CYCLIC INTERCONVERSION OF VITAMIN K₁ AND VITAMIN K₁ 2,3-EPOXIDE IN MAN

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1 The disposition of a single intravenous bolus dose of 10 mg vitamin K₁ and vitamin K₁-2,3-epoxide were studied in two healthy subjects without and with 12 h pretreatment dose of phenprocoumon (0.4 mg/kg).

2 For each compound administered alone the plasma concentration-time profile was adequately fitted by a biexponential equation, with an average terminal half-life of 2.0 and 1.15 h for the administered vitamin K and its 2,3-epoxide respectively.

3 While vitamin K₁ was measurable in plasma following administration of vitamin K₁-2,3-epoxide, the epoxide was not detectable following administration of vitamin K₁.

4 Following pretreatment with phenprocoumon and after intravenous administration of vitamin K₁, both the average half-life and area under the plasma concentration-time profile of vitamin K₁ were marginally reduced to 1.5 h and 1.76 mg l⁻¹ h respectively, while the plasma concentration of vitamin K₁-2,3-epoxide was readily measurable and its half-life markedly prolonged to 14.7 h.

5 Following pretreatment with phenprocoumon and after oral administration of vitamin K₁-2,3-epoxide, no vitamin K₁ was detectable in plasma and the half-life of the epoxide was 13.8 h.

6 Based on area considerations the data suggest that either phenprocoumon does more than just inhibit the reduction of vitamin K₁-2,3-epoxide to vitamin K₁, or that the simple model describing the interconversion between vitamin K₁ and its epoxide is inadequate. The same conclusion is drawn from the analysis of comparable data in dogs, obtained by Carlisle & Blaschke (1981).

Keywords vitamin K₁ vitamin K₁-2,3-epoxide interconversion

Introduction

Vitamin K₁ is required for the γ -carboxylation of glutamyl residues of precursor proteins which results in the formation of active clotting factors (Jones *et al.*, 1977; Stenflo & Suttie, 1977). During the carboxylation reaction vitamin K₁ is oxidized to its biological inactive metabolite vitamin K₁-2,3-epoxide (Willingham & Matschiner, 1974; Willingham *et al.*, 1976; Ren *et al.*, 1977; Sadowski *et al.*, 1977). Subsequently, this metabolite is reduced back to vitamin K₁ by an enzyme called phyloquinone epoxide reductase (Bell & Matschiner, 1972; Willingham & Matschiner, 1974). Oral anticoagulant drugs are proposed to exert their hypoprothrombinaemic effect by inhibition of the epoxide reductase (Bell & Matschiner, 1972; Bell *et al.*, 1972; Caldwell *et al.*, 1974; Matschiner *et al.*, 1974; Whitton *et al.*, 1978) and thereby interrupt the regeneration of vitamin K₁.

Most of the evidence of this cyclic interconversion of vitamin K₁ and vitamin K₁-epoxide comes from the studies in animals (Bell, 1978; Carlisle & Blaschke,

1981); experiments in man are limited. The kinetics of vitamin K₁ have been determined in man using the radioactive labelled vitamin (Shearer *et al.*, 1974, 1977; Bjornsson *et al.*, 1979). It was also shown that administration of oral anticoagulant drugs causes an accumulation of vitamin K₁-epoxide in the plasma (Shearer *et al.*, 1977; Bechtold & Jähnchen, 1979; Bjornssen *et al.*, 1979) which most likely reflects inhibition of epoxide reductase by these drugs. However, no information is available on the disposition of vitamin K₁-epoxide and the effect of oral anticoagulant drugs on the kinetics of the administered metabolite. Such data are required to provide greater insight into the cyclic interconversion between vitamin K₁ and vitamin K₁-epoxide in man.

Methods

In two healthy male volunteers (subject 1: 38 years,

bodyweight 83 kg; subject 2: 33 years, bodyweight 73 kg), who gave their informed consent, four studies were performed on separate occasions at least 4 weeks apart:

Study 1: 10 mg vitamin K₁ was injected intravenously.

Study 2: 10 mg vitamin K₁ was injected intravenously 12 h after pretreatment with a single oral dose of phenprocoumon (0.4 mg/kg).

Study 3: 10 mg vitamin K₁-2,3-epoxide was injected intravenously.

Study 4: 20 mg vitamin K₁-2,3-epoxide was given orally 12 h after pretreatment with a single oral dose of phenprocoumon (0.4 mg/kg). Heparinized blood samples were drawn from a forearm vein before and frequently after administration of vitamin K₁ and vitamin K₁ epoxide. Plasma was separated and stored at -20°C until assayed. Analysis of vitamin K₁ and vitamin K₁-epoxide were performed by a gas-liquid chromatographic method (Bechtold & Jähnchen, 1979).

