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The Bioavailability of a Mixed Micellar Preparation of Vitamin K_1, and its Procoagulant Effect in Anticoagulated Rabbits

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Abstract—We have investigated the pharmacokinetics and procoagulant activity of a new, mixed-micellar preparation of vitamin K_1 (MM-K) in male New Zealand White rabbits. Oral administration of MM-K alone caused a significant ($P < 0.01$) increase in the plasma concentrations of vitamin K_1 as measured by normal-phase high-performance liquid chromatography (HPLC). Maximum plasma concentrations of vitamin K_1 (450 ng mL^{-1}, range 133–824 ng mL^{-1}) were recorded at 3·3 h (range 3–5 h), and were significantly ($P < 0.05$) greater than those seen after administration of an existing polyethoxylated castor oil preparation (PE-K; Konakion), which were 260 ng mL^{-1}, range 198–390 ng mL^{-1} (t_{max} 0·8 h, range 0·4–1·2 h). AUC after MM-K (4·6 $\mu g\ mL^{-1}\ h^{-1}$, range 2·1–6·3 $\mu g\ mL^{-1}\ h^{-1}$) was also significantly ($P < 0.05$) greater than after PE-K (1·6 $\mu g\ mL^{-1}\ h^{-1}$, range 1·0–2·1 $\mu g\ mL^{-1}\ h^{-1}$). However, the bioavailability of vitamin K_1 after administration of MM-K was poor (9·4%), and there was considerable intra-individual variability between the concentrations of vitamin K_1 recorded in the plasma samples.

Both preparations of vitamin K_1 stimulated clotting factor synthesis in rabbits anticoagulated with the potent and long-acting coumarin, brodifacoum. Maximum stimulation of clotting factor synthesis by vitamin K_1 after MM-K was 87%, range 44–124% (%PCA). The maximum was seen later (t_{max} 12 h) than after PE-K (PCA 82%, range 47–125%; t_{max} 5 h). However, there was considerable intra-individual variability in response to both MM-K and PE-K. Furthermore, there was no difference between the maximum PCA produced by the two preparations. It is concluded that the incorporation of vitamin K_1 into bile salt micelles does not enhance either the absorption or efficacy of the vitamin after oral administration.

Vitamin K_1 is an essential cofactor in the postribosomal γ-carboxylation of blood clotting factors II, VII, IX and X (Jackson & Suttie 1977). The active form of the vitamin, vitamin K_1 hydroquinone, is produced by reduction of the parent molecule (Willingham & Matschiner 1974), and participates in the γ-carboxylation of glutamic acid residues located in the *N*-terminal region of the clotting factor precursor proteins. Concurrent with this process, the hydroquinone is oxidized to the inactive metabolite vitamin K_1 2,3-epoxide (Bell 1978). Reduction of the epoxide back to vitamin K by vitamin K_1 2,3-epoxide reductase maintains existing stores of the vitamin in the liver, and this recycling process accounts for the very low plasma concentrations and dietary requirements for vitamin K_1 under normal conditions (Frick et al 1967).

Coumarin anticoagulants block the vitamin K_1 epoxide cycle by inhibition of vitamin K_1 2,3-epoxide reductase and vitamin K_1 quinone reductase (Bell & Matschiner 1972; Whitlon et al 1978). Inhibition prevents recycling of the vitamin and leads to a fall in the circulating concentrations of clotting factor precursor proteins. However, anticoagulation can be overcome by the administration of pharmacological doses of vitamin K_1. According to recent data, this is due to the formation of vitamin K_1 hydroquinone via a pathway independent of the vitamin K_1 epoxide cycle, and which is unaffected by coumarins (Wallin & Martin 1987). However, the latter process is driven only by much higher concentrations of vitamin K_1 than normally required (Wallin & Martin 1987).

