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Disposition of vitamin K_1 after intravenous and oral administration to subjects on phenprocoumon therapy

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Summary

The disposition and prothrombin-complex activity of a new i.v. dosage form of vitamin K_1 using mixed micelles of glycocholic acid and lecithin as a vitamin K_1 solution was studied in 9 volunteers on an oral phenprocoumon dosage regimen. Each of two volunteers received 10, 20, 40 or 60 mg i.v. and oral doses in a cross-over fashion. An additional subject received a single 60 mg i.v. bolus dose. The intravenous doses were well tolerated with no subjective or objective side-effects. Using a sensitive gas chromatographic assay (detection limit ~ 5 ng/ml), the concentrations of vitamin K_1 could be followed for 24–36 h after the i.v. doses and for 12–33 h after the oral doses. The steady-state apparent volume of distribution was 20 ± 6 liters and the clearance was 70 ± 19 ml/min. The bioavailability demonstrated large inter-individual variation ranging from 3.5% to 60%. After the i.v. dose, vitamin K_1 showed multi-compartmental characteristics with a terminal half-life of 14 ± 6 h. No dose dependency was detected in any of the pharmacokinetic parameters. The pharmacologic activity of vitamin K_1 after i.v. and oral dosing, defined as the area under the curve of the increase in the prothrombin-complex activity over baseline values during phenprocoumon therapy, correlated well with the logarithmic value of the area under the plasma concentration–time of vitamin K_1 ($r^2 = 0.677$, $t = 5.42$, $P < 0.001$).

Introduction

The absorption of vitamin K_1 appears to vary significantly among individuals. The fractional absorption has been reported to range between 4% to more than 80% in individual subjects as determined from fecal accumulation of unchanged vitamin K_1, plasma concentrations or continuous sampling of thoracic duct lymph (Blomstrand and Forsgren, 1968; Shearer et al., 1972, 1974; Park et al., 1984).

The absorption of vitamin K_1 is thought to involve an energy-dependent saturable process in the upper small intestine (Hollander and Rein, 1973) suggesting that absorption of vitamin K_1 may be dose-dependent. More importantly, however, is the influence of bile salts upon absorption (Shearer et a., 1972, 1974). Bile salts act as emulsifiers of vitamin K_1 in the gastrointestinal tract and variability in their production or release may cause significant variability in the degree and rate of absorption. Because of the reported varia-

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ble bioavailability of vitamin K_1, intravenous administration of vitamin K_1 is expected to give more reproducible plasma concentrations than oral dosing regimens. However, intravenous administration of vitamin K_1 is often associated with flushing, dyspnea, and chest pain. In severe cases cardiovascular collapse has been known to occur. These adverse reactions are believed to relate to the emulsifying/dispersion agent used in the parenteral preparation (Cremophor), an ethylated castor oil product (Mandel and Cohn, 1985). The recent introduction of a new colloidal system based on mixed micelles formed from lecithin and glycocholic acid and which are capable of solubilizing highly lipophilic agents (Steffen and Schmidt, 1979) has stimulated the hope that these adverse reactions of intravenous administration of vitamin K_1 may be reduced or eliminated. These micelles are thermodynamically stable, and because of their size in the 10 nm region forms optically clear solution. When diluted, the glycocholic acid is partially lost and the remaining lecithin bilayers likely associated with the LDH fraction in vivo (Tall, 1980). Drug associated with these micelles is therefore thought to be rapidly released in vivo and preliminary studies suggest an onset of effect of diazepam by i.v. administration in mixed micelles as rapid as when administered i.v. in a propylene glycol and ethanol solution (Hoffman LaRoche & Co., unpublished results).

The present study study was undertaken to gain information regarding the disposition of a new mixed micelle i.v. solution of vitamin K_1 in subjects on anticoagulant therapy, and to assess the efficacy of i.v. versus oral dosing of vitamin K_1.

Materials and Methods

Subjects

Nine healthy male subjects between 22 and 36 years of age were enrolled in the study. Due to personal reasons one of the subjects (subject no. 4) discontinued his participation after completing only the intravenous part of the study. All subjects had normal hematologic and blood chemistry values, normal renal function values, normal electrocardiographic (EKG) readings and a normal physical examination. Subject information is given in Table 1.

