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Plasma disposition of vitamin K_1 in relation to anticoagulant poisoning

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1 The disposition of vitamin K_1, after intravenous (10 mg) and oral doses (10 mg and 50 mg) was studied in six healthy male subjects.
2 After intravenous administration, the plasma concentration-time profile was adequately fitted with an average terminal half-life of 1.7 h.
3 After oral administration (10 mg and 50 mg) the availability of vitamin K showed marked inter-individual variation (10–63%). With the higher dose intra-individual variation was also observed.
4 Experiments in brodifacoum-anticoagulated rabbits demonstrate that the duration of action of a pharmacological dose (10 mg/kg) is short (9 h) and that high plasma concentrations (*ca* 1 μg/ml) of the vitamin are required to drive clotting factor synthesis during maximum coumarin anticoagulation.
5 Taken collectively, these data indicate that the short duration of action of vitamin K, frequently observed in cases of coumarin poisoning, is a consequence of requirements for high vitamin K concentrations and rapid clearance of the vitamin.

Keywords vitamin K_1 coumarin anticoagulants 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). Coumarin anticoagulants such as warfarin, difenacoum and brodifacoum are thought to produce their hypothrombinaemic effect by inhibition of the epoxide reductase (Bell & Matschiner, 1972; Whitlon *et al.*, 1978; Park *et al.*, 1979). For this reason coumarin anticoagulants are referred to as indirect antagonists of vitamin K.

Daily requirements for the vitamin are low (*ca.* 1 μg/kg) (Frick *et al.*, 1967; Barkhan & Shearer, 1977), but vitamin K_1 administration, in pharmacological doses, is necessary in cases of intentional or accidental poisoning with coumarin anticoagulants. In such circumstances, the duration of action of the vitamin may be short and frequent, repeated administration is necessary to restore clotting factor synthesis (Bjornsson & Blaschke, 1978; Shearer & Barkhan, 1979). The short duration of action of vitamin K_1 during coumarin overdose has become more evident with the development of new rodenticidal coumarin anticoagulants, such as difenacoum and brodifacoum, which are more potent and persistent vitamin K_1 antagonists than warfarin (Park & Leck, 1982).

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Thus, repeated administration of vitamin K_1 (10 mg; four times daily) was required to correct prothrombin time in an individual poisoned with difenacoum (Barlow *et al.*, 1982).

At present there is no standard regime for the administration of vitamin K_1 as an antidote to coumarin poisoning. In this report we describe the plasma disposition of vitamin K_1 after oral and intravenous administration of pharmacological doses of the vitamin to healthy volunteers, and relate these findings to vitamin K_1 requirements in chronically anticoagulated rabbits.

Methods

Subjects

Male volunteers, aged 24–32 years were investigated. All subjects were in good health, shown by history, physical examination and laboratory screening. The protocol of the investigation was approved by the Mersey Regional Ethical Committee, and written informed consent obtained from all participants.

Plan of study

The plasma pharmacokinetics of *trans*-vitamin K_1 were determined in six male volunteers after a single intravenous infusion over a period of 20 min of Konakion® (10 mg) in saline (20 ml) after an overnight fast. Konakion® contains both *trans*-vitamin K_1 and *cis*-vitamin K_1 in a ratio of 88: 12 (Wilson & Park, 1983). Blood samples were obtained at 0, 0.08, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 9, 12, and 24 h after administration of vitamin K_1, collected in heparinised tubes. Plasma was obtained by centrifugation (2000 *g*) and stored frozen (−20°C) until assayed. Vitamin K_1 concentrations were determined using a normal phase high performance liquid chromatography method which has been developed for the estimation of pharmacological concentrations of *trans*-vitamin K_1 in plasma (Wilson & Park, 1983). In the same volunteers, plasma concentrations of vitamin K_1 were measured at the same time points after oral administration of 10 mg and 50 mg doses of Konakion tablets (10 mg). Tablets were chewed before swallowing, as recommended by the manufacturer (Roche Products Ltd, Welwyn Garden City, England). The order of administration of the one i.v. dose and the two oral doses was randomised.