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Administration of the large doses of vitamin K_1 required during coumarin poisoning is problematic. Vitamin K_1 is extremely lipid soluble, and the intestinal absorption is highly variable (Park et al 1984; Uchida & Komeno 1988). Intravenous administration of the vitamin is used, but this can lead to serious cardiovascular complications due to the polyethoxylated castor oil base used to suspend the vitamin (Barash et al 1976; Rich & Drage 1982). A new commercial formulation of vitamin K_1, a colloidal preparation based on mixed micelles formed from lecithin and glycolic acid is believed to be less toxic than the polyethoxylated preparation after intravenous use (A. W. Kormann, personal communication). In theory, the mixed micellar preparation (MM-K) may also enhance the bioavailability of vitamin K_1 after oral administration, as it has been shown that uptake of vitamin K_1 from the gut is dependent on the presence of bile salts (Shearer et al 1974). Therefore, the aim of this study was to compare the pharmacokinetic and pharmacodynamic effect of MM-K with that of the present polyethoxylated formulation (PE-K) in untreated rabbits, and in rabbits anticoagulated with the potent coumarin, brodifacoum.

Materials and Methods

Male New Zealand White rabbits (2·0–3·5 kg) were obtained from Hyline Rabbit Farms (Cheshire, UK). Rabbits were housed in the departmental animal house for at least a week before the experiments were begun. Animals had free access

to both food and water throughout, and were maintained on diet R14 (Labshore Animal Foods, Poole, UK). PE-K (Konakion) was obtained from Hoffman-La Roche, Welwyn Garden City, UK. MM-K (Konakion-MM) was a gift from Hoffman-La Roche, Basel. 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, UK; and menaquinone-4 (MK-4), a gift from Dr. M. J. Shearer (Guys Hospital, London). The concentrations of vitamin K_1 in plasma were determined by normal-phase HPLC as described previously (Wilson & Park 1983). General reagents were obtained from BDH, Poole, UK; and solvents (HPLC grade) from Fisons, Loughborough, UK. Rabbit brain thromboplastin was obtained from Manchester Comparative Reagents (Manchester, UK).

Determination of the plasma concentrations of vitamin K_1

The pharmacokinetics of MM-K were determined after oral and intravenous administration of a pharmacological dose (10 mg kg^{-1}) of vitamin K_1 to a group of 4 rabbits. Further rabbits (n=4) were dosed orally with 10 mg kg^{-1} of PE-K. Oral dosing was carried out by gastric gavage. Intravenous administration was via the left marginal ear vein, and blood samples were taken from the right marginal ear vein. Blood samples were collected at 0, 1, 2, 3, 5, 7 and 12 h after dosing, for the measurement of plasma concentrations of vitamin K_1 (a further sample was taken 24 h after MM-K). Blood was collected into heparinized tubes, centrifuged at 3000 *g* for 10 min, and the plasma was stored frozen (−20°C) until assayed.

Procoagulant activity of the two formulations

Twenty-four h before the administration of vitamin K_1, two groups of rabbits (n=4 in each group) were given brodifacoum dissolved in polyethylene glycol 200 (10 mg kg^{-1}; 0·5 mg mL^{-1}). Immediately before the administration of vitamin K_1, blood samples were collected as described above, and a further 0·9 mL of blood was collected into trisodium citrate (10% by volume) for the measurement of prothrombin time and prothrombin complex activity (PCA). Further blood samples (5 mL in heparin) were collected at 1, 2, 3, 5, 7, 12 and 24 h. Prothrombin time was determined by the method of Quick (1957) using a Schnitger & Gross automatic coagulometer (Park et al 1979). PCA (%) was related to prothrombin time by reference to standard curves which were determined by the accurate dilution of normal rabbit plasma with plasma obtained from severely anticoagulated rabbits (clotting times of the latter animals were >500 s, indicating the absence of active vitamin K_1-dependent clotting factors).

Results were used to calculate plasma half-lives (by linear regression of the terminal phase), AUC (by the trapezoidal rule), and bioavailability of vitamin K_1. Statistical significance was determined by the Mann-Whitney U-test.

