Pharmacokinetics and efficacy of oral versus intravenous mixed-micellar phylloquinone (vitamin K$_1$) in severe acute liver diseasefailure

Running head: Vitamin K$_1$ in acute liver failure

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Abbreviations used in this paper:

ALF, acute liver failure

AST, aspartate aminotransferase

HPLC, high-performance liquid chromatography

INR, international normalised ratio

MM K_1, mixed-micellar vitamin K_1

PIVKA, *P*roteins *I*nduced by *V*itamin *K* *A*bsence or *A*ntagonism

PIVKA-II, undercarboxylated prothrombin (factor II)

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ABSTRACT

Background & Aims: In patients with severe acute liver dysfunction, i.v. phylloquinone (vitamin K_1) is ~~often~~may be given to exclude vitamin K deficiency, rather than impaired hepatic synthesis of coagulation factors alone, as the cause of the coagulopathy. However, there have been no studies of the pharmacokinetics or efficacy of i.v. or oral K_1 in such patients. *Methods*: 49 adults with severe acute liver disease were randomised double-blind to a single 10 mg dose of i.v. or oral mixed-micellar K_1, or placebo. Serum levels of phylloquinone and undercarboxylated prothrombin (PIVKA-II) were assessed before and after treatment. *Results*: At admission, 13 patients (27%) had either low serum K_1 levels or elevated PIVKA-II concentrations, indicative of subclinical vitamin K deficiency. In the 16 patients who received i.v. K_1, there was one (6%) treatment failure (K_1 rise < 10 ng/ml above baseline), compared with 12 of the 15 (80%) who received oral K_1 ($p<0.0001$). One patient in the placebo group developed overt vitamin K deficiency. *Conclusions*: A minority of patients with severe acute liver dysfunction have subclinical vitamin K deficiency at the time of presentation, which is corrected by a single dose of i.v. K_1. The intestinal absorption of mixed-micellar K_1 is unreliable in adults with severe acute liver dysfunction.

INTRODUCTION

Vitamin K is an essential cofactor for the production in the liver of biologically active forms of the coagulation factors II (prothrombin), VII, IX, and X. Its role is to promote the conversion of certain glutamic acid residues in the protein precursors to γ-carboxyglutamic acid (Gla). The Gla-residues of these coagulation factors provide

an efficient chelating site for calcium ions and are essential for protein-membrane interaction and effective haemostatic function [11]. When γ-carboxylation is impaired because of deficiency or antagonism of vitamin K, inert precursors of vitamin K-dependent proteins — known as *P*roteins *I*nduced by *V*itamin *K A*bsence or *A*ntagonism (PIVKA) — are synthesised in the liver and released into the blood [2,32,3]. In the case of prothrombin, specific and sensitive immunoaassays have been developed which can detect small decreases in the Gla content of prothrombin before any changes occur in conventional coagulation tests such as the International Normalised Ratio (INR) [3-63-6].

The major dietary form of vitamin K is phylloquinone (vitamin K_1; K_1), which is derived predominantly from green leafy vegetables. Its intestinal absorption involves the intraluminal solubilisation of vitamin K into mixed micelles composed of bile salts and the products of pancreatic lipolysis. This absorption is known to be impaired in patients with extrahepatic biliary obstruction and severe pancreatic insufficiency [77], but there are no data in patients with liver disease. Although impaired hepatic synthesis is almost certainly the dominant factor in the development of the usually severe coagulopathy seen in patients with acute liver failure (ALF), such patients may also have multiple risk factors for vitamin K deficiency, including poor oral intake, severe cholestasis and treatment with broad spectrum antibiotics — which inhibit production of menaquinones (vitamin K_2) by certain intestinal bacteria and their subsequent ileal absorption [88]. Consequently, it has been recommended that intravenous (i.v.) K_1 be given empirically to exclude a contribution of vitamin K deficiency to the coagulopathy [99].