Animal study

The pharmacological response to vitamin K_1 was determined in male New Zealand White rabbits (2.5–3.0 kg; Bantin & Kingman, Hull, U.K.) anticoagulated with brodifacoum (10 mg/kg; Sorex Ltd, Widnes, U.K.) as previously described (Park & Leck, 1982). Vitamin K_1 (10 mg/kg; Konakion®) was given intravenously (diluted to 1 ml in saline), into the marginal ear vein 24 h after i.v. administration of brodifacoum (10 mg/kg) in polyethylene glycol (0.5 ml/kg). Blood samples were obtained at 0, 1, 2, 3, 5, 7, 9, 12, 16 & 24 h for determination of plasma *trans*-vitamin K_1 concentrations (Wilson & Park, 1983) and at 0, 1, 2, 3, 4, 5, 7, 9, 12, 16 and 24 h for the determination of prothrombin complex activity (Park *et al.*, 1979). Results are presented as the mean ± s.d.

Pharmacological response to vitamin K_1 in man

The pharmacological response to vitamin K_1 in a patient poisoned with an unknown quantity of brodifacoum was investigated. Full details of this clinical case will be presented elsewhere. The plasma concentrations of vitamin K_1 2,3-epoxide were determined (Wilson & Park, 1983) after intravenous administration of vitamin K_1 as described in the volunteer study. In addition plasma samples were also obtained for the measurement of prothrombin time by a one stage procedure using Manchester comparative thromboplastin and an automated coagulometer, from which prothrombin complex activity was determined (Serlin *et al.*, 1979). A prothombin time of 65 s was recorded immediately before administration of vitamin K_1.

Pharmacokinetic analysis

A biexponential equation was fitted to the plasma concentration *vs* time data obtained after a short intravenous infusion of vitamin K_1 using a regression analysis programme (Nielsen-Kudsk, 1980), and by a weighted non linear least squares regression method (NONLIN). In each case the correlation coefficients for the two first order rate constants were greater than 0.95. After both oral and intravenous administration, 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 component. The extrapolated area constituted less than 10% of the observed area in all instances apart from the 10 mg oral dose for volunteer 6 where it constituted 14%. The plasma concentrations obtained were much lower than those observed for the other five

volunteers with the same dose. The volume of distribution at steady state (V_{ss}) was calculated by standard methods (Gibaldi & Perrier, 1975) from the coefficients and exponents of the best fit biexponential equation to the data.

Results

Vitamin K_1 disposition in man

After intravenous administration of vitamin K_1 (10 mg), plasma concentrations of *trans*-vitamin K_1, the biologically active form of the vitamin, declined biexponentially with time in healthy volunteers (Figure 1); the relevant pharmacokinetic parameters are presented in Table 1. Little (< 20 ng/ml) or no vitamin K_1 2,3-epoxide was detected in plasma. The plasma concentration-time profile for *trans*-vitamin K_1 was similar in an individual poisoned with brodifacoum, although in this individual high concentrations (Cp_{max} 580 ng/ml) of vitamin K_1 2,3-epoxide were measured (Figure 2). After oral adminstration of the vitamin (10 mg) maximum concentrations occurred about 3 to 4 h after dosing (Figure 1 and Table 2). The availability (F) showed wide variability (10–63%). After oral administration of a larger dose (50 mg) to the same volunteers, concentrations of the vitamin reached a maximum at between 3 and 5 h after dosing. The availability of the two doses was similar for volunteers 1, 2, 3 and 4, but was markedly reduced with the 50 mg dose for volunteers 5 and 6. On repeating the study, availability remained constant for volunteer 5, but increased from 4% to 51% for volunteer 6. The vitamin was administered at the same time and under the same conditions (i.e. after an overnight fast) on both occasions.