Study design

The subjects received daily doses of phenprocoumon and were titrated to a prothrombin complex activity of approximately 15–20% of normal values measured by the Quick-method (Quick, 1938), reached in about 10 days. The individual daily doses needed to achieve these values were subsequently maintained for the remainder of the study. On days 15 and 29 after initiation of the study, the subjects received single oral or intravenous doses of vitamin K_1 in a cross-over fashion. Of the 8 subjects completing the study, two subjects each received vitamin K_1 orally and

TABLE 1

Subject information

Subject	Age (years)	Weight (kg)	Height (cm)	Cigarettes per day	Measured Vitamin K_1 Dose	
					day 15	day 29
1	30	76	192	20	10.3 mg i.v.	8.4 mg p.o.
2	22	72	178	–	20.9 mg i.v.	17.6 mg p.o.
3	28	77	185	18	43.3 mg i.v.	36.3 mg p.o.
4	35	81	186	–	66.9 mg i.v.	
5	30	68	174	–	8.6 mg p.o.	10.0 mg i.v.
6	36	66	176	–	18.8 mg p.o.	20.1 mg i.v.
7	28	67	174	–	39.3 mg p.o.	40.3 mg i.v.
8	22	73	170	10	58.8 mg p.o.	64.9 mg i.v.
9	26	67	173	2	56.4 mg i.v.	56.3 mg p.o.

intravenously in doses of 10, 20, 40 and 60 mg. Fourteen 10 ml blood samples were obtained at various times during the intravenous dose study up to 36 h after administration. The blood was collected via an indwelling catheter in a contralateral arm vein to the i.v. infusion. After the oral dose twelve 10 ml blood samples were obtained up to 33 h after administration. The subjects were hospitalized during the total study and food withheld for 2 h after drug administration.

Drug administration

Intravenous dosing. One to 6 ampules of vitamin K_1 in mixed micelle solution * (each ampule containing 10 mg vitamin K_1/ml) were diluted to 55 ml with sterile 5% glucose solution. Fifty ml of this solution was then infused at a constant rate over 30 min into an arm vein. During drug infusion the subjects remained in a supine position. An aliquot of the solution was analyzed for determination of the actual dose administered.

Oral dosing. Various volumes of a commercial solution of vitamin K_1 (Konakion) were diluted to 105 ml with water to yield concentrations of 10, 20, 40 and 60 mg/dl vitamin K_1. One hundred ml of the solution was ingested on an empty stomach with the subjects in a sitting position. An aliquot of the solution was analyzed for determination of the actual dose administered.

The subjects remained seated for 2 hours after both the oral and intravenous administration.

Ethical consideration

The study was approved by the institutional ethics committee. The subjects were told that they were free to discontinue participation in the study at any time.

Analysis

The analysis of vitamin K_1 and K_1-2,3-epoxide was carried out using a modification of the gas-chromatographic assay described by Bechtold et al. (1984). In short, 0.4 ml plasma was mixed with 10 µl of vitamin $K_{2(20)}$ solution (4 µg/ml in ethanol) as internal standard and 1 ml water and extracted into 5 ml *n*-hexane/ethanol (1 : 1). The organic phase was evaporated to dryness, redissolved in 200 µl *n*-heptane and 1 µl injected into a gas chromatograph via the Grob split–splitless injection technique. Column: 25 m × 0.32 mm i.d. fused fused silica capillary column (CP Sil 5 CB, Chrompack, Middleburg, The Netherlands). Injection temperature: 80 °C. After 40 s the injector was flushed with carrier gas (split ratio 1 : 50) and the column oven was heated to 285 °C with maximum rate to its final temperature of 285 °C. Detector temperature: 330 °C. Oxygen-free nitrogen was used as make-up gas for the detector (inlet pressure: 1.0 bar). Detector: electron-capture (Ni^{63}—10 mCi). Detection limit: ~ 5 ng/ml.

Data analysis

To determine the terminal half-life of vitamin K_1 the concentrations vs time data were fitted to a 2- or 3-compartment pharmacokinetic model without (i.v. dose) and with absorption (oral dose) including a lag-time using the Topfit pharmacokinetic computer program * (Bozlar and Van Rossum, 1982). The vitamin K_1-epoxide concentration–time data, both after the i.v. and oral administration were adequately fitted with a 2-compartment model with delayed formation or absorption with delayed formation. The fit was considered adequate when the residual weighted sums of squares represented less than 5% of the total weighted sums of squares and a plot of the relative difference between the measured and computer-fitted concentrations versus time showed no trends.