Results

Determination of the plasma concentrations of vitamin K_1

The mean plasma concentrations of vitamin K_1 after intravenous and oral administration of MM-K in the absence of other interventions are shown in Fig. 1. The maximum plasma concentration after intravenous dosing was 31·2 μg mL^{-1} (range 18·3–47·1 μg mL^{-1}). Plasma concentrations of vitamin K_1 then declined rapidly, with an elimination half-life of 2·7 h (range 1·6–3·2 h). Plasma concentrations of vitamin K_1 after oral administration of MM-K or PE-K are shown in Fig. 2. The most noticeable difference between the two preparations of vitamin K_1 was the time to peak plasma concentrations of the vitamin. Maximum plasma concentrations after MM-K were seen at 3·3 h (range 3–5) h. Time to peak concentration after PE-K was significantly ($P<0·05$) shorter (0·8 h, range 0·4–1·2 h). Maximum plasma concentrations were greater after administration of MM-K (450 ng mL^{-1}, range 133–824 ng mL^{-1}) than after PE-K (260 ng mL^{-1}, range 198–390 ng mL^{-1}). However, there was a considerable degree of variation between the plasma concentrations of vitamin K_1 in all rabbits, although this was most marked after MM-K.

Procoagulant activity of the two formulations

Change in PCA after administration of the two preparations of vitamin K_1 is shown in Fig. 3. Consistent with its known effects (Park et al 1979), brodifacoum reduced PCA to 50% of normal within 24 h. There were no significant changes in PCA within the first 2 h of administration of either preparation of vitamin K_1. However, clotting factor synthesis had begun after 3 h in rabbits given PE-K, and peak PCA (82%, range 47–125%) was seen 5 h after administration of this formulation. Whilst there was considerable intra-individual variability, there was a linear relationship between maximum plasma concentrations of vitamin K_1 (after logarithmic transformation of the data) and peak PCA measure-

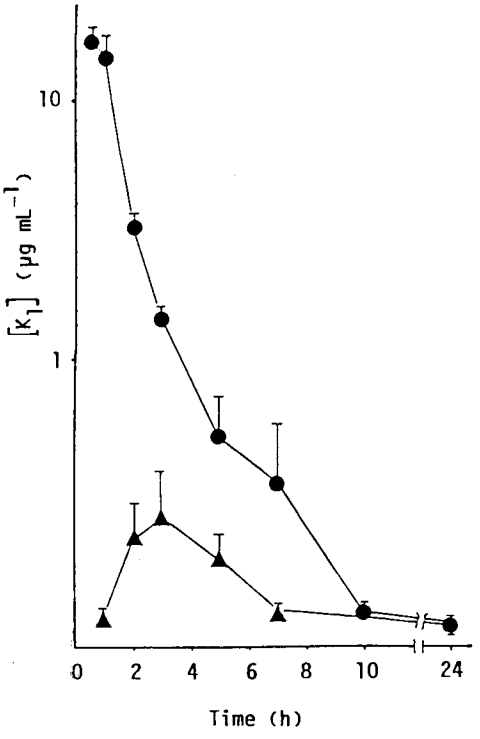

FIG. 1. Plasma concentrations of vitamin K_1 (mean ± s.e. mean) after intravenous (●) and oral (▲) administration of 10 mg kg^{-1} MM-K.

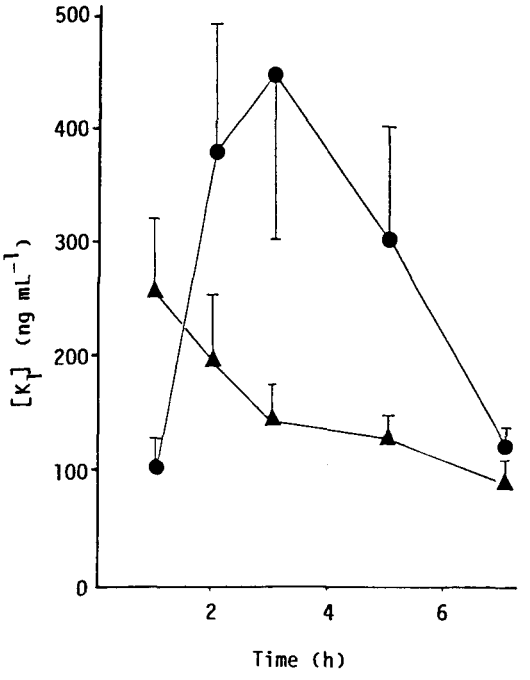

FIG. 2. Plasma concentrations of vitamin K_1 (mean ± s.e. mean) after oral administration of PE-K, 10 mg kg^{-1} (▲), and MM-K, 10 mg kg^{-1} (●).

