg^{-1}, i.v.). Livers were then removed from the first group 4 h later ($n = 4$), and from the second group 24 h later ($n = 3$). In the remaining two groups of rabbits vitamin K_1 (10 mg kg^{-1} i.v.) was administered 24 h after the administration of brodifacoum (10 mg kg^{-1}, i.p.). Livers were subsequently removed at 4 h ($n = 4$) and 24 h, ($n = 3$) respectively. Hepatic concentrations of vitamin K_1 were then determined by h.p.l.c.-EC. The extraction method has been described previously (Cholerton & Park, 1986; Winn *et al.*, 1987). The limit of sensitivity was 0.5 ng g^{-1}, and linear regression determined from the standard curve of peak height vitamin K_1: MK-6 was $1.392 \times + 0.404$, $r = 0.998$; intra-assay variation 6.4%.

Determination of the concentration of vitamin K_1 in rabbit liver microsomes

Three groups of rabbits were used. In the first group the liver was removed from untreated animals for measurement of normal concentrations of vitamin K_1 ($n = 6$). In the second group of rabbits, the liver was removed 4 h after the administration of vitamin K_1, alone ($n = 9$). In the final group of rabbits, the liver was removed 4 h after the administration of vitamin K_1 in the presence of brodifacoum ($n = 7$).

Approximately 12 g of liver was used from each rabbit for this assay. Microsomes were prepared according to the method of Maggs *et al.* (1983). When prepared, microsomes were stored frozen at −70°C. At the time of assay, each sample was suspended in distilled water and a measured volume of the suspension was removed and extracted for determination of the concentrations of vitamin K_1 (see above). Concentrations of vitamin K_1 in rabbit liver microsomes were related to microsomal protein concentration by the method of Lowry *et al.* (1951).

Materials

Vitamin K_1 (Konakion) was obtained from Hoffmann La Roche, Welwyn Garden City. Brodifacoum (3-(3-[4′bromo(1-1′-biphenyl)]-4-yl-1,2,3,4,-tetrahydro-1-naphthalenyl)-4-hydroxy-2H-1-benzopyran-2-one) was a gift from Sorex Laboratories, Widnes. Menaquinone 6 (MK6; 2-methyl-3-farnesylfarnesyl-1,4-naphthoquinone) was a gift from Dr M.J. Shearer. All general reagents were obtained from B.D.H., Poole, Dorset; and all solvents (h.p.l.c. grade) were obtained from Fisons, Loughborough. Rabbit brain thromboplastin was obtained from Manchester Comparative Reagents, Manchester.

Pharmacokinetic and statistical analysis

A bioexponential equation was fitted to the vitamin K_1 plasma concentration-time data (excluding the observation made at $t = 30$ min), by use of a regression analysis programme (Neilsen-Kudsk, 1980). The two first-order rate constants were then used to calculate the β and γ-half-lives, from the following equation.

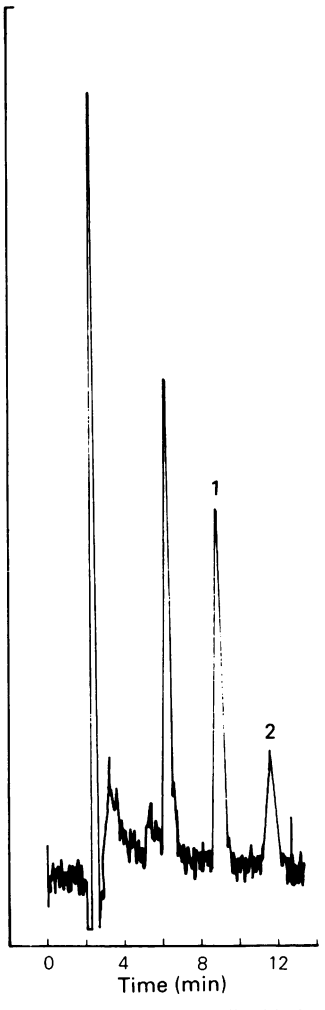

Figure 1 Typical high performance liquid chromatography (h.p.l.c.) trace of vitamin K_1 (1) and MK-6 (2) after electrochemical detection (Coulochem model 5100A) in the redox mode of operation.