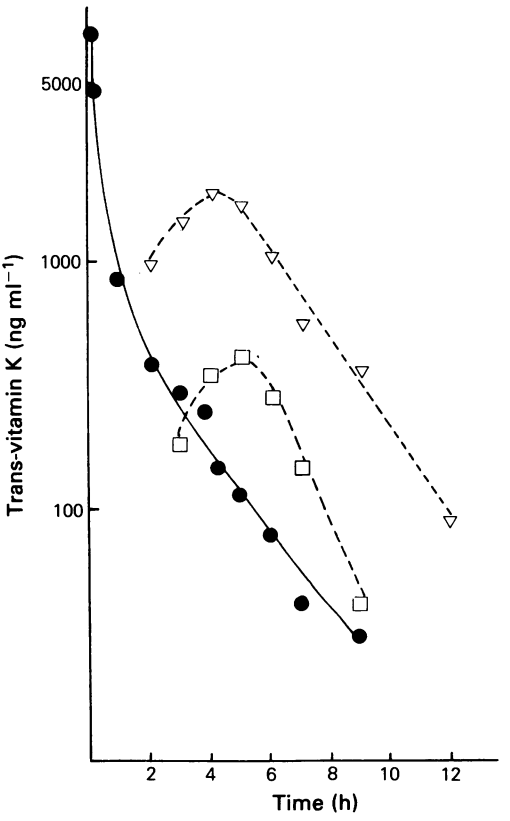

Figure 1 Plasma concentrations of trans-vitamin K_1 in a healthy volunteer after administration of vitamin K_1; 10 mg i.v. (●), 10 mg oral (□) and 50 mg oral (▽).

Relationship between plasma vitamin K_1 concentrations and duration of pharmacological response

The pharmacological response to vitamin K_1 was investigated by measuring prothrombin

Table 1 Disposition kinetics of *trans*-vitamin K_1 after intravenous administration of vitamin K_1 (10 mg) in volunteers.

Volunteer	*Plasma concentration-time profile* [a] *(C, mg l^{-1}; t = h)*	*Terminal half-life ($0.693/\lambda_z$; h)*	*V (l)*	*Area (mg l^{-1} h)*
	$C = C_1e^{-\lambda 1t} + C_2e^{-\lambda zt}$			
1	$12.0e^{-3.78t} + 1.01e^{-0.44t}$	1.7	1.9	5.15
2	$3.05e^{-2.38t} + 0.82e^{-0.50t}$	1.4	3.9	2.76
3	$5.11e^{-2.71t} + 0.64e^{-0.51t}$	1.4	2.8	3.09
4	$2.20e^{-2.24t} + 0.70e^{-0.31t}$	2.2	6.5	3.41
5	$3.90e^{-5.54t} + 0.27e^{-0.37t}$	1.9	8.9	1.39
6	$5.78e^{-2.77t} + 0.88e^{-0.50t}$	1.4	2.5	3.83

[a] *Estimated parameters following an intravenous bolus dose.*

The intercepts C_1, C_2, were calculated from the intercepts obtained at the time when the infusion was stopped, C_1^1, C_2^1, using the standard equation $C_i = C_i{}^1T(1 - e^{-\lambda 1T})$ where T is the duration of the infusion.

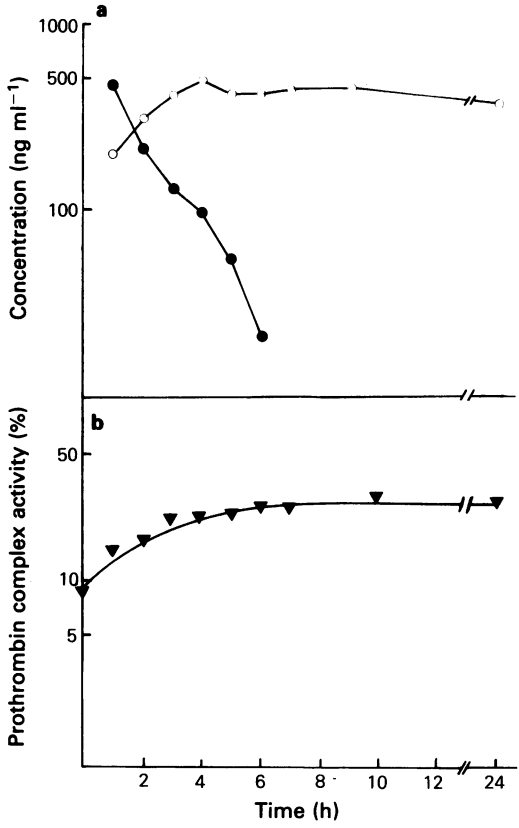

Figure 2 (a) Plasma concentration of *trans*-vitamin K_1 (●) and vitamin K_1 epoxide (○) after i.v. administration of vitamin K_1 (10 mg) to a patient poisoned with brodifacoum and (b) prothrombin complex activity (▼) in the same individual.