A commonly prescribed formulation of K_1 is Konakion® MM (F. Hoffmann-La Roche Ltd, Basle, Switzerland), which contains phylloquinone solubilised in glycocholate-phosphatidylcholine mixed micelles. We have shown that the mixed-micellar K_1 (MM K_1) formulation is associated with a lower risk of anaphylactoid reactions after i.v. administration than the original Konakion, which contained the nonionic detergent Cremophor EL® as solubiliser [10], but that [10]the intestinal absorption of MM K_1 in infants with conjugated hyperbilirubinaemia, given as prophylaxis against vitamin K deficiency bleeding of the newborn, is unreliable [11][12]. There is no information on the efficiency of intestinal absorption of MM K_1 in adults with liver disease.

The aims of this randomised, double-blind, placebo-controlled study were to: (i) assess the possible contribution of vitamin K deficiency to the coagulation disorder found in patients with severe acute liver damage, and (ii) compare the pharmacokinetics and efficacy of oral versus i.v. MM K_1 in correcting any underlying vitamin K deficiency.

PATIENTS AND METHODS

Over an eight-month period, 49 patients (24 women, 25 men; mean age 34 years, range 16-73 years) with, or at high risk of developing, ALF [12][13], were transferred to the Liver Failure Unit of King's College Hospital and enrolled into the study by one of two investigators (SPP and DR). As part of the eligibility criteria, all of the patients had an INR > 2 at admission, and none had received K_1 within the preceding three days. The commonest cause of acute liver dysfunction was acetaminophen paracetamol hepatotoxicity (n=37; 76%), followed by other drugs (ecstasy in four,

paroxetine in one) in 10%. The final diagnoses in the remainder were presumed nonA-E viral hepatitishepatitis of unknown origin in two, malignant hepatic liver infiltration in two, and one case each of acute fatty liver of pregnancy and *Amanita phalloides* poisoning.

At admission, 27 patients (55%) had already developed ALF and were encephalopathic and/or mechanically ventilated, and eight (16%) had a marked metabolic acidosis (arterial pH <7.3). All patients were managed according to a standard protocol for acute liver failure [13][14]. Continuous veno-venous haemodiafiltration was instituted in anuric or oliguric patients with a serum creatinine level of > 400 μmol/l. Intravenous N-acetylcysteine and broad-spectrum antibiotics were administered routinely. Patients who developed grade III-IV encephalopathy were intubated and ventilated electively, and a nasogastric tube was inserted for enteral nutrition and drug administration.

The study medications (MM K_1 and placebo in identical-appearing ampoules) were paired and coded by the trial pharmacist, and stored at 4°C. The trial pharmacist also provided the random allocation sequence, in opaque consecutively numbered envelopes. On the day of admission, patients were randomised double-blind to: (i) placebo by mouth or nasogastric tube (made up to 10 ml in water) and 10 mg MM K_1 i.v. (given over 1 min), (ii) 10 mg MM K_1 by mouth/nasogastric tube and placebo i.v., or (iii) placebo/placebo. The study medications were administered by the patient's nurse at 12:00 on the day of admission. The clinical status of the patients, and the results of standard biochemical and haematological tests, were assessed daily.

Assessment of vitamin K status

Vitamin K status was assessed from serum measurements of K_1 and by a sensitive functional assay based on the detection of undercarboxylated species of prothrombin (PIVKA-II).

Blood samples were collected before administration of the study medications (baseline sample) and on the three consecutive mornings after admission, with further samples on days five and seven unless the patient had died or been discharged. At each sampling time, 10 ml of venous blood was collected into plain tubes, protected from light and allowed to clot at 4°C. Serum was separated by centrifugation and stored at -70°C.

Serum vitamin K_1 assay: Serum K_1 was measured by high performance liquid chromatography (HPLC) with electrochemical detection in the redox mode, as described previously [14,15]. Volumes of 0.05 ml serum were first analysed by a protocol designed to measure K_1 concentrations in the pharmacological range (>5 ng/ml) [14,15]. If the level was <10 ng/ml by this protocol, volumes of 0.2-0.5 ml serum were subjected to a protocol for endogenous concentrations [14,15]. Menaquinone-6 was used as the internal standard. For 0.5 ml serum extracted, the lower limit of detection was 0.05 ng/ml. The mean intra- and inter-assay precision values (RSD %) were 3% and 9%, respectively, for concentrations in the pharmacological range, and 5% and 10% for endogenous concentrations. The normal adult reference range by the same assay was 0.17-0.68 ng/ml (median 0.37 ng/ml) in the fasting state, and 0.15-1.55 ng/ml (median 0.53 ng/ml) in non-fasting subjects [15,16].