The clearances were calculated from the ratio of the dose and the area under the plasma concentration–time curve (AUC) from the i.v. doses. The area was determined from time zero after the i.v. dose and from the estimated lag time after the oral dose as determined from the fitting procedure to the last concentration–time point using the trapezoidal rule. To this area was added the estimated area from the last time-point to infinity using the ratio: last concentration/terminal rate constant.

* Ro-016722/120 – Hoffmann-LaRoche consisting of 54.6 mg glycocholic acid and 75.6 mg lecithin/ml solution (batch PT 9177 HO4).

* Thomae, Biberach, F.R.G.

The steady-state apparent volume of distribution was calculated from the parameters dose, AUC and area under the moment curve as described by Benet and Galeazzi (1979) adjusted for the infusion time after the i.v. dose.

The bioavailability was determined from the ratio of the area under the plasma concentration–time curve for vitamin K_1 for the oral to i.v. dose adjusted for the difference in dose for each individual subject.

The area under the effect curve was calculated from the temporary increase in the prothrombin complex activity as a result of the vitamin K_1 administration using the linear trapezoidal rule (Hartmann et al., 1985). The pre-dose prothrombin activity at day 15 and day 29 were used as baseline values.

Results

The plasma concentration could be followed for 24–36 h post i.v. administration and 12–33 h after the oral dose. The obtained pharmacokinetic parameters for vitamin K_1 and the areas under the vitamin K_1-epoxide plasma concentration–time curves for the individual subjects after the i.v. and oral doses are given in Tables 2 and 3. The maximum measured concentrations (C_{peak}) of vitamin K_1 and vitamin K_1-epoxide and the time to reach the peak concentrations (t_{peak}) are also given in Tables 2 and 3.

TABLE 3

Pharmacokinetic information of vitamin K_1-epoxide after i.v. and oral administration of vitamin K_1

Subject	i.v.			Oral		
	AUC (ng·h/ml)	C_{peak} (ng/s) ml)	t_{peak} (h)	AUC (ng·h/ml)	C_{peak} (ng/ml)	t_{peak} (h)
1	4800	353	4	3820	203	4
2	18200	1380	4	5320	213	9
3	32800	2700	4	5620	225	9
4	43900	3020	4			
5	9310	326	4	3730	174	9
6	13180	1060	4	3600	174	6
7	44400	3480	6	1530	84	9
8	53600	3840	6	170030	824	9
9	36900	3020	2	29500	1280	12

The plasma concentrations of vitamin K_1 and vitamin K_1-epoxide after i.v. and oral doses are given for a representative subject (subject 2) in Fig. 1.

After an initial rapid decline, the concentrations after the i.v. doses reached a log-linear terminal phase after 8–12 h with an average half-life of

TABLE 2

Pharmacokinetic parameters of vitamin K1 after i.v. and oral administration

I.v. dose						Oral dose					
Subject no.	V_c L	V_{ss} L	Terminal $t_{1/2}$ (h)	CL (ml/min)	AUC (ng·h/ml)	Terminal $t_{1/2}$ (h)	AUC (ng·h/ml)	F	C_{peak} (ng/ml)	t_{peak} (h)	t_{lag} [a] (h)
1	5.0	26.2	13	58	2970	2.2	1330	0.55	296	4	2
2	3.6	22.7	8	77	4540	6	1540	0.40	394	3	2
3	3.6	20.4	9	88	8180	12	1550	0.23	453	3	2
4	3.0	16.2	14	94	11805						
5	2.9	15.8	22	38	4430	5	1620	0.43	227	3	3
6	3.7	15.3	7	89	3750	7	750	0.21	129	4	3
7	3.6	11.3	12	52	12890	16	446	0.035	95	4	3
8	3.2	28.9	21	75	14400	12	2220	0.17	597	3	1
9	2.8	25.2	22	61	17730	19	10640	0.60	1780	4	1
Mean	3.5	20.2	14	70		10		0.33		3.5	2.1
S.D.	0.7	5.9	6	19		6		0.20		0.5	0.8

[a] Time of appearance of drug in plasma. Obtained from the compartmental fitting.