$t_{1/2} = 0.693/K$ (where K is the elimination rate constant). Results are expressed as mean ± s.e.mean. Statistical significance was determined by the Mann-Whitney U-test for non-parametric data.

Results

Determination of endogenous concentrations of vitamin K_1

In order to determine the normal physiological concentrations of vitamin K_1 in the rabbit, 8 rabbits were studied for the 4 consecutive days before the administration of brodifacoum. The results obtained (Figure 2) show that the plasma concentrations of the vitamin remained between 10–25 ng ml^{-1} throughout the course of this experiment.

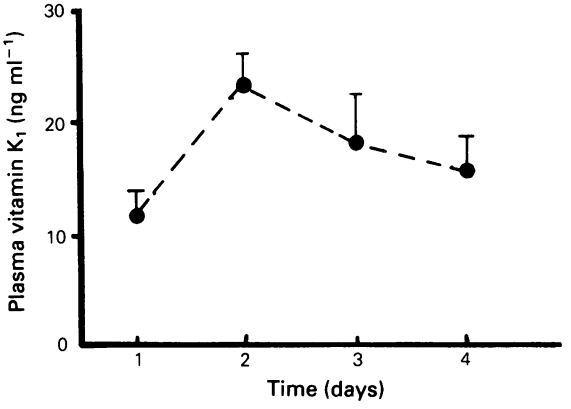

Figure 2 Physiological concentrations of vitamin K_1 in the rabbit, showing the variability recorded over 4 successive days (mean with s.e.mean shown by vertical bars; $n = 8$).

Determination of the plasma concentrations of vitamin K_1 in the presence and absence of brodifacoum

Twenty-four hours after the administration of brodifacoum (10 mg kg^{-1}), the endogenous levels of vitamin K_1 in plasma (9.2 ± 2.3 ng ml^{-1}) were significantly lower ($P \leq 0.05$) than those in the rabbits given polyethylene glycol 200 alone (15.7 ± 3.9 ng ml^{-1}). Following administration of an exogenous dose of vitamin K_1 (10 mg kg^{-1}, i.v.), there was a marked increase in the plasma concentrations of the vitamin (Figure 3). Thirty minutes after the administration of vitamin K_1, the plasma concentrations of the vitamin were 70 ± 30 μg ml^{-1} (vehicle), and 102 ± 10 μg ml^{-1} (brodifacoum). In both groups of rabbits, there was a rapid decline in the plasma concentrations of vitamin K_1 between 30 min and 2 h of administration of the vitamin. Previous workers have defined this as the α-phase of elimination of vitamin K_1, and the half-life is less than 1 h (Shearer *et al.*, 1977; Park *et al.*, 1979; Hart *et al.*, 1984). In order to reduce the number of blood samples taken, the α-phase was not measured in the present experiments.

The α-phase of elimination was followed by a second phase of elimination (β-phase). This was apparent between 2–10 h after the administration of vitamin K_1 in both groups of animals. The plasma half-lives of the β-phase of elimination were

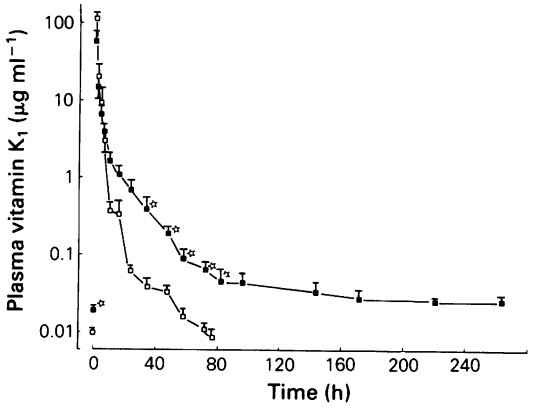

Figure 3 Plasma concentrations of vitamin K_1 before and after the administration of a pharmacological dose of the vitamin (10 mg kg^{-1}, i.v.) in both vehicle-treated (■) and brodifacoum-treated (□) rabbits (mean with s.e.mean shown by vertical bars; $n = 6$) in each group. * Denotes statistical significance ($P < 0.05$).