Serum PIVKA-II assay: Serum PIVKA-II was assayed using a conformation-specific monoclonal antibody (which does not cross-react with fully carboxylated native prothrombin) in a modification of a previously described ELISA-based assay [55]. Serum PIVKA-II by immunoassay is conventionally expressed as Arbitrary Units per ml (AU/ml) since, in states of vitamin K deficiency, circulating PIVKA-II may comprise multiple forms of partially carboxylated prothrombin and neither their relative abundance in plasma nor their relative affinity for the antibody is known. Calibration is therefore made against PIVKA-II species purified from patients treated with oral anticoagulants. Using electrophoretic techniques, 1 AU is equivalent to 1 μg of purified PIVKA-II [55]. The minimum limit of detection for our assay was 0.15 AU/ml. In 43 adult patients on warfarin therapy (INR > 1.5), the mean value in our laboratory was 40.0 AU/ml (range 6.9-99.5 AU/ml) [1617]. As previously reported [1718], there was good agreement between the specificity of this assay and a commercial kit (Eitest mono-II; Eisai Co Ltd, Tokyo) which has been used widely for the detection of subclinical vitamin K deficiency [18-2019-21].

Statistical analysis

Data are expressed as means ± SEM. Group means were compared using the the Student's *t* test (two-tailed), and differences in proportions were assessed using the χ^2 test. Pearson's correlation coefficients were calculated to assess the strength of relations between the serum K_1 terminal half-lives (determined by log-linear regression analysis of the 24 h and 48 h data) and the severity of liver and renal dysfunction.

Written, informed consent was obtained from the patients or their next-of-kin. The

study was approved by the Research Ethics Committee of King's College Hospital.

RESULTS

The clinical details and laboratory values at admission of the three study groups are given in Table 1. The three groups were well-matched for sex, aetiology and severity of liver dysfunction, although patients in the oral group were somewhat younger than those in the other two groups (27 *v* 37 yr; p=0.03). The mean INR at admission was 6.4 (range 2.0 to 15), which fell improved progressively thereafter to 4.2 at 48 h and 1.6 at 96 h. The corresponding mean admission values for serum creatinine and bilirubin were 202 (49-700) mmol/l and 154 (6-916) mmol/l, respectively, which rose to peak values of 311 (45-752) mmol/l and 243 (16-886) mmol/l on day five of admission. A total of 32 patients (65%) developed ALF, either before their transfer to King's (n=27) or during their subsequent admission (n=5).

Twenty-seven of the 49 patients (55%) made a full recovery with intensive care support, and eight others (16%) who fulfilled the King's criteria for transplantation in ALF [1213] were transplanted on day 3-30 (median six) and later discharged. Fourteen other patients (29%) died 3-10 days (median six) after admission, generally due to chest sepsis with multiorgan failure or cerebral oedema. Ten patients in this group fulfilled transplant criteria, but either had medical or psychiatric contraindications to transplantation (n=7) or died before transplantation could be carried out (n=3).

Table 1:

Clinical data and laboratory values at admission in the three study groups.

Results are expressed as means with ranges.

		Vitamin K$_1$		Placebo
		Oral	Intravenous	
	No.	15	16	18
	Sex	8M, 7F	7M, 9F	10M, 8F
	Age (range)	27 (16-49)*†	37 (18-73)*	38 (19-73)†
	Cause of ALF: AcetaminophenParacetamol Others	13 2		11 5 13 5
	Serum bilirubin (normal, 3-20 μmol/l)	186 (21-916)	153 (6-483)	131 (55-483)
	Aspartate aminotransferase (10-50 IU/l)	5839 (348-22000)	3199 (25-9369)	4941 (29-26500)
	Alkaline phosphatase (30-150 IU/l)	122 (58-277)	208 (40-1665)	108 (57-196)
	Gamma-glutamyl transferase (5-55 IU/l)	78 (31-190)	167 (18-1249)	78 (23-174)
	International Normalised Ratio (0.7-1.2)	6.2 (2.8-12.1)	6.0 (2.0-15)	6.9 (2.0-15)

*p=0.03 oral v i.v. group. †p=0.03 oral v placebo group. There were no significant differences between the three groups with respect to the other variables.