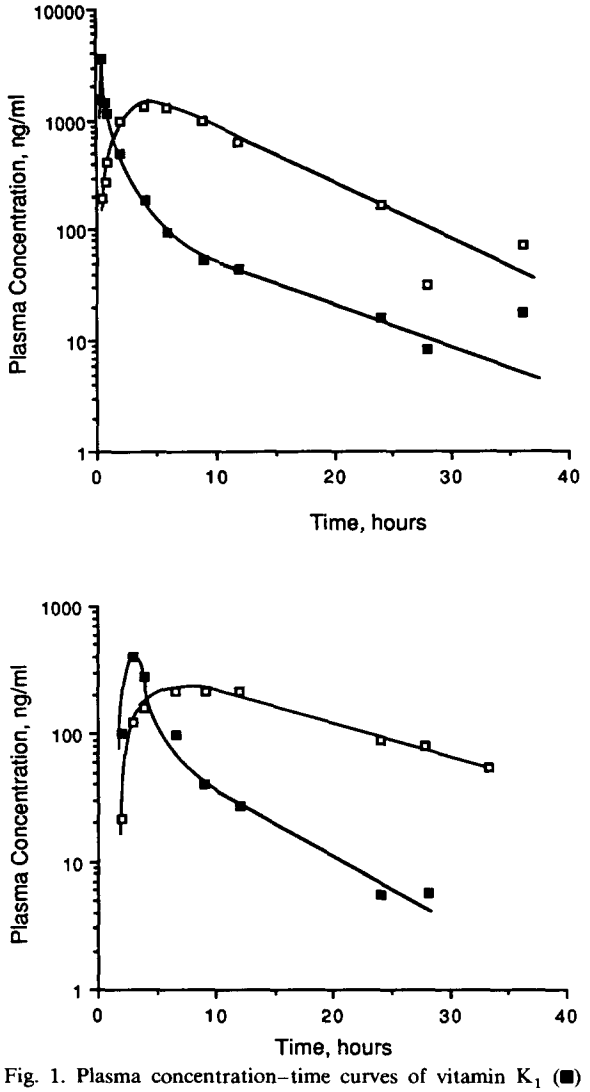

Fig. 1. Plasma concentration–time curves of vitamin K_1 (■) and vitamin K_1-epoxide (□) after 20.9 mg i.v. (upper panel) and 17.6 mg oral (lower panel) doses to patient no. 2. The i.v. dose was infused over 30 min. The data were fitted with 2- or 3-compartment models as described in the text.

14 ± 6 h (Table 2). After the oral dose no plasma concentrations were detectable for the first 1–3 h (t_{lag}, Table 2). Because the patients were fasting for the first 2 h after administration, this delay may relate to a delayed release of bile acids that occurred first at meal time. The plasma concentrations peaked 3–4 h after administration (t_{peak}, Table 2). After the oral administration the concentration could only be followed for 12–33 h and for some subjects (1 and 5) the measured half-life was much shorter than after the i.v. dose suggesting the terminal elimination phase had not been reached before reaching the sensitivity limit of the assay method, potentially underestimating the bioavailability for these subjects.

The vitamin K_1-epoxide concentrations were expectedly high after both routes of administration (Fig. 1, Table 3) consistent with an inhibition of the epoxide-reductase by phenprocoumon, thereby decreasing the clearance of vitamin K_1-epoxide. These results are similar to those found by other authors (Hollander and Rein, 1973; Choonara et al., 1985; Shearer et al., 1977; Park et al., 1986) and consistent with the conversion model presented by Bechtold et al. (1983). In the absence of anticoagulation therapy no or only trace concentrations of vitamin K_1-epoxide are usually seen (Bechtold et al., 1983; Bechtold, 1985).

The prothrombin complex activity prior to initiation of the vitamin K_1-administration varied between 15% and 20% of normal values. The maximum prothrombin complex activity after vitamin K_1 administration varied substantially in the individual subjects but correlated statistically significantly with the logarithm of the systemically available dose ($r^2 = 0.786$, $t = 7.17$, $P < 0.001$) (Fig. 2).

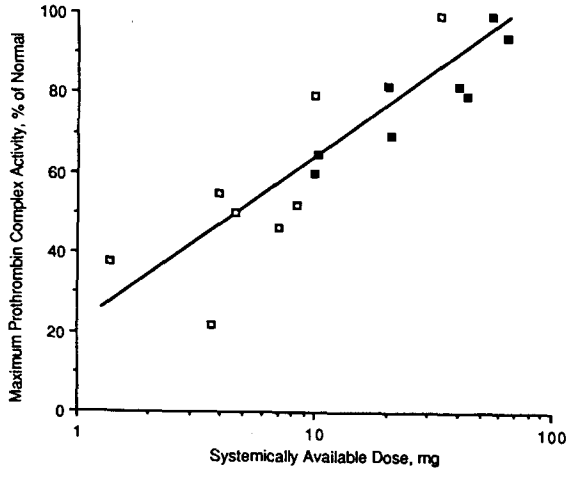

Fig. 2. Correlation between the maximal prothrombin complex activity achieved and the systemically available vitamin K_1 in subjects on constant phenprocoumon therapy. Baseline values of the prothrombin complex activity prior to the vitamin K_1 study ranged between 15 and 20% of normal. ■, i.v. dose; □, oral dose. $r^2 = 0.786$, $t = 7.17$, $P < 0.001$.