0.95 ± 0.28 h and 1.54 ± 0.78 h in the rabbits given vehicle and brodifacoum, respectively. There was no significant difference between these results in the two groups of rabbits.

Following the β-phase, both groups of rabbits displayed a further, much slower phase of elimination of vitamin K_1 from the plasma (γ-phase of elimination). The plasma half-lives of the γ-phase in the two groups were estimated to be $13.5 + 5.1$ h (rabbits given vehicle) and $15.1 + 3.0$ h (rabbits given brodifacoum). The half-lives of the γ-phase of elimination in the two groups of rabbits were not significantly different. Calculation of apparent volume of distribution, area under the curve and clearance also revealed no significant differences between the two groups of rabbits. However, between 24 h and the end of the experiment, the plasma concentrations of vitamin K_1 were significantly reduced ($P \leqslant 0.05$) in rabbits given brodifacoum, compared with rabbits given vehicle alone.

Determination of prothrombin complex activity

In accordance with convention, (Nagashima *et al.*, 1969; Park *et al.*, 1979), perturbation of PCA is depicted on a logarithmic scale (Figure 4). The resting PCA of rabbits given brodifacoum was 100%. Twenty-four hours after the administration of brodifacoum there was a significant fall in PCA ($P \leqslant 0.005$); PCA fell to 26 ± 6% of normal at this time (Figure 4). For the first 6 h after the subsequent administration of vitamin K_1 PCA rose rapidly, reaching a maximum of 129 ± 9%. PCA then declined for the remainder of the experiment and

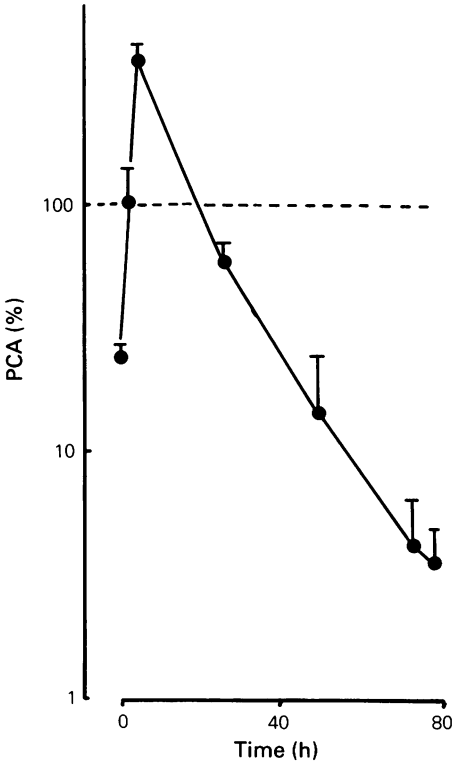

Figure 4 Change in prothrombin complex activity (PCA) against time following the administration of vitamin K_1 (10 mg kg^{-1}, i.v.) in rabbits pretreated with brodifacoum. PCA values are expressed as a percentage of normal (100%) and the dotted line represents 100% PCA. Note logarithmic scale.

77 h after the administration of vitamin K_1, had reached a level of only 4 ± 1% of normal.

In contrast with these data, administration of the same dose of vitamin K_1 had no effect on the normal PCA recorded in rabbits pretreated with vehicle.