Serum vitamin K$_1$

Mean baseline serum concentrations of K$_1$ were similar in the three groups, being 1.6 ±0.4 ng/ml (range, 0.2-6.5 ng/ml) in the i.v. MM K$_1$ group, 2.4±0.8 ng/ml (0.1-10.0 ng/ml) in those given MM K$_1$ orally, and 2.2±0.08 ng/ml (0.03-6.5 ng/ml) in the placebo group (NS). Three of the 49 patients (6%) had pretreatment serum K$_1$ levels below the normal limit of 0.15 ng/ml (one in the oral group and two in the placebo group), and a further two patients (both in the placebo group) developed low levels within 48 h after admission despite clinical improvement in both patients. In those given placebo, there was a progressive fall in mean serum K$_1$ concentrations with

time, from 2.2 ± 0.08 ng/ml (0.03-6.5 ng/ml) at admission, to 1.8 ng/ml (0.1-4.5 ng/ml) at 48 h, 1.0 ng/ml (0.1-2.7 ng/ml) at five days, and to 0.7 ng/ml (0.5-1.3 ng/ml) on day seven.

Individual and mean serum concentrations of K_1 in the two supplemented groups, expressed on a logarithmic scale, are shown in Fig. 1. At 24 h after i.v. MM K_1, the peak serum K_1 concentrations was 91.5 ± 39.2 ng/ml (range 7.2-650 ng/ml), compared with 17.2 ± 8.4 ng/ml (range 0.3-91.8 ng/ml) after oral MM K_1 ($p=0.08$ i.v. v oral). When treatment failure was defined as an incremental K_1 rise < 10 ng/ml, one instance was recorded in the 16 patients (6%) given i.v. MM K_1, compared with 12 treatment failures in the 15 (80%) who received oral MM K_1 ($p<0.0001$).
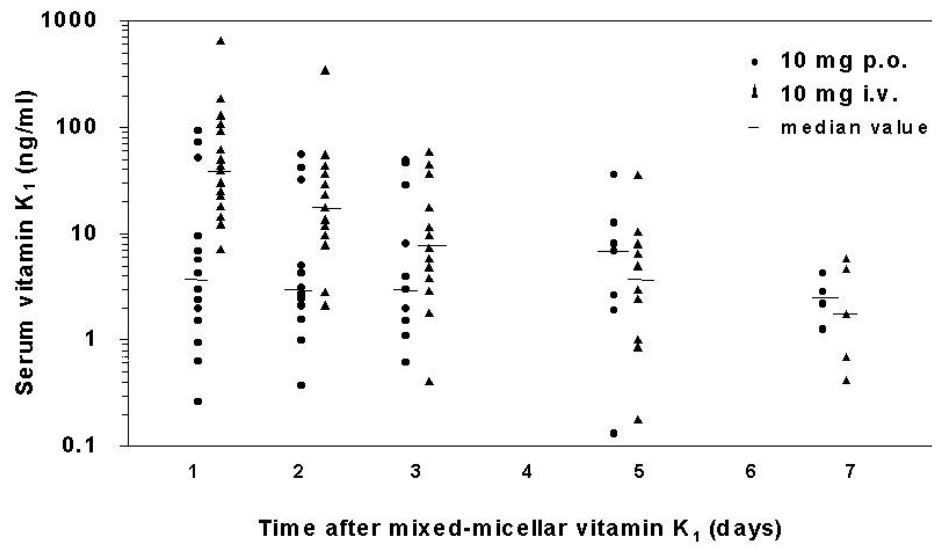
Figure 1   Serum concentrations of vitamin K_1 after oral or i.v. mixed-micellar vitamin K_1.

Serum PIVKA-II

At admission, nine of the 49 patients (18%) had mildly raised PIVKA-II concentrations of 0.15-0.91 AU/ml, consistent with subclinical vitamin K deficiency. These elevated levels fell by at least 50% within 48 h in two of the four patients given oral MM K_1, in both of the patients given i.v. MM K_1, and in none of the three in the placebo group (Fig. 2). In the two patients from the oral group whose serum PIVKA-II levels rose further after vitamin K, the maximum incremental K_1 rise of 2.3 ng/ml indicated poor intestinal absorption in both.

Two other patients had markedly raised PIVKA-II levels (> 10 AU/ml and 8 AU/ml), which were in the same range as that found in patients with overt vitamin K deficiency or during oral anticoagulation therapy. One of these patients was given i.v. K_1, and the PIVKA-II level fell from > 10 AU/ml to 3.3 AU/ml after 48 h (and to 0.50 AU/ml on day 7), with a corresponding fall improvement in the INR from 2.2 to 1.0. The other patient was in the placebo group, and the PIVKA-II level rose from 8 AU/ml to > 10 AU/ml at all subsequent time points between 24 h and 7 days. Following an initial fall improvement in the INR from 2.6 at admission to 1.3 at 48 h, the INR again increased to 2.6 on day 7 despite improving liver function tests, and subsequently returned to normal 48 h after the patient was removed from the trial and given i.v. MM K_1.
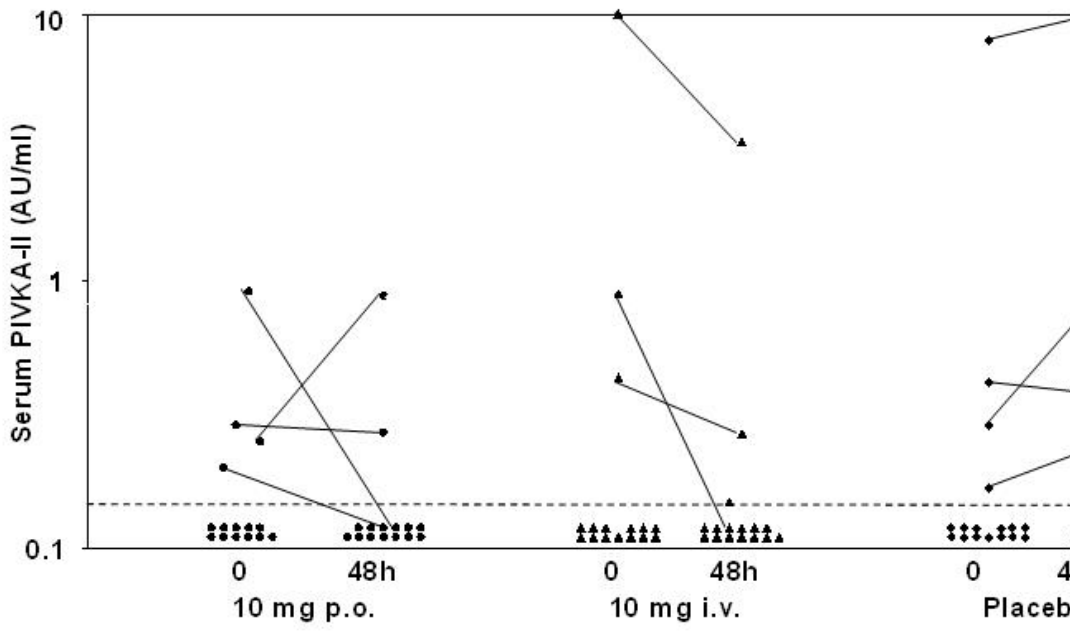
Figure 2 Serum concentrations of undercarboxylated prothrombin (PIVKA-II;

logarithmic scale) before and 48 h after mixed-micellar vitamin K_1 or placebo. The dashed horizontal line at 0.15 AU/ml represents the upper limit of normal.

Vitamin K_1 pharmacokinetics

In the 15 patients (12 in the i.v. group) with peak incremental serum K_1 levels > 10 ng/ml in whom half-lives could be determined, the mean serum terminal half-life of MM K_1 was 25.3±3.7 h (range, 6.6-55.9 h). Seventeen of the patients (35%) required renal support during their admission, with continuous veno-venous haemodiafiltration in 15 and haemodialysis in two. Amongst the patients requiring renal support, the mean serum K_1 half-life was 26.3±6.7 h, compared with a half-life of 24.7±4.6 h in those with preserved renal function (NS). There were no significant correlations between serum K_1 half-life and admission or peak serum creatinine concentrations, severity of liver disease (as assessed by the INR and serum bilirubin) or need for ventilation.

DISCUSSION

In severe acute liver disease, there are reductions in the plasma concentrations of several coagulation proteins, including the procoagulant vitamin K-dependent factors II, VII, IX and X and the anticoagulant protein C [9,219,22]. The hepatic synthesis of these vitamin K-dependent factors differs from other coagulation factors in that their functional activity is also dependent on the integrity of the microsomal γ-glutamyl carboxylation system, which converts the inactive protein precursors to their active

zymogens, with a Thus,reduced ratio of functional to antigenic levels of prothrombin being a hallmark of an impaired carboxylation resulting in the hepatic secretion of non-functional PIVKA-II [22,2324]. In ALF, it has been commonly assumed that the reduced levels of prothrombin (and other vitamin K-dependent proteins) are due to impaired hepatic protein synthesis [2223]. However, Kerr *et al* [2425] showed recently that the factors that fall to the lowest levels in ALF are those which are directly activated after tissue factor generation — pointing to a tissue factor-initiated consumption of factors II, V, VII and X (but not factor IX) as a major cause of their depletion in ALF.

The present study has shown that over a quarter of patients with severe acute liver dysfunction have subclinical vitamin K deficiency at presentation which, if untreated, may progress to overt vitamin K deficiency with a raised INR and bleeding tendency. The PIVKA-II analyses showed that 11/49 patients (22%) presented with a functional defect of prothrombin γ-carboxylation. In most, PIVKA-II was mildly raised (0.15-1.0 AU/ml), consistent with subclinical vitamin K deficiency, but in two patients the PIVKA-II was in the range found in warfarin-anticoagulated patients (>7.0 AU/ml), indicating that a component of their raised INR was due to overt vitamin K deficiency. A smaller proportion of 3/49 patients (6%) had a low pre-treatment serum K_1 (<0.15 ng/ml), suggestive of a low tissue vitamin K status. As in previous reports [20,2521,30], we did not detect an association between baseline serum K_1 and PIVKA-II levels. Serum K_1 concentrations at admission varied widely, presumably related in part to variations in dietary intake [2635] and inter-individual differences between circulating vitamin K and liver stores (which may include bacterial menaquinones; K_2 vitamins). Another possible reason for the lack of association between serum K_1 and PIVKA-II is that some patients may have received vitamin K more than three days before transfer to

King's College Hospital. At admission, 10 (20%) had K_1 levels above the upper limit of the non-fasting range (1.6 ng/ml). PIVKA-II has a long terminal half-life and may remain detectable in plasma for several days after correction of vitamin K deficiency.

To the authors' knowledge, this is the first detailed study of vitamin K status in severe acute liver dysfunction and the first placebo-controlled study of vitamin K prophylaxis using either the intravenous or oral route. Under normal physiological conditions, 40-70% of an oral dose of K_1 is absorbed from the jejunum and ileum of the upper intestine and as for other fat-soluble vitamins and highly lipid-soluble nutrients, the intraluminal phase of absorption involves the solubilisation of K_1 into mixed micelles [77]. In the present study, we found that only 20% of patients given the oral MM formulation developed an incremental serum K_1 rise ≥ 10 ng/ml at 24 h, compared with all but one (94%) of those given i.v. K_1.

In the patients in whom serum half-lives could be estimated, the mean K_1 terminal half-life of 25.3 ± 3.7 h was about approximaely twofold longer than that found in subjects with normal hepatic function given pharmacological doses of the same MM preparation [2738]. This finding of a prolonged terminal half-life in patients with severe acute liver dysfunction is not unexpected, since tracer experiments with labelled phylloquinone in normal individuals have shown that a sizable fraction of a single dose is rapidly catabolised in the liver, with at least 40-50% excreted in the faeces via the bile and 20% excreted in the urine within three days [77].

In summary, over a quarter of our patients presenting with severe acute liver damage had evidence of subclinical K_1 deficiency at admission, which was corrected by a

single dose of i.v. MM K_1. Intestinal absorption of MM K_1 in these patients was unreliable, so that there is no rationale to change our current practice of recommending i.v. K_1 to patients with severe liver disease and an associated coagulopathy. Based on the findings of a markedly prolonged serum K_1 half-life in these patients, a single dose of 10 mg K_1 given i.v. appears to be sufficient to maintain supraphysiological vitamin K blood levels for at least a week.

Acknowledgements

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