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Vitamin K_1 metabolism in relation to pharmacodynamic response in anticoagulated patients

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1 The disposition of, and pharmacological response to, a single intravenous dose of vitamin K_1 (10 mg) was studied in eleven patients on daily warfarin therapy.

2 The pharmacokinetics of vitamin K_1 in patients were similar to those reported previously in healthy volunteers, terminal half-life 1.7 h.

3 All patients had been taking warfarin for at least 3 months. Steady state warfarin plasma concentrations ranged from 0.5 to 1.4 $\mu g\ ml^{-1}$. Prothrombin complex activity ranged from 15 to 28.5%.

4 There was considerable inter-individual variation in pharmacodynamic response as expressed by prothrombin complex activity (PCA) and Factor VII.

5 The maximum values for PCA and Factor VII were reached at 24–96 h and 24–48 h, respectively, after the administration of vitamin K_1.

6 Vitamin K_1 (10 mg) has a long duration of action (> 168 h) in terms of clotting factor synthesis in patients on steady state warfarin.

7 All the patients on warfarin had measurable levels (Cp_{max} 0.3–1.2 $\mu g\ ml^{-1}$) of vitamin K_1 2, 3-epoxide.

8 There was a significant correlation between the pharmacodynamic response as expressed by change in % PCA and the AUC for vitamin K_1 2,3-epoxide ($P < 0.05$).

Keywords vitamin K_1 2,3-epoxide warfarin clotting factor activity

Introduction

Vitamin K is essential for normal coagulation because it is a co-factor for the post-ribosomal synthesis of clotting factors II, VII, IX and X (Stenflo & Suttie, 1977). The vitamin K dependent step in clotting factor synthesis involves the post-ribosomal conversion of glutamyl residues into γ-carboxyglutamyl residues in clotting factor precursors. During the γ-carboxylation reaction, vitamin K_1 is converted into a biologically inactive metabolite vitamin K_1 2,3-epoxide. The epoxide is reduced back to the vitamin by a microsomal epoxide reductase and the cyclic interconversion of vitamin and epoxide is referred to as the vitamin K_1-epoxide cycle (Willingham & Matschiner, 1974; Bell, 1978).

Warfarin is thought to act by inhibition of the epoxide reductase (Bell & Matschiner, 1972), resulting in an inhibition of synthesis of clotting factors II, VII, IX and X. Previous studies have shown that administration of a physiological dose of radiolabelled vitamin K_1 alongside warfarin results in detectable levels of vitamin K_1, 2,3-epoxide (Shearer *et al.*, 1973, 1977). There is a widely held view that patients taking

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warfarin who are given vitamin K_1 because of excessively prolonged prothrombin times, are difficult to control for several days afterwards. However, there is no firm evidence for such a 'hangover' effect of vitamin K_1. The aim of this study was to investigate the relationship between the pharmacodynamic response to vitamin K_1 and the plasma concentrations of vitamin K_1 2,3-epoxide in patients on daily warfarin therapy and to determine whether or not vitamin K_1 has a prolonged effect.

Method

Plan of study

Eleven patients (four females) who were due to finish their course of warfarin therapy were studied. Written informed consent and approval from the local ethics committees was obtained. The clinical details of the patients are shown in Table 1.

On day 0 each patient received a single intravenous dose of vitamin K_1 (10 mg), diluted in 10 ml 0.9% saline, over a 10 min period. Blood samples were collected prior to the vitamin K_1 and at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 24, 48, 72, 96, 120, 144 and 168 h. Seven of the patients were studied for more than 168 h, range 216–312 h. All patients remained on their normal daily dose of warfarin throughout the study.

Analytical methods

Prothrombin times were determined by the one stage technique using Manchester Comparative Thromboplastin and an automated coagulometer. Prothrombin complex activity (PCA) was calculated from a standard curve, obtained by determining prothrombin times of pooled normal citrated human plasma diluted in adsorbed plasma (deficient in Factors II, VII, IX and X) at concentrations from 6.25 to 100%, as previously described (Park *et al.*, 1979). Factor VII activity was determined by a one stage technique using beagle dog Factor VII deficient plasma.

Vitamin K_1 (the biologically active *trans*-isomer) and vitamin K_1 2,3-epoxide plasma concentrations were determined using a normal phase high performance liquid chromatography assay (Wilson & Park, 1983).

Plasma warfarin concentrations were determined using a normal phase high performance liquid chromatography assay (Breckenridge *et al.* 1985).