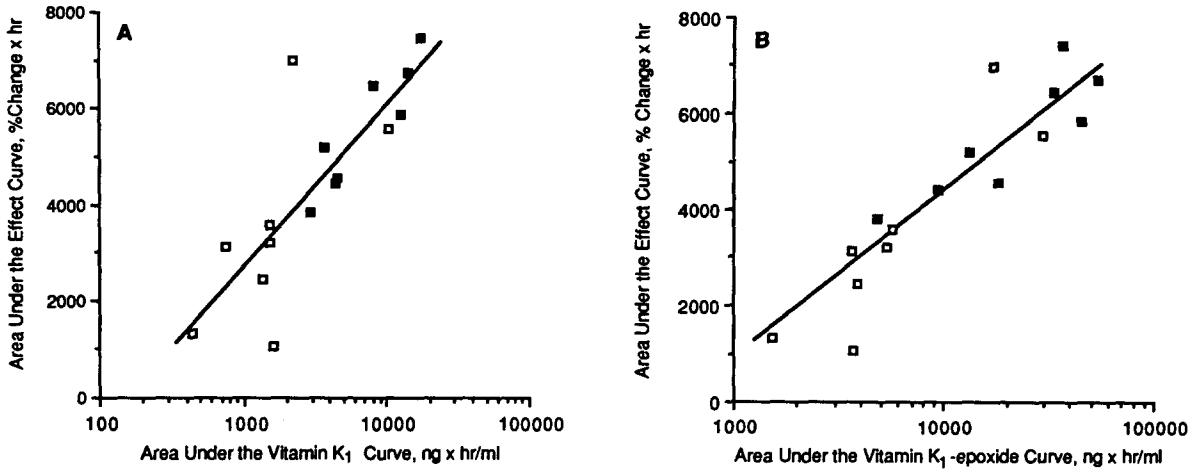

Fig. 3. Correlation between the area under the effect curve (see Materials and Methods) and the area under the vitamin K_1 plasma concentration–time curve (left) and the area under the vitamin K_1-epoxide plasma concentration–time curve (right). ■, i.v. dose; □, oral dose. $r^2 = 0.677$, $t = 5.42$, $P < 0.001$ for vitamin K_1, and $r^2 = 0.836$, $t = 8.36$, $P < 0.001$ for vitamin K_1-epoxide.

The pharmacologic activity, reported here as the area under the curve of the increase in the prothrombin complex activity over baseline values during phenprocoumon therapy, correlated well with the logarithmic value of the area under the plasma concentration–time curve of vitamin K_1 ($r^2 = 0.677$, $t = 5.42$, $P < 0.001$) (Fig. 3A). The effect, however, correlated even stronger with the logarithmic values of the area under the vitamin K_1-epoxide curve ($r^2 = 0.833$, $t = 8.36$, $P < 0.001$) (Fig. 3B).

Discussion

The intravenous doses were well tolerated with no subjective or objective side-effects. With the limitation that only two subjects were studied at each dose level, no dose-dependency was observed in any of the pharmacokinetic parameters of vitamin K_1 in this study in contrast to what has been found for other vitamins (Marcus and Coulston, 1985). Although the absorption of vitamin K_1 is thought to occur by an active, saturable process, no dose-dependency in the bioavailability was indicated. This is consistent with results by Park et al. (1984) who also did not find a dose-dependent bioavailability giving doses of 10 and 50 mg. The bioavailability in this study varied substantially from a low of 3.5% to a high of 60%. This large variability is similar to the finding of others (Blomstrand and Forsgren, 1968; Shearer et al. 1972, 1974; Park et al., 1984). Park et al. (1984) have also reported a large intra-subject variability in total amount absorbed indicating that the bioavailability is not reproducible in individual subjects. It has been suggested that the absorption of vitamin K_1 is mainly determined by the presence of bile acids in the upper gastrointestinal tract (Hollander and Rein, 1973); the bile acids acting as solubilizers of vitamin K_1. Variable release of bile acids may therefore be a key factor in the variation in the bioavailability. This is consistent with the fact that our subjects were fasting for the first 2 h after vitamin K_1 administration and that no significant amount of vitamin K_1 appeared in plasma until 2–3 h after administration, and that peak concentrations were achieved after 3–4 h. In fact, one may speculate that the variable absorption may in part be related to a variable passage of the oral preparation in the gastrointestinal tract in the 2 h before the meal and the release of bile acids during the meal. In part it may also relate to a variable amount of bile acids released. If so, one may expect the variability to be reduced by administering vitamin K_1 together with meals.