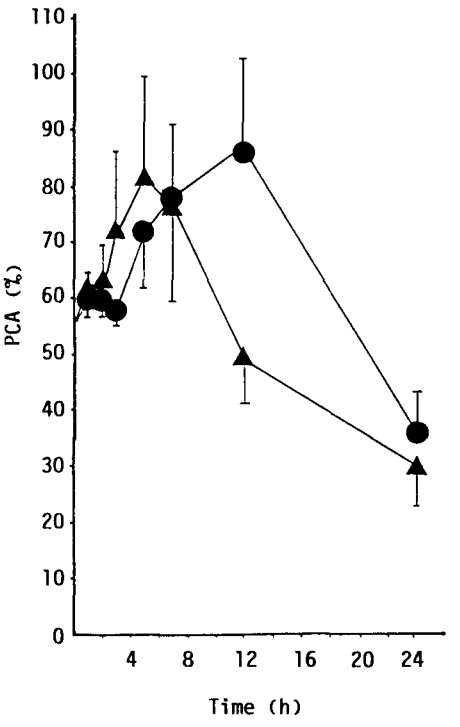

FIG. 3. PCA (%; mean+s.e. mean) versus time after the oral administration of either PE-K (▲), or MM-K (●), at doses of 10 mg kg^{-1}.

ments. However, peak PCA was recorded consistently later than peak plasma concentration of vitamin K_1. Five hours after administration of the vitamin, the PCA of rabbits given MM-K had begun to rise, and clotting factor synthesis was greatest 12 h after the administration of this formulation (87%, range 44–124%). Peak PCA and (log) vitamin K_1 concentrations were again correlated, and again peak PCA was achieved later than the maximum concentrations of vitamin K_1. The PCA of rabbits given PE-K had fallen by the time PCA of rabbits given MM-K was maximal. There was, however, no significant difference between maximum PCA in the two groups.

In addition to the measurement of concentrations of vitamin K_1, plasma concentrations of the metabolite of vitamin K_1, vitamin K_1 2,3-epoxide were measured in these experiments (Fig. 4). Consistent with the increases in PCA and vitamin K_1, there was also a linear relationship between the maximum plasma concentrations of vitamin K_1 and vitamin K_1 2,3-epoxide; and between vitamin K_1 epoxide and PCA. Moreover, the maximum concentrations of vitamin K_1 epoxide were seen later (t_{max} 5 h, range 3–7 h) than the maximum concentration of vitamin K_1 (above).

Discussion

The need for frequent administration of pharmacological doses of vitamin K_1 after coumarin poisoning is due to inhibition of the vitamin K_1 epoxide cycle, and the reduction