Plasma protein binding of warfarin was determined by equilibrium dialysis as previously described (Serlin *et al.*, 1979). Tracer amounts (< 0.1 μg) of [^{14}C]-warfarin (Radiochemical Centre, Amersham, specific activity 55 mCi mmol^{-1}) were added to plasma samples, which were dialysed at 37°C against a 0.1 mol l^{-1} phosphate buffer for 4 h using a Dianorm apparatus.

Calculations

A biexponential equation was fitted to the vitamin K_1 plasma concentration *vs* time curve data using a regression analysis programme (Nielsen-Kudsk, 1980). For both vitamin K_1 and the 2,3-epoxide, the area under the plasma concentration–time curve was determined by the trapezoidal rule up to the last observation, and the terminal area until infinity by extrapolation,

Table 1 Clinical details of patients on steady state warfarin

Patient	*Age (years)*	*Diagnosis*	*Dose (mg daily)*	*Duration (months)*	*Plasma warfarin concentration (μg mg^{-1})*	*Free plasma warfarin concentration (ng ml^{-1})*
PR	40	DVT	6	4	0.57	3.59
AWO	42	PE	7*	8	0.70	6.27
AWA	44	PE	4	6	0.51	6.22
RA	50	PE	4	6	0.60	9.14
WH	63	DVT	4	6	0.95	12.87
AM	64	Prophylaxis	7*	3	1.37	18.75
PE	68	PE	2.5/5*	7	0.75	11.43
JM	66	Prophylaxis	4*	3	0.88	10.18
ES	64	Prophylaxis	4*	6	0.92	10.68
AK	63	PE	3*	6	1.00	16.27
DG	63	PE	11	36	1.19	15.11

* Receiving other medication as well
DVT – Deep vein thrombosis
PE – Pulmonary embolus

by dividing the last observation by the terminal component. The area extrapolated was under 10% in all cases, with the single exception of patient RA where the extrapolated area for vitamin K_1 2,3-epoxide was 11.1%.

Spearman's rank correlation was used to compare the pharmacodynamic response as expressed by change in % PCA and the AUC for vitamin K_1 2,3-epoxide. Spearman's rank correlation was also used to compare the plasma warfarin concentration (both free and total) with the initial PCA, the change in % PCA and the AUC for vitamin K_1 2,3-epoxide. The Factor VII data was not used in any statistical analysis as it was only possible to obtain Factor VII levels in 6 of the 11 patients studied. All data is presented as the mean ± s.d.

Results

All patients were anticoagulated within the therapeutic range (BCR 1.8–3.5). Prothrombin times ranged from 22 to 37.2 s and PCA from 15 to 28.5%.

The mean elimination half-life for vitamin K_1 was 1.7 ± 0.7 h (Figure 1). After 10 h, plasma vitamin K_1 levels were below 20 ng ml^{-1} (limit of sensitivity) in all 11 patients. All the patients had detectable levels of vitamin K_1 2,3-epoxide, Cp_{max} 540 ± 252 ng ml^{-1}, 2–6 h after the dose of vitamin K_1. The large variation in total AUC for vitamin K_1 2,3-epoxide is shown in Table 2. In volunteers vitamin K_1 2,3-epoxide could not be detected for 24 h following the same pharmacological dose of vitamin K_1 (10 mg), limit of sensitivity 20 ng ml^{-1} (Park *et al.*, 1984).

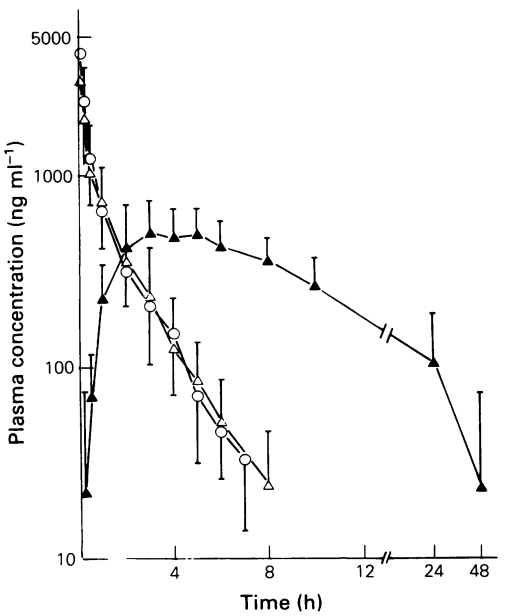

Figure 1 Plasma concentrations of vitamin K_1 (△) and vitamin K_1 2,3-epoxide (▲) in anticoagulated patients, after intravenous administration of vitamin K_1 (10 mg). Control values for vitamin K_1 (○) were obtained from healthy volunteers.