Determination of vitamin K_1 in rabbit liver homogenate

The concentration of vitamin K_1 in normal rabbit liver was 127.7 ± 44.3 ng g^{-1} (wet weight liver). Three hours after administration of vitamin K_1, the concentration of the vitamin was significantly $P \leqslant 0.001$) greater than resting levels (Table 1), but there was no significant difference at this time between the concentrations of vitamin K_1 in rabbits given vehicle (32.8 ± 6.4 μg g^{-1}), compared with rabbits given brodifacoum (46.6 ± 4.3 μg g^{-1}; Table 1). The concentrations of vitamin K_1 in liver had fallen significantly ($P \leqslant 0.001$) after 24 h, but were

Table 1 Concentration of vitamin K_1 in whole liver homogenate from normal rabbits and rabbits treated with anticoagulant after administration of a dose of the vitamin ($10\,mg\,kg^{-1}$, i.v.)

Time	Concentration of vitamin K_1	
	Vehicle treated	*Brodifacoum treated*
3 h after K_1	$32.8 \pm 6.4\,\mu g\,g^{-1}$	$46.6 \pm 4.3\,\mu g\,g^{-1}$
24 h after K_1	$4.5 \pm 0.92\,\mu g\,g^{-1}$	$2.67 \pm 0.86\,\mu g\,g^{-1}$
72 h after K_1	$0.116 \pm 0.026\,\mu g\,g^{-1}$	$0.045 \pm 0.018\,\mu g\,g^{-1}$

still above normal and there were no significant differences between the hepatic concentrations of the vitamin in the two groups of rabbits ($4.50 \pm 0.92\,\mu g\,g^{-1}$ in rabbits given vehicle; $2.67 \pm 0.86\,\mu g\,g^{-1}$ in rabbits given brodifacoum; Table 1). The concentration of vitamin K_1 continued to fall, until by 72 h after its administration, the concentration was $44.7 \pm 18.4\,ng\,g^{-1}$ (wet weight liver) in rabbits given brodifacoum. This was significantly ($P \leqslant 0.05$) lower than the concentrations of vitamin K_1 recorded in either normal rabbit livers (see above), or in rabbits given vehicle ($116.1 \pm 26.0\,ng\,g^{-1}$).

Determination of the concentration of vitamin K_1 in rabbit liver microsomes

Normal microsomal concentrations of vitamin K_1 were $16.0\,ng\,mg^{-1}$ protein. Three hours after the administration of vitamin K_1 ($10\,mg\,kg^{-1}$, i.v.) there was a significant ($P \leqslant 0.001$) rise in the concentration of vitamin K_1 in the microsomes. The concentration of vitamin K_1 in microsomes prepared from the livers of rabbits given brodifacoum was $4.00 \pm 2.28\,\mu g\,mg^{-1}$ protein. This was not significantly different from the concentration of vitamin K_1 in microsomes prepared from vehicle-treated rabbits ($2.66 \pm 1.01\,\mu g\,mg^{-1}$ protein).

Discussion

Clotting factor synthesis occurs in the rough endoplasmic reticulum of the liver (Suttie, 1985) and under normal conditions is not related directly to plasma concentrations of vitamin K_1. Since high dietary concentrations of vitamin K_1 appear to have little influence on coagulation in the short-term (Karlson *et al.*, 1986) the variation in the physiological concentrations of vitamin K_1 observed in the present study is unlikely to influence clotting factor synthesis. Nevertheless, plasma concentrations of the vitamin are part of the total body pool of vitamin K_1 and may reflect changes in the disposition of the vitamin, and in particular the vitamin K_1 epoxide cycle, under conditions in which vitamin K_1 metabolism is compromised. In this respect, 24 h after administration of brodifacoum in the present study, there was a significant fall in the normal physiological plasma concentration of vitamin K_1. According to current concepts this is due to inhibition of vitamin K_1 quinone reductase and epoxide reductase (Bell & Matschiner, 1972; Whitlon *et al.*, 1978), preventing recycling and thereby the conservation of vitamin K_1.