complex activity in rabbits anticoagulated (P.C.A. < 20%) with a dose of brodifacoum which produces maximum antagonism of vitamin K_1 for at least 1 week (Park & Leck, 1982). Brodifacoum is a useful research tool for this purpose; chronic administration of warfarin 63 mg/kg was required to produce comparable antagonism (Park, unpublished data). After intravenous administration of vitamin K_1, prothrombin complex activity (P.C.A.) rose from 14 ± 7% to 105 ± 30% at 9 h after which P.C.A. declined monoexponentially with a half-life of 6.0 ± 0.3 h (Figure 3b), which indicates complete inhibition of clotting factor synthesis (Park *et al.*, 1979). Plasma concentrations of the vitamin declined bi-exponentially, after intravenous administration (Figure 3a). The minimum effective concentration of the vitamin was determined to be 1.00 ± 0.38 μg/ml at 9 h, after which P.C.A. declined at a rate

Table 2 Disposition kinetics of *trans*-vitamin K_1 after oral administration of vitamin K_1 (10 mg or 50 mg) in volunteers.

	10 mg dose			*50 mg dose*			*Relative availability*
Volunteer	*Area (mg l⁻¹ h)*	F (%)	$C_p max$ *(ng ml⁻¹)*	*Area (mg l⁻¹ h)*	F (%)	$C_p max$ *(ngml⁻¹)*	F *(50 mg)/F (10 mg)*
1	1.77	34	407	9.45	36	1795	1.06
2	1.57	57	328	6.69	48	917	0.85
3	0.85	27	201	4.04	26	786	0.96
4	1.53	45	337	5.96	35	589	0.78
5[a]	0.90	65	255	1.05	15	204	0.24
				0.69	10	185	0.15
6[a]	0.39	10	115	0.79	4	144	0.40
				9.80	51	2693	5.67

[a]The plasma disposition of vitamin K_1 after oral administration of a 50 mg dose was determined on two separate occasions. The availability (F) was determined from areas obtained after oral and intravenous administration of the vitamin.