Treatment with vitamin K_1 increased the mean % PCA from 22.4 ± 4.0 to 66.4 ± 10.1 (Table 3 and Figure 2). In six patients Factor VII was also measured and the mean activity rose from 20.3 ± 10.2 to 85.7 ± 37.1 % following the vitamin K_1. In four patients, the PCA returned to its initial value—at 168, 216, 240 and 264 h, respectively. In one of these patients (WH), the Factor VII activity also returned to its initial value at 144 h.

The mean warfarin plasma concentration was 0.86 ± 0.27 μg ml^{-1}. All samples were collected between 0 and 12 h prior to the daily dose of warfarin. The mean % free warfarin was 1.25 ± 0.29. The concentrations of total and free warfarin are shown in Table 1.

There was a direct correlation between change in % PCA and the AUC for vitamin K_1 2,3-epoxide, using Spearman's rank correlation (ρ 0.666, $P < 0.05$). There was no correlation ($P > 0.10$) between the AUC for vitamin K_1 2,3-epoxide, initial PCA or change in % PCA and plasma warfarin concentrations (either free or total).

Discussion

After intravenous administration, vitamin K_1 plasma concentrations declined in a biexponential fashion. The pharmacokinetic values of vitamin K_1 were similar to those obtained in healthy volunteers (Park *et al.*, 1984). Previous studies using radiolabelled vitamin K_1 (Shearer *et al.*, 1977; Bjornsson *et al.*, 1979) have shown that warfarin does not interfere with the pharmacokinetics of vitamin K_1, but this is the first detailed study in patients who in contrast to young healthy volunteers are older, suffer from various illnesses and also are receiving other medication. Furthermore, in this study a pharmacological dose (10 mg) was employed, whereas previously, a more physiological dose (45–300 μg) was used.

The duration of action of vitamin K_1 has not been defined. Previous work in patients poisoned with 4-hydroxycoumarins suggests that the duration of action is short (Barlow *et al.*, 1982), and this is supported by experimental work in the

Table 2 Pharmacokinetics of vitamin K_1 and K_1 2,3-epoxide

Patient	*Vitamin K_1* *Elimination half-life (h)*	*AUC ($\mu g\ ml^{-1}\ h$)*	*Vitamin K_1 2,3-epoxide AUC ($\mu g\ ml^{-1}\ h$)*
PR	1.23	1.74	2.48
AWO	2.69	2.43	18.86
AWA	0.93	5.23	10.33
RA	1.37	5.05	6.44
WH	2.78	4.68	10.66
AM	1.12	1.88	10.68
PE	1.58	2.62	7.41
JM	1.62	2.54	2.88
ES	1.78	2.57	10.60
AK	2.58	2.28	3.86
DG	1.19	2.60	9.28
Mean	1.72	3.06	8.50
s.d.	0.67	1.28	4.69

rabbit (Park *et al.*, 1984). In patients however, there is only partial inhibition of clotting factor synthesis and it is thought that the duration of action may be longer. The British National Formulary in fact gives a duration of action of 2 weeks.

Warfarin inhibits clotting factor synthesis and because of this its action on clotting factor activity is not immediate. This makes it difficult to determine the precise duration of response to a single pharmacological dose of vitamin K_1 by direct measurement of PCA alone. We have therefore had to use a theoretical model to determine clotting factor synthesis following the administration of vitamin K_1 (Nagashima *et al.*, 1969).

$$R_{syn} = R_{net} + k_d P$$

where R_{syn} is the rate of clotting factor synthesis (II, VII, IX and X), R_{net} is the rate of net change in PCA, k_d is the apparent first order degradation constant for PCA and P is the PCA value. In order to determine k_d it is necessary to completely inhibit clotting factor synthesis and this was considered to be unethical in the patients studied. We therefore used the mean data from the 13 volunteers studied by Nagashima *et al.* (1969), as there appeared to be

Table 3 Pharmacodynamic response to vitamin K_1

Patient	*Initial PCA (%)*	*Maximum PCA (%)*	*Change in % PCA*	*Final PCA (%) (time (h) **)*	*Initial Factor VII (%)*	*Maximum Factor VII (%)*	*Final Factor VII (%) (time (h) **)*
PR	24.5	50.5	26	23 (240)	—	—	—
AWO	17.5	77	59.5	31*	—	—	—
AWA	22	63	41	22 (216)	—	—	—
RA	24	68	44	54*	—	—	—
WH	22.5	70	47.5	23*	19	130	14*
AM	23	67	44	48+	22	98	43+
PE	19	83	64	55.5 (312)	35	120	49 (312)
JM	28.5	61	32.5	43+	11	55	23+
ES	23	68	45	55+	27	76	60+
AK	15	50	35	29+	7.5	35	19+
DG	27	73	46	23.5*	—	—	—
Mean	22.4	66.4	44.0		20.3	85.7	
s.d.	4.0	10.1	11.0		10.2	37.1	