Despite inhibition of vitamin K_1 quinone reductase and epoxide reductase, the administration of pharmacological doses of vitamin K_1 can overcome the action of coumarins (Lowenthal & MacFarlane, 1964; Park & Leck, 1982). The present experiments were consistent with these earlier findings, since there was an immediate sharp rise in PCA in rabbits given brodifacoum (but no change in the PCA of rabbits given vehicle). The rapid rise in PCA has been attributed to the γ-carboxylation of clotting factor precursor proteins which have accumulated during the period of anticoagulation (Suttie, 1970). However, although vitamin K_1 from the pharmacological dose promoted clotting factor synthesis, the rise in PCA was short-lived (6 h). The cessation of clotting factor synthesis occurred at plasma concentrations of vitamin K_1 that were greater by at least an order of magnitude than those seen under normal conditions.

Calculation of further pharmacokinetic variables derived from the present data indicate that there were no significant differences between the two groups of rabbits in terms of half-life, volume of distribution and clearance of the vitamin. Nevertheless, between 24 h and the end of the experiment the plasma concentrations of vitamin K_1 in rabbits given anticoagulants were significantly below those recorded in rabbits given vehicle, suggesting that the very high initial plasma concentrations of vitamin K_1 may have obscured subtle changes in the disposition of the vitamin. Accordingly, after administration of an exogenous dose it is interesting to note that differences between the two groups were only discernible once plasma concentrations of the vitamin returned to near physiological levels. Similarly, it was also clear that brodifacoum depleted endogenous plasma vitamin K_1. Therefore, in order to determine whether the effect of brodifacoum was due to changes in the accessibility of vitamin K_1 to either liver cells, or the liver endoplasmic reticulum directly, we determined the concentrations of the vitamin in whole liver homogenate and in liver microsomes from both normal rabbits and rabbits treated with anticoagulant.

Three hours after vitamin K_1 was given, its concentration in whole liver was much greater than that recorded in untreated rabbits, but there was no difference between the concentrations recorded in

rabbits given anticoagulant compared with rabbits given vehicle. The hepatic concentrations of vitamin K_1 had fallen significantly after 24 h, but remained above normal, whilst clotting factor synthesis had ceased at this time. This was consistent with the relationship between plasma concentrations of vitamin K_1 and clotting factor synthesis in the two groups of rabbits, and indicates that neither plasma nor whole liver distribution of a pharmacological dose of vitamin K_1 is affected by coumarins.

The microsomal concentrations of vitamin K_1 were measured only at the 3 h timepoint after its administration, but at this time microsomal concentrations in rabbits given the vitamin were significantly greater than normal rabbits. Furthermore, the concentration of vitamin K_1 in microsomes prepared from the livers of rabbits pretreated with brodifacoum was not different from rabbits given vehicle. Therefore, it appears that the presence of coumarins does not limit the uptake of vitamin K_1 into liver microsomal tissue.

In conclusion, whilst brodifacoum significantly reduced the physiological concentrations of vitamin K_1, there appeared to be no significant difference between the disposition of a pharmacological dose of vitamin K_1 in the presence or absence of coumarin anticoagulation at concentrations at which clotting factor synthesis is promoted. At the present time there is no clear resolution of the need for large and repeated doses of vitamin K_1 after coumarin poisoning. Evidence from *in vitro* studies shows that there is a pathway for the reduction of vitamin K_1 to vitamin K_1 hydroquinone that exists in liver, independent of the vitamin K_1 epoxide cycle (Wallin, 1986). This pathway requires much larger concentrations of the vitamin than those used by the vitamin K_1 epoxide cycle, and it has been suggested that the latter mechanism may be of importance during coumarin poisoning (Wallin & Martin, 1987). Our data appear to be consistent with this suggestion, since we have found that clotting factor synthesis in rabbits anticoagulated with brodifacoum only occurs when plasma, liver and liver microsomal concentrations of vitamin K_1 are much higher than those recorded under normal conditions. It is evident that during coumarin poisoning it may be necessary to maintain plasma concentrations of vitamin K_1 markedly above normal, physiological concentrations, thereby maintaining liver and microsomal concentrations at a level sufficient to ensure adequate clotting factor synthesis. These findings in experimental animals are consistent with the requirements for vitamin K_1 observed in clinical cases of coumarin poisoning.

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