The injection solution of vitamin K₁ was prepared by dilution of a commercially available vitamin K₁ preparation (Konakion®, Hoffman-LaRoche Ltd., Grenzach, FRG) with 250 ml of laevulose (5%). This solution was infused over a period of 20–30 min, accurately recorded. Vitamin K₁-epoxide was synthesized according to the method of Tishler *et al.* (1940). Purity (95%) was checked by gas chromatography. For intravenous administration, vitamin K₁-epoxide (0.08 g) was dissolved in 0.4 g Cremophor EL (BASF, Ludwigshafen, FRG), to which was added 8.0 ml of water for injection and the preparation was sterilized by ultrafiltration and diluted with 250 ml laevulose (5%). An appropriate quantity of this solution was infused over 20–30 min, too. For oral administration, vitamin K₁-epoxide was dissolved in Tween 20 and ingested after dilution with 100 ml of water. Phenprocoumon was administered as Marcumar® -tablets (Hoffman-LaRoche Ltd,

Grenzach, FRG). Both subjects had fasted at least 6 h before administration of vitamin K₁ or its epoxide, respectively.

Pharmacokinetic analysis

A biexponential equation was fitted to the plasma concentration-time data of the injected compound following the short intravenous infusion of vitamin K₁ or vitamin K₁-epoxide, using a non-linear regression program (Metzler *et al.*, 1974). The area under the plasma concentration-time curve, up to the last observation, was determined by the trapezoidal rule and the terminal area until infinity by extrapolation, by dividing the last observation by the terminal exponent.

Results

After intravenous administration of vitamin K₁ alone, the plasma concentration declined biexponentially with time. The relevant parameters defining the curve are summarized in Table 1. The extrapolated area only constituted between 1.2–2.8% of the observed area. No or only traces of vitamin K₁-epoxide could be detected in the plasma (Figure 1a). After intravenous administration of vitamin K₁, following pretreatment with a single dose of phenprocoumon, the area under the vitamin K₁ concentration-time curve decreased by about 20 to 30%, while the plasma concentration of vitamin K₁-epoxide was high and sustained in plasma. The maximum concentration of the epoxide occurred about 3 to 4 h after administration of vitamin K₁ and declined with half-lives of 21.8 and 7.6 h in subject 1 and 2, respectively (Figure 1b). The area under the plasma concentration-time curve

Table 1 Disposition kinetics of vitamin K and its 2,3-epoxide in man

		Plasma-time profile of administered compound ^a $C = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t}$ [C, mg/l; t = h]	Terminal half-life of administered compound (0.693/λ ₂ ; h)	Area (mg l ⁻¹ h)		Area ratio (KO/K)
Without phenprocoumon				Vit K	Vit KO	
After i.v.	Subject 1	2.10e ^{-1.83t} + 0.27e ^{-0.26t}	2.7	1.96	—	—
Vit K (10 mg)	Subject 2	13.89e ^{-4.22t} + 1.02e ^{-0.54t}	1.3	2.56	—	—
After i.v.	Subject 1	2.87e ^{-5.15t} + 0.45e ^{-0.62t}	1.1	0.53	0.98	1.8
Vit KO (10 mg)	Subject 2	4.67e ^{-4.47t} + 0.43e ^{-0.61t}	1.2	0.41	1.22	3.0
With phenprocoumon						
After i.v.	Subject 1	3.26e ^{-2.43t} + 0.27e ^{-0.42t}	1.7	1.43	7.86	5.5
Vit K (10 mg)	Subject 2	5.56e ^{-2.76t} + 0.66e ^{-0.55t}	1.3	2.09	8.27	4.0

^a Estimated parameters following an intravenous bolus dose. The intercepts, C₁, C₂, were calculated from the intercepts obtained at the time when the infusion was stopped, C₁', C₂', using the standard form C_i = C_i'λ_iτ/(1-e^{-λ_iτ}), where τ is the duration of the infusion.

Figure 1 Plasma concentrations of vitamin K₁ (●) and vitamin K₁-2,3-epoxide (▽) in two healthy subjects following intravenous injection of 10 mg vitamin K₁. (a) without pretreatment, (b) after 12 h pretreatment with a dose of phenprocoumon (0.4 mg/kg).

of formed vitamin K₁-epoxide was about 4 to 5.5 times greater than that of vitamin K₁.

Following intravenous administration of vitamin K₁-epoxide to the same subjects, the plasma concentration of the epoxide also appeared to decline biexponentially with time. The terminal half-life (1.1, 1.2 h) of the epoxide was a little shorter than that of vitamin K₁, and the area under the concentration time curve was approximately half that of vitamin K₁ (Table 1). Formation of vitamin K₁ from the epoxide was clearly evident in both subjects (Figure 2a). The maximum plasma concentration of the formed vitamin K₁ occurred 1 to 2 h after injection of the epoxide and then declined with apparent half-lives of 6.8 and 2.0 h in subject 1 and 2, respectively. The area under the

formed vitamin K₁ amounted to 55% (subject 1) and 33% (subject 2) of that of vitamin K₁-epoxide.