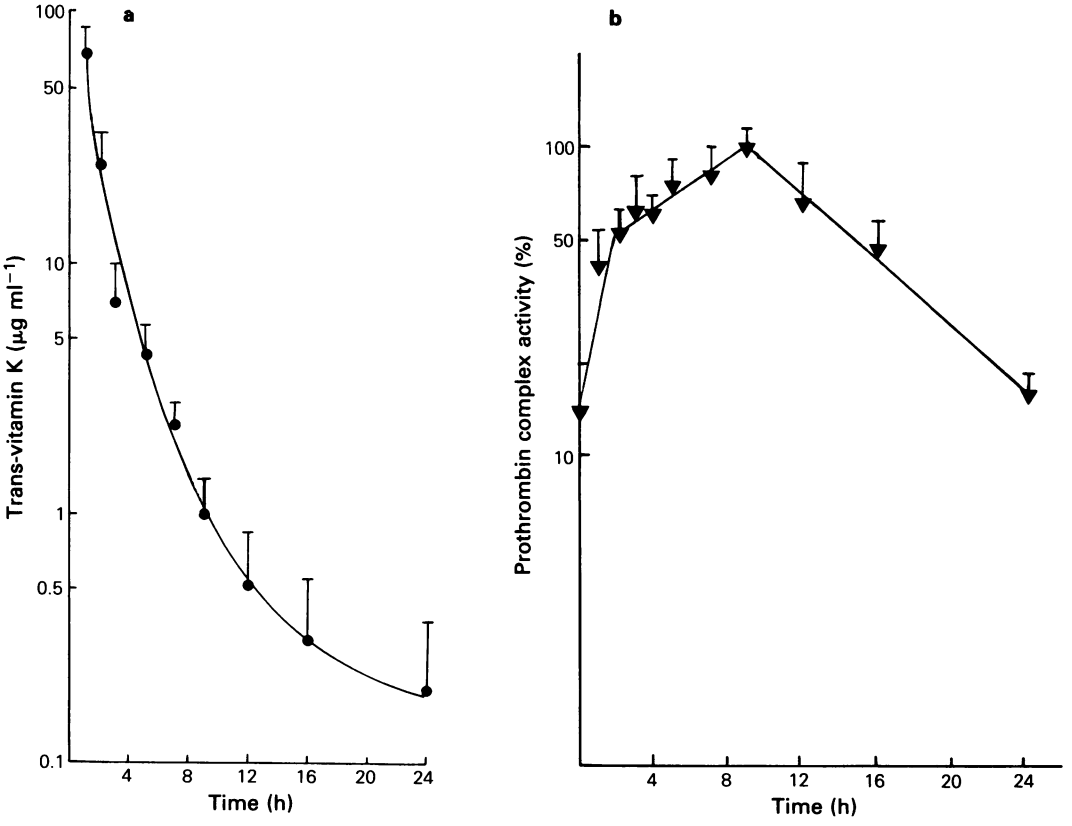

Figure 3 The relationship between (a) plasma concentrations of trans-vitamin K_1 (●) and (b) prothrombin complex activity (▼), after intravenous administration of vitamin K_1 (10 mg/kg) to male New Zealand White rabbits anticoagulated with brodifacoum (10 mg/kg).

corresponding to complete inhibition of clotting factor synthesis. Prothrombin complex activity reflects the synthesis of clotting factors II, VII and X during brodifacoum anticoagulation (Leck & Park, 1981).

The relationship between the pharmacological response to vitamin K_1 and plasma concentrations of the vitamin, in an individual poisoned with brodifacoum, is shown in Figure 2. After a sharp initial rise P.C.A. appeared to plateau. This pattern of response suggests incomplete antagonism of vitamin K_1 (Park & Leck, 1982).

Discussion

Vitamin K_1 is the antidote most frequently used in the treatment of coumarin overdosage, therapeutic or deliberate (O'Reilly & Aggeler, 1976). Current therapeutic advice is that intravenous administation of the vitamin is used initially, but an oral formulation may be used later (British National Formulary). Administration of the vitamin is continued *ad hoc* until the prothrombin time returns to normal. There is no information available which relates dose and route of administration to pharmacological effect. Furthermore, plasma concentration–pharmacological effect relationships for the vitamin have not been determined. It is only by obtaining such information that a rational regime for treating coumarin anticoagulant overdosage can be established. In this paper, we focus attention on the short duration of action of vitamin K_1 when used as an antidote for coumarin anticoagulant poisoning.

To investigate the relationship between plasma concentrations of vitamin K_1 and duration of pharmacological response, we measured prothombin complex activity (P.C.A.) in rabbits anticoagulated with a dose of brodifacoum which produces maximum antagonism of vitamin K_1 (Leck & Park, 1982). Chronic administration of warfarin, in doses 10-fold greater on a molar basis is required to produce comparable antagonism. Therefore this animal

model may be used to assess *maximum* vitamin K_1 requirements for coumarin poisoning. From Figure 3 it can be seen that plasma concentrations in excess of 1.0 μg/ml are required to drive clotting factor synthesis in brodifacoum anticoagulated rabbits. Such concentrations were maintained for only 9 h after i.v. administration of vitamin K_1 (10 mg/kg) after which P.C.A. declined at a rate corresponding to complete inhibition of clotting factor synthesis.

The high plasma concentrations of vitamin K_1 required for clotting factor synthesis in chronic coumarin poisoning contrast with normal hepatic concentrations of the vitamin (44 ng/g in the rat; Haroon & Hauschka 1983) and the estimated body pool of about 100 μg/kg (Bjornsson *et al.*, 1980; Duello & Matschiner, 1972). However, the small pool of vitamin K_1 associated with clotting factor synthesis is normally conserved in the vitamin K_1-epoxide cycle and may contain high molecular weight forms of the vitamin (menaquinones −7, −9 and −10) which are more active on a molar basis than vitamin K_1 (Wiss & Gloor, 1966; Matschiner & Taggart, 1968; O'Reilly, 1976). Thus in the absence of a functional epoxide reductase (i.e. during chronic brodifacoum anticoagulation), recycling of vitamin K_1 does not occur and therefore vitamin K_1 requirements are much greater than normal.