The values of clearance found in this study (70 ml/min) are similar to those calculated from the studies by Park et al. (1984) (60 ml/min) and by Choonara et al. (1985) (62 ml/min) in subjects on warfarin therapy but somewhat smaller than the values found by Bjornsson et al. (1979) in subjects on warfarin therapy (111 ml/min) and the values reported by Bechtold (1985) in subjects on phenprocoumon therapy (128 ml/min).

The apparent steady-state volume of distribution of 20 liters is substantially higher than the values reported by Bechtold (1985) (11 liters in subjects on phenprocoumon and 6.1 liters in non-coagulated subjects) and what can be calculated from the data by Park et al. (1984) 5 liters), but is similar to the values of 17 liters reported by Bjornsson et al. (1979). These differences probably lie in the fact that we employed a sensitive assay method of vitamin K_1. Because of the sensitive assay method the plasma concentrations could be followed after the i.v. dose to 36 hours resulting in a terminal half-life on the average of 14 ± 6 h which is substantially longer than reported in previous studies (54–260 min (Shearer et al., 1972, 1974,); Hollander and Rein, 1973) where the concentrations were followed for 12–24 h. This longer half-life suggests a wider distribution than previously reported.

There was a strong correlation between the maximal prothrombin complex activity on continuous phenprocoumon dosing and the logarithm of the systemically available dose (Fig. 2). The correlation suggests that an amount between 10 and 20 mg needs to be systemically available to reach a goal of a minimum return to 70% of normal anticoagulation. When the bioavailability is therefore variable, the maximum effect becomes variable as well as unpredictable. Due to the variable bioavailability found by us and others (Blomstrand and Forsgren, 1968; Shearer et al., 1972, 1974; Park et al., 1984) it appears that i.v. dosing may be the preferred route of administration of vitamin K_1 if predictable alteration in the coagulation is to be achieved.

There was also a high correlation between the cumulative effect (the area under the effect curve), and the logarithmic value of the area under the plasma concentration–time curve of vitamin K_1, again indicating that the disposition of the drug is crucial for the pharmacologic response. However, interestingly the correlation was better between the cumulative effect and the logarithmic value of the area under the vitamin K_1-epoxide plasma concentration–time curve than the cumulative effect and the log value of K_1 plasma concentration–time curve. Because the correlation is similar for vitamin K_1 and vitamin K_1-epoxide when using the i.v. data only ($r^2 = 0.841$, $t = 5.64$ and $r^2 = 0.765$, $t = 4.42$, respectively) the above observation may relate to the possibility that part of vitamin K_1 is exerting its effect – with subsequent metabolism to the epoxide – before it reaches the site of measurement after oral absorption. The vitamin K_1-epoxide concentration, therefore, becomes a better indicator of the amount of vitamin K_1 reaching the site of action after the oral dose than the amount of vitamin K_1 reaching the site of measurement. The effect of vitamin K_1 on the coagulation process has been suggested to be through an initial step of reduction to vitamin K_1-hydroquinone which is important for a γ-carboxylation process in forming prothrombin (Uotila and Suttie (1982)). In this process, vitamin K_1-hydroquinone is oxidized to vitamin K_1-epoxide. Because anticoagulants inhibit the epoxide-reductase important for reforming vitamin K_1-hydroquinone from vitamin K_1-epoxide (Fasco and Principe, 1980) and the elimination of vitamin K_1-epoxide is also drastically reduced (Shearer et al., 1972; Bechtold, 1985), the area under the vitamin K_1-epoxide curve may therefore be a reasonable representative of the cumulative amount of vitamin K_1 that has stimulated the prothrombin formation. As the cumulative concentration of vitamin K_1 observed after the i.v. dose at the measurement site is a reasonable approximation of the cumulative arterial concentrations of vitamin K_1 reaching the effect site, vitamin K_1 and the vitamin K_1-epoxide will predict the effect to a similar degree after i.v. dosing.

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