After oral administration of vitamin K₁-epoxide, pretreatment with phenprocoumon markedly prolonged the terminal half-life of the epoxide, from about 1.1 to 15.9 h (subject 1) and 11.6 h (subject 2), and totally suppressed the appearance of measurable concentrations of vitamin K₁ in plasma (Figure 2b).

Discussion

Both the intravenous and oral data show that the interconversion between vitamin K₁ and its 2,3-epoxide, and the effect of oral anticoagulant drugs on

Figure 2 Plasma concentrations of vitamin K₁-2,3-epoxide (▽) and vitamin K₁ (●) in two healthy subjects following a single dose of vitamin K₁-2,3-epoxide. (a) 10 mg vitamin K₁-2,3-epoxide i.v., (b) 20 mg vitamin K₁-2,3-epoxide orally 12 h after pretreatment with phenprocoumon (0.4 mg/kg).

this interconversion, can be demonstrated in man by following the plasma concentrations of both compounds.

After intravenous injection of therapeutic doses (10 mg) of vitamin K₁ the obtained pharmacokinetic parameters were comparable in magnitude to those reported following injection of tracer amounts (300 µg) of radiolabelled vitamin K₁ (Bjornssen *et al.*, 1979). No pronounced dose-dependency in the pharmacokinetics of this vitamin is therefore evidenced. No or only traces of vitamin K₁-epoxide were detected in the plasma following injection of vitamin K₁. This is also in agreement with results obtained in animals (Park *et al.*, 1979; Schmidt *et al.*, 1979; Trenk *et al.*, 1980; Carlisle & Blaschke, 1981) and in man (Shearer *et al.*, 1977; Bjornssen *et al.*, 1979) following administration of tracer amounts of radiolabelled vitamin K₁. The lack of accumulation of

vitamin K₁-epoxide, after administration of vitamin K₁, is most likely associated with its more rapid conversion to vitamin K₁, than the reverse reaction.

There are an increasing number of documented examples of *in vivo* interconversion, amongst these are the interconversion between cortisone and cortisol (Jenkins & Sampson, 1967), and between prednisone and prednisolone (Gambertoglio *et al.*, 1980). Analysis of the quantitative aspects of such interconversions requires independent i.v. administration of both species and measurement of them in plasma (Wagner *et al.*, 1981). This was done in the case of the present study.

The simplest system, and the one most commonly employed, to characterise interconversions, is depicted in Figure 3. In this model, the interconversion between the two species is direct, elimination by other pathways is permitted, whilst distribution of the

Figure 3 Simple scheme for the interconversion between two species, A and B. $CL_{A,B}$ and $CL_{B,A}$ denote the clearance terms associated with the interconversion, and $CL_{A,O}$, $CL_{B,O}$ are associated with irreversible loss of these compounds from the body.

species within the body can be of variable complexity. The mass balance equations, relating intravenous dose to corresponding areas under the concentration-time (AUC) curves, if applicable to the vitamin K₁: vitamin K₁-epoxide system, are given by (Appendix 1)

For dose of vitamin K₁

$$\text{Dose}^K = CL_{K,O} \cdot \text{AUC}(K)^K + CL_{KO,O} \cdot \text{AUC}(KO)^K \quad (1)$$

and for dose of vitamin K-epoxide

$$\text{Dose}^{KO} = CL_{K,O} \cdot \text{AUC}(K)^{KO} + CL_{KO,O} \cdot \text{AUC}(KO)^{KO} \quad (2)$$

Here the superscript refers to the species being administered, vitamin K₁ (K) or its epoxide (KO), AUC (i) refers to the *i*th species (K,KO) being measured and $CL_{K,O}$ and $CL_{KO,O}$ refer to the clearances associated with the irreversible loss from the body of vitamin K and its epoxide, respectively.

Equations 1 and 2 are simultaneous equations with two unknowns, $CL_{K,O}$ and $CL_{KO,O}$. However, the absence of measurable concentration of the epoxide after intravenous administration of vitamin K, $\text{AUC}(KO)^K$, prevents a unique solution for both clearance parameters. Nonetheless, the approach does allow a statement to be made as to whether in this model phenprocoumon acts solely by inhibiting the conversion of vitamin K-epoxide to vitamin K. If this were so, although the clearance associated with this interconversion process ($CL_{KO,K}$) would be reduced, those associated with irreversible loss ($CL_{K,O}$; $CL_{KO,O}$) would not. Accordingly, following i.v. vitamin K, in the presence of the anticoagulant, the associated mass balance equation

$$\text{Dose}^{K*} = CL_{K,O} \text{AUC}(K)^{K*} + CL_{KO,O} \text{AUC}(KO)^{K*} \quad (3)$$

should hold, where the asterisk denotes events following pretreatment with phenprocoumon.