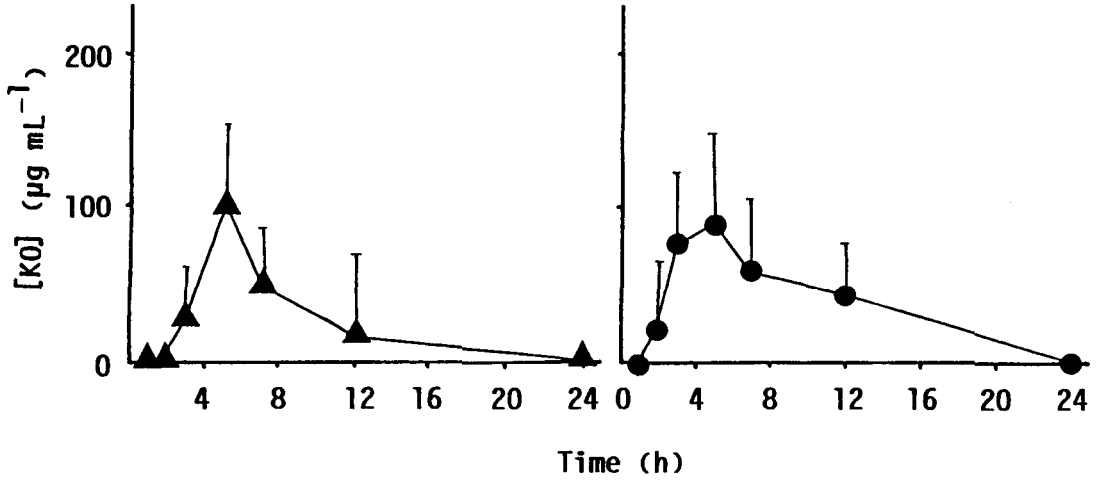

FIG. 4. Plasma concentrations of vitamin K_1 2,3-epoxide (mean ± s.e. mean) after administration of either PE-K (▲), or MM-K (●) in the presence of brodifacoum.

of vitamin K_1 via an NADPH-dependent pathway (Wallin & Martin 1987). To overcome the toxic effects associated with intravenous administration of the existing preparation of vitamin K_1, a new formulation, based on mixed micelles (glycolate and lecithin) has been developed. Whilst development was primarily for intravenous use, the preparation of vitamin K_1 into micelles might be expected to enhance total absorption of the vitamin from the gastrointestinal tract, since uptake of vitamin K_1 from the gut is dependent on the presence of bile salts in the gastrointestinal tract (Shearer et al 1974).

It has been shown that oral administration of the existing preparation of vitamin K_1 is accompanied by a considerable variability and poor bioavailability. For example, bioavailability in man after the administration of a pharmacological dose of the vitamin was 10–63% (Park et al 1984); and 13% (Uchida & Komeno 1988). The uptake of vitamin K_1 was not dependent on the concentration of the vitamin in the gut, since increasing the dose from 10 to 50 mg kg^{-1} served only to increase the variability of the uptake (Park et al 1984). However, incorporation of vitamin K_1 into mixed-micelles produced no greater uptake than that seen with PE-K. AUC after MM-K was only slightly greater than the AUC after PE-K, and bioavailability remained very limited (less than 10%). Moreover, the variability seen with PE-K was not reduced by the incorporation of vitamin K_1 into mixed micelles.

The major difference between the two preparations was the time taken to achieve maximum plasma concentrations of vitamin K_1. Appearance of vitamin K_1 in the plasma after MM-K was significantly slower than after PE-K. The reasons for this difference are unclear. The difference is unlikely to be explained by indirect absorption of PE-K into the hepatic portal blood, compared with lymphatic absorption of the colloidal preparation (Blomstrand & Forsgren 1968; Hollander 1973), since previous evidence has shown that other suspensions of vitamin K_1 do undergo lymphatic absorption (Jolly et al 1977). It would appear, therefore, that the breakdown of mixed micelles before the release of vitamin K_1 into plasma takes significantly longer than absorption from an oil-based suspension.

The difference between rates of absorption of the two preparations was reflected in the changes in PCA recorded after the administration of vitamin K_1 in rabbits previously anticoagulated with brodifacoum. Previous work from these laboratories has shown that doses of vitamin K_1 which produce plasma concentrations above 300–400 ng mL^{-1} stimulate clotting factor synthesis after coumarin poisoning (Choonara et al 1985). The plasma concentrations recorded in the present experiments suggested that oral administration of MM-K may have been sufficient to act as a reliable antidote to coumarin poisoning. However, there was no significant difference between the maximum PCA in either group. Furthermore, the marked degree of intra-individual variability between plasma concentrations of the vitamin was also reflected in the changes in PCA that were recorded in the latter series of experiments.

In conclusion, incorporation of vitamin K_1 into mixed micelles had little effect on the uptake of the vitamin from the gastrointestinal tract, except to delay absorption by between 1 and 3 h. In anticoagulated rabbits MM-K did not promote clotting factor synthesis further than PE-K. The clinical benefit of the new formulation of vitamin K_1 is, therefore, likely to derive from the reduced toxicity after intravenous administration.