* 264 h + 168 h

** Time (h) is the time of the last sample collected for each individual patient

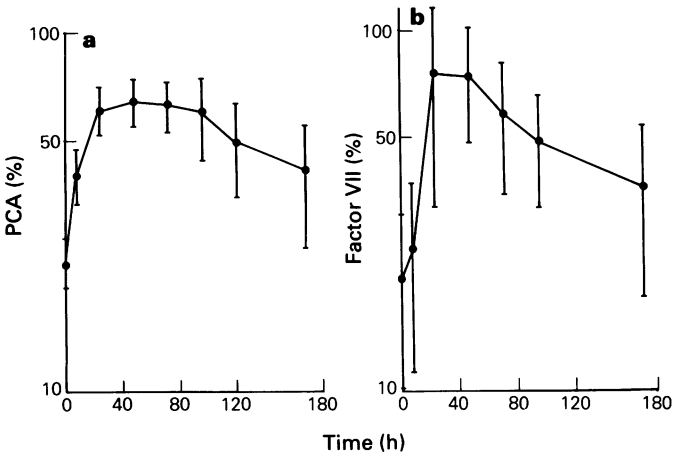

Figure 2 Changes in (a) prothrombin complex activity (PCA), and (b) Factor VII activity in anticoagulated patients, after intravenous administration of vitamin K_1 (10 mg).

little inter-individual variation of the level of k_d.

The mean values for clotting factor synthesis are shown in Table 4, assuming a mean k_d of 1.21. It can be seen that the duration of action of vitamin K_1 in relation to clotting factor synthesis is greater than 168 h in the 11 patients studied. Clotting factor synthesis peaked at 24 h and this is reflected in the maximum PCA values (50–85%) which were present between 24 and 96 h. It is therefore clear that the duration of action of vitamin K_1 is considerably longer in patients undergoing controlled, therapeutic anti-coagulation, than during chronic coumarin poisoning (Barlow *et al.*, 1982; Park *et al.*, 1984). Previous animal work based on the limiting situation of maximum vitamin K_1 antagonism (Park *et al.*, 1984) had suggested that high plasma concentrations (> 500 ng ml^{-1}) of vitamin K_1 are required for clotting factor synthesis. However, we have shown that in patients on warfarin, vitamin K_1 requirements both in terms of dose and plasma levels are considerably less. Clotting factor synthesis occurred in the presence of plasma vitamin K_1 concentrations below 20 ng ml^{-1}; the limit of sensitivity for the assay employed. Therefore it was not possible to determine a plasma concentration-effect relationship for vitamin K_1 in these patients.

In two individuals the Factor VII activities rose to greater than 100%. This has been described previously and the clinical significance is uncertain (Meer *et al.*, 1968). In the patients studied there was no clinical evidence of rebound hypercoagulability.

As in previous studies (Yacobi *et al.*, 1976) we found no correlation between plasma warfarin concentrations (free or total) and pharmacological effect in the patients studied. There was considerable inter-patient variation in warfarin levels, despite a similar degree of anticoagulation. Furthermore, there was no correlation between plasma warfarin concentrations and perturbation of vitamin K_1 metabolism, as measured by the AUC for vitamin K_1 2,3-epoxide.

Nevertheless, there was a significant correlation between the net increase in % PCA and the AUC for vitamin K_1 2,3-epoxide. A possible explanation for this observation is that the AUC for vitamin K_1 2,3-epoxide reflects not only inhibition of the epoxide reductase but also utilization of vitamin K_1 within the vitamin K_1-epoxide cycle.

The direct correlation between the change in % PCA and the AUC for vitamin K_1 2,3-epoxide is strong evidence that in patients on therapeutic doses of warfarin, i.e. with partial inhibition of the epoxide reductase, the process of conversion of vitamin K_1 2,3-epoxide and the γ-carboxylation of vitamin K dependent clotting factors are closely related. This relationship has been

Table 4 Clotting factor synthesis following vitamin K_1

Time (h)	R_{syn}
0	27.1
24	88.6
48	78.8
72	75.1
96	70.5
120	54.5
168	49.6

previously shown in animals (Bell, 1978; Friedman & Smith, 1979) and it has been suggested that the epoxidase and carboxylase enzymes are in fact the same. Further studies are required to determine the exact relationship between these two processes in man.

In conclusion, we have found a long duration of action (> 168 h) for a single pharmacological dose of vitamin K_1 (10 mg) in patients anticoagulated with warfarin despite the rapid clearance of the vitamin from plasma. This is in agreement with the clinical suspicion of a 'hangover' effect to vitamin K.

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