As values for AUC of both vitamin K and its epoxide exist, following intravenous vitamin K-epoxide alone and following intravenous vitamin K after pretreat-

Table 2 Areas and corresponding estimates of clearance associated with elimination of vitamin K₁ and its epoxide from the dog¹

		Dose (d·min × 10 ⁻⁶)	AUC(K) (d·min/ml·min × 10 ⁶)	AUC(KO) (d·min/ml·min × 10 ⁶)	Clearance terms ² (ml/min)		Calculated ³ dose given
					$CL_{K,O}$	$CL_{KO,O}$	
Without warfarin	Vitamin K	Dog A	4.12	0.350	59	37	
		Dog B	4.20	0.498			
	Vitamin K-epoxide	Dog A	2.12	2.86	67	33	
		Dog B	1.75	6.04			
With warfarin	Vitamin K	Dog A	3.37	12.6			659
		Dog B	3.60	10.2			584
	Vitamin K-epoxide	Dog A	—	17.0			623
		Dog B	—	13.6			461

¹Taken from the data of Carlisle & Blaschke (1981).

²Calculated through appropriate substitution of AUC and Dose into equation 1 and 2.

³Estimated by substitution of $CL_{K,O}$ and $CL_{KO,O}$ values (column 5) and AUC values (column 4) into equation 3 and the analogous equation for the epoxide during warfarin treatment.

ment with phenprocoumon (Table 1), simultaneous solution of equations 2 and 3 is possible, yielding values for the clearance terms $CL_{K,O}$ and $CL_{KO,O}$. The calculated values for $CL_{K,O}$ are 24.7 and -83 l/h in subjects 1 and 2 respectively, whilst those for $CL_{KO,O}$ are calculated to be -3.2 and -19.9 l/h respectively. The existence of a negative value for $CL_{KO,O}$ is a physical impossibility. Either the model used to describe the vitamin K_1 -epoxide system (Figure 3) is incorrect or phenprocoumon does more than inhibit the reduction of vitamin K_1 -epoxide to vitamin K_1 .

A similar conclusion is reached through analysis of the data in dogs observed by Carlisle & Blaschke (1981), who measured the AUC of K and KO, following independent administration of an intravenous bolus dose of K and KO, before and after pretreatment with another oral anticoagulant, warfarin. The pertinent data are summarised in Table 2. Thus while solution of equations 1 and 2 yielded positive values of $CL_{K,O}$ and $CL_{KO,O}$ substituting these clearance values into equation 3 and the corresponding equation, when vitamin K_1 -epoxide is administered after pretreatment with warfarin, gave larger-than-administered values for the doses of both vitamin K_1 and vitamin K_1 -epoxide, during the warfarin pretreatment experiments (Table 2). These data in dogs imply that either $CL_{K,O}$, $CL_{KO,O}$ or both are depressed during warfarin treatment.

Appendix 1

Consider the simple scheme depicted in Figure 3. The corresponding rate equations for the two compounds A and B are

$$V_A \frac{dC(A)}{dt} = -CL_{A,B} \cdot C(A) - CL_{A,O} C(A) + CL_{B,A} C(B) \quad (1a)$$

$$V_B \frac{dC(B)}{dt} = -CL_{A,B} \cdot C(A) - CL_{B,O} C(B) - CL_{B,A} C(B) \quad (2a)$$

where $C(A)$, $C(B)$ are the plasma concentrations of compounds A and B respectively, V_A and V_B are the corresponding volumes of distribution, $CL_{A,B}$ and $CL_{B,A}$ the interconversion clearances and $CL_{A,O}$ and $CL_{B,O}$ the corresponding clearance terms associated with irreversible loss of compounds A and B from the body.

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- If at time $t = 0$, only compound A was administered, that is, $C(A,0) = \text{Dose}^A/V_A$; $C(B,0) = 0$, then integration of equations 1a and 2a, between $t = 0$, $t = \infty$, and addition yields.
- $$\text{Dose}^A = CL_{A,O} \cdot \text{AUC}(A)^A + CL_{B,O} \cdot \text{AUC}(B)^A \quad (3a)$$
- where the superscript denotes events following administration of drug A alone. The corresponding equation when compound B alone is given intravenously is
- $$\text{Dose}^B = CL_{A,O} \cdot \text{AUC}(A)^B + CL_{B,O} \cdot \text{AUC}(B)^B \quad (4a)$$
- Addition of further distributional parameters, to characterise multi-compartmental models, will be cause for elaboration of the rate equations, but equations 3a and 4a will always be the resultant mass balance equations.

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