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References

Barash, P., Kitahata, L. M., Mandel, S. (1976) Acute cardiovascular collapse after intravenous phytonadione. Anaesth. Analg. 55: 304–306

Bell, R. G. (1978) Metabolism of vitamin K and prothrombin synthesis: anticoagulants and the vitamin K-epoxide cycle. Fed. Proc. 37: 2599–2604

Bell, R. G., Matschiner, J. T. (1972) Warfarin and the inhibition of vitamin K activity by an oxide metabolite. Nature 237: 32–33

Blomstrand, R., Forsgren, L. (1968) Vitamin K_1 ^3H in man: its intestinal absorption and transport in the thoracic duct lymph. Int. Z. Vit. Forsch. 38: 45–64

Choonara, I. A., Scott, A. K., Haynes, B. P., Cholerton, S., Breckenridge, A. M., Park, B. K. (1985) Vitamin K_1 metabolism in relation to pharmacodynamic response in anticoagulated patients. Br. J. Clin. Pharmacol. 20: 643–648.

Frick, P. G., Riedler, G., Brogli, H. (1967) Dose response and minimal daily requirement for vitamin K in man. J. Appl. Physiol. 23: 387–389

Hollander, D. (1973) Vitamin K_1 absorption by everted intestinal sacs of the rat. Am. J. Physiol. 225: 360–364

Jackson, C. M., Suttie, J. W. (1977) Recent developments in understanding the mechanism of vitamin K and vitamin K antagonist drug actions and the consequences of vitamin K action in blood coagulation. Prog. Haematol. 10: 333–359

Jolly, D. W., Craig, C., Nelson, T. E. Jr (1977) Estrogen and prothrombin synthesis: effect of estrogen on absorption of vitamin K_1. Am. J. Physiol. 232: H12–H17

Park, B. K., Leck, J. B., Wilson, A. C., Serlin, M. J., Breckenridge, A. M. (1979) A study of the effect of anticoagulants on [^3H]-vitamin K_1 metabolism and prothrombin complex activity in the rabbit. Biochem. Pharmacol. 28: 1323–1329

Park, B. K., Scott, A. K., Wilson, A. C., Haynes, B. P., Breckenridge, A. M. (1984) Plasma disposition of vitamin K_1 in relation to anticoagulant poisoning. Br. J. Clin. Pharmacol. 18: 655–662

Quick, A. J. (1957) In Haemorrhagic diseases Ed. Quick, A.J. p 379. Lea & Febiger, Philadelphia.

Rich, E. C., Drage, C. W. (1982) Severe complications of intravenous phytonadione therapy. Two cases with one fatality. Postgrad. Med. 72: 303–306

Shearer, M. J., McBurney, A., Barkhan P (1974) Studies of the absorption and metabolism of phylloquinone (vitamin K_1) in man. Vitamins & Hormones 32: 513–542

Uchida, K., Komeno, T. (1988) In "Current Advances in Vitamin K Research". Ed. Suttie, J. W., 477–492. Elsevier, New York

Wallin, R., Martin, L. F. (1987) Warfarin poisoning and vitamin K_1 antagonism in rat and human liver. Design of a system *in vitro* that mimics the situation *in vivo*. Biochem. J. 241: 389–396

Whitlon, D. S., Sadowski, J. A., Suttie, J. W. (1978) Mechanism of coumarin action: significance of vitamin K epoxide reductase inhibition. Biochemistry 17: 1371–1377

Willingham, A. K., Matschiner, J. T. (1974) Changes in phylloquinone epoxidase activity related to prothrombin synthesis and microsomal clotting activity in the rat. Biochem. J. 140: 435–441

Wilson, A. C., Park, B. K. (1983) Quantitative analysis of pharmacological levels of vitamin K_1 and vitamin K_1 2,3-epoxide in rabbit plasma by high performance liquid chromatography. J. Chromatogr. 277: 292–299