Vitamin K_1 concentrations decline bi-exponentially after intravenous administration of a radiotracer dose in man (Shearer *et al.*, 1972; Bjornsson *et al.*, 1979) and the rabbit (Park *et al.*, 1980) with a terminal half-life in the range 1–4 h. The pharmacokinetic parameters obtained in the present work, with a pharmacological dose (10 mg), are similar to those reported previously with more physiological doses (e.g. 0.3 mg; Bjornsson *et al.*, 1979). Thus there is no evidence for pronounced dose-dependency in the pharmacokinetics of the vitamin (Bechtold *et al.*, 1983). Coumarin anticoagulants do not alter the plasma disposition of vitamin K_1 in either man (Shearer *et al.*, 1977) or the rabbit (Park *et al.*, 1980). Therefore the pharmacokinetic parameters for vitamin K_1 obtained for healthy volunteers will be the same as those obtained from individuals poisoned with coumarin anticoagulants.

The availability of vitamin K_1 was investigated after 10 and 50 mg doses in healthy volunteers. The availability of the lower dose ranged from 10 to 63%, while with the higher dose we encountered both marked inter- and intra-individual variation in the availability of the vitamin. Shearer *et al.* (1974) estimated the maximum absorption of a tracer dose of the vitamin to be 80%. Vitamin K_1 is adequately absorbed from the gastrointestinal tract only if bile salts are present (Shearer *et al.*, 1974). The vitamin is absorbed by an energy-dependent saturable process in the proximal portions of the small intestine (Hollander, 1973) and is then transported via the thoracic lymph duct. Thus, a number of factors may contribute to inter- and intrasubject variation in the absorption of vitamin K_1. In addition, the rate of dissolution of the vitamin K_1 tablets may be variable.

From a toxicological point of view we require knowledge of vitamin K_1 requirements in terms of route of administration and dose after coumarin poisoning. Our animal experiments indicate that, in the *limiting* situation of *maximum* vitamin K_1 antagonism, plasma concentrations of the order of 1.0 μg/ml are required to drive clotting factor synthesis. This requirement for such high plasma concentrations, taken together with the steep decline in plasma vitamin K_1 concentrations after intravenous administration of a pharmacological dose, provides an explanation for the short duration of action of vitamin K_1 in such circumstances. The minimum effective plasma concentration of vitamin K_1 will obviously be dependent upon the degree of coumarin poisoning and may vary between species. It is stressed that the animal model used here represents the extreme situation of maximum vitamin K_1 antagonism. Plasma concentration-duration of effect relationships for vitamin K_1 cannot be obtained from volunteer studies. Such information can only be gleaned from individual cases of coumarin poisoning.

The effectiveness of vitamin K_1 in man is illustrated by the case of an individual poisoned with an unknown quantity of brodifacoum. After intravenous administration of vitamin K_1 (10 mg) P.C.A. rose from 8% to 22% within four hours, but then remained below 30% activity until further vitamin K_1 administration. This pattern of response suggests incomplete antagonism of vitamin K_1 (Park & Leck, 1982). The high plasma-concentrations of vitamin K_1 2,3-epoxide observed in this individual are in accord with the proposed mechanism of action of brodifacoum (Park *et al.*, 1979).

In man high (> 0.5 μg/ml) plasma concentrations of vitamin K_1 are maintained for short periods after intravenous administration of a single (10 mg) intravenous dose, but were not achieved with an equivalent oral dose. Increasing the oral dose to 50 mg produced a wide, 10–fold variation in maximum vitamin K_1 plasma concentrations which occurred 2–5 h after dosing. Thus although rapid intravenous administration of vitamin K_1 may be associated with

side-effects (facial flushing, chest constriction, cyanosis), it is essential if immediate high concentrations of the vitamin are required to drive clotting factor synthesis.

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