Bioavailability of phylloquinone from an intravenous lipid emulsion1–4

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ABSTRACT This randomized, controlled study evaluated the bioavailability of phylloquinone from an intravenous lipid emulsion. A mild vitamin K deficiency was induced in 12 healthy adult men and women by dietary restriction of phylloquinone (40 μg/d, days 1–11) and by administration of warfarin (1.0 mg/d, days 5–11). On day 11, subjects received a 500-mL intravenous solution of either lipid or saline, both of which contained 154 μg phylloquinone. Bioavailability was assessed by serial measurements of plasma phylloquinone, vitamin K₁,2,3-epoxide, PIVKA-II (proteins induced by vitamin K absence or antagonists–II), and percentage undercarboxylated osteocalcin. As a result of vitamin K deficiency and minidose warfarin, vitamin K₁,2,3-epoxide, PIVKA-II, and percentage undercarboxylated osteocalcin increased significantly between days 1 and 11 (P = 0.05, 0.016, and 0.001, respectively). With the infusions, plasma phylloquinone increased in both groups (P = 0.001). After the infusions vitamin K₁,2,3-epoxide decreased in both groups (P = 0.002). Changes in plasma phylloquinone and vitamin K₁,2,3-epoxide were no different in the two groups (mean areas under the curves ± SEM; 116 ± 13 nmol·h/L for the saline group and 102 ± 20 nmol·h/L for the lipid group for phylloquinone; 38.6 ± 7.5 nmol·h/L for the saline group and 31.3 ± 9.0 nmol·h/L for the lipid group for vitamin K₁,2,3-epoxide). PIVKA-II decreased significantly from baseline values (P = 0.005) in both groups after the infusions. Intraavenous lipid reversed the effects of minidose warfarin and of dietary restriction of phylloquinone on hemostasis and vitamin K nutritional status. This reversal was no different from that seen with the infusion of phylloquinone in a saline solution. Am J Clin Nutr 1998;67:716–21.

KEY WORDS Phylloquinone, bioavailability, lipid emulsions, vitamin K, osteocalcin, parenteral nutrition, adults, warfarin

INTRODUCTION

Commercial intravenous lipid emulsions contain large quantities of phylloquinone (1). Such emulsions are widely used in parenteral nutrition, and they can contain as much as twice the daily recommended dietary allowance of phylloquinone in 500 mL of a 10% emulsion, or ~150 μg (2). However, it has not yet been established whether this source of phylloquinone is used in host metabolism and utilized as a cofactor in the carboxylation of vitamin K-dependent proteins. Two observations have lent indirect evidence that it is. A recent report of warfarin resistance with the concurrent infusion of a lipid emulsion implied that the phylloquinone in these preparations is biologically active, or bioavailable (3). A second, earlier study observed stable concentrations of PIVKA-II (proteins induced by vitamin K absence or antagonists–II) in children who received intravenous lipid emulsions. However, with no restriction of other sources of vitamin K and no control group, this study was also inconclusive (4). Thus, definitive evidence of bioavailability is still lacking.

It is important to determine whether the phylloquinone from intravenous lipids is bioavailable. Although low doses of warfarin are widely used to prevent thrombosis (5–7), it is unclear whether this intervention is effective in patients who receive intravenous lipids. If the phylloquinone in lipid emulsions is in fact bioavailable, the prophylactic effects of warfarin might be inhibited by the infusion of a lipid emulsion. Furthermore, in addition to being a cofactor for the posttranslational synthesis of γ-carboxyglutamic acid (Gla) in several clotting factors, vitamin K mediates the carboxylation of many other proteins found in a variety of tissues. These proteins include osteocalcin, matrix Gla protein, a protein encoded by a growth arrest–specific gene (GAS6), a vascular smooth muscle cell–derived Gla containing growth potentiating factor (VSMC GPF), and nephrocalcin (8, 9). Because triacylglycerol-rich particles in intravenous lipids are known to act as endogenous chylomicrons in many respects (10), they might thereby deliver vitamin K to a variety of extrahepatic target tissues. Finally, patients who receive long-term total parenteral nutrition with lipid emulsions routinely receive supplemental intravenous phylloquinone in their formulas (11).

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SUBJECTS AND METHODS

Subjects

Twelve participants were selected from a group of healthy volunteers. Each of the two study groups (lipid and saline) consisted of six volunteers (four men and two women) with similar mean ages (lipid: 25.7 ± 3.6 y; saline: 26.7 ± 7.6 y) and body mass indexes (in kg/m²) (lipid: 25.3 ± 2.7; saline: 23.8 ± 2.3). Each subject’s weight remained stable throughout the study. All subjects were in good health before the study as determined by a medical history, physical examination, and routine laboratory examination of serum electrolytes, blood urea nitrogen, creatinine, alanineaminotransferase, aspartateaminotransferase, triacylglycerol, and cholesterol.

Prospective subjects were excluded from participation if they had any of the following: 1) any serious or chronic medical illness, such as a bleeding disorder, hyperlipidemia, hypertension, a collagen vascular disease, recent infection, or cancer; 2) ongoing treatment with a drug that might affect their response to warfarin or preclude the safe infusion of a lipid product; 3) a history of nonsteroidal antiinflammatory use over the preceding month; 4) use of oral anticoagulants over the preceding year; 5) surgery of any type over the preceding year; 6) pregnancy; or 7) a history of smoking or heavy alcohol use.

The study was approved by the Human Investigation Review Committee and the Clinical Study Unit (CSU) at the New England Medical Center and Tufts University, Boston. An informed consent document was signed by each subject before admission to the study.

Throughout the 13-d study period, participants resided in the CSU. Subjects were allowed to leave the CSU to fulfill their regular daily activities. Subjects consumed only those foods prepared in the CSU’s metabolic kitchen.

Experimental protocol

On arrival at the CSU, each volunteer entered a 4-d baseline period during which they consumed a diet that was low in vitamin K (40 µg phylloquinone/d). Prepared in the metabolic kitchen, this diet consisted of a 2-d cycle menu. It was designed to meet the recommended dietary allowances for energy, protein, minerals, and vitamins other than vitamin K. This vitamin K–deficient diet, a modification of a diet we designed earlier (13), included ~15% of energy from fat yet had little effect on absorption of fat-soluble vitamins, as shown previously. To verify that phylloquinone intake did not exceed 40 µg/d, a composite dietary analysis of sample homogenized diets was done for phylloquinone at the beginning of the study (14). This diet was maintained during the 12 d of the study.

Fixed minidose warfarin period

From days 5 to 11, in addition to the low-phylloquinone diet, subjects received a daily dose of 1.0 mg sodium warfarin (Coumadin; Dupont Pharma, Wilmington, DE) at breakfast. The prothrombin time and the activated partial thromboplastin time (APTT) were measured every other day throughout the study. Both the prothrombin time and APTT remained within the normal laboratory ranges (10.9–13.7 and 22.8–33.1 s, respectively) in all subjects throughout the study.

Intravenous infusions

On the 11th day of the study, subjects were randomly assigned to one of two experimental groups. The study group (lipid) received a 500-mL intravenous infusion of a 10%-lipid emulsion (Intralipid; Pharmacia Inc) and the control group (saline) received 500 mL saline solution, which contained the same amount of phylloquinone (AquaMephyton; Merck and Company, West Point, PA) that had previously been determined from the lot of lipid used for the study (154 µg phylloquinone). The saline solution, which contained detergent-dispersed phylloquinone, was prepared by a research pharmacist immediately before the start of the infusion. Both the glass bottle and the infusion set were wrapped in aluminum foil to prevent light exposure. Assays for phylloquinone were performed in triplicate directly on a sample of infused saline solution and lipid emulsion to confirm that there had been no degradation of phylloquinone before the infusions (7 ± SEM: 134 ± 4.3 mg/L for saline; 156 ± 3.7 mg/L for lipid).

On day 11 at 0800, after subjects ate breakfast and after they ingested 1.0 mg warfarin, an intravenous cannula was inserted into an antecubital vein of each subject. Through this cannula the intravenous infusions were administered at a constant, pump-assisted rate over 12 h. In the opposite arm, another intravenous line was inserted for the purpose of serial blood draws. Subjects rested in a recumbent position throughout the infusion. Blood samples were drawn periodically until 24 h after the end of each infusion. Subjects were discharged on the morning of the 13th day of the study after a regular breakfast, which was supplemented with 5 mg oral phylloquinone (Mephyton; Merck and Company, Inc).

Blood samples

Venous blood samples were drawn after a 12-h overnight fast. Samples were obtained between 0800 and 0900 on days 1, 5, 6, 8, 10, 11, and 12. On day 11, blood was drawn immediately before the start of the intravenous infusion. Additional blood samples were drawn starting on day 11 at 6, 12, 15, 18, 21, 24, 30, and 36 h after the start of the infusions. Samples were collected in plain glass vacuum tubes with no additives and in glass vacuum tubes with EDTA. Tubes were wrapped in aluminum foil and trans-
ported to the laboratory. A portion of the plasma or serum from each subject was immediately frozen and stored at −70 °C.

Urine samples

Urine samples were collected in refrigerated containers over 24-h periods on days 1–13 of the study for quantitation of urinary Gla and creatinine. Samples were collected by the investigators at the end of every 24-h period.

Analytic procedures

All blood and urine samples were analyzed by investigators who were blinded to the randomization code. Phylloquinone and its metabolite, vitamin K$_{1}$-2,3-epoxide, were measured by reversed-phase HPLC by postcolumn solid-phase reduction of phylloquinone to its hydroquinone followed by fluorometric detection as described previously (15). Urinary Gla was assayed by reversed-phase HPLC of the ortho-phthalaldehyde derivative as described earlier (16). Urinary creatinine was analyzed by a colorimetric method on a Cobas Mira analyzer (Roche Instruments, Belleville, NJ). The phylloquinone content of aliquots of sample lipid and saline solutions were also analyzed by HPLC methods (1), as were phylloquinone contents of homogenized sample diets.

Prothrombin time and APTT were determined with the use of an MLA Electra 800 automated clot timer (Medical Laboratory Automation, Inc, Pleasantville, NY) with thromboplastin C and actin reagents (Dade Diagnostic, Miami). PIVKA-II antigen concentrations in plasma were quantitatively determined by a sandwich enzyme-linked immunosorbent assay with commercial kits from American Bioproducts Co (Parsippany, NJ). Serum osteocalcin was measured by radioimmunoassay with a rabbit polyclonal antibody raised against purified bovine bone osteocalcin (17). The antibody recognizes both native and undercarboxylated osteocalcin. Undercarboxylated osteocalcin was measured in the osteocalcin radioimmunoassay after separation of osteocalcin and undercarboxylated osteocalcin by adsorption of the carboxylated osteocalcin to hydroxyapatite (18). Cholesterol and triacylglycerol were assayed with a Cobas Fara centrifugal analyzer (Roche Diagnostic Systems, Totowa, NJ).

Both R (+) and S (−) enantiomers of warfarin were measured in extracts of plasma with the use of a stereoselective HPLC assay. The warfarin isomers were extracted with the method described by Anderson et al (19). Briefly, R, S, and p-chlorowarfarin (the internal standard) were extracted from 1 mL acidified plasma into methylene chloride:hexane (1:5, by vol). The extract was evaporated to dryness and reconstituted into a mobile phase (10% isopropanol in 0.01 mol phosphate buffer/L) and injected into the HPLC apparatus. Separation of the isomers was achieved by isocratic elution on an Alpha-1-acid glycoprotein chiral analytic column (Chiral-AGP; Chrom Tech., Inc, Apple Valley, MN) by modification of the method of De Vries and Schmitz-Kummer (20).

Statistical analysis

Results are expressed as means ± SEMs. Statistical analyses were performed with SYSTAT (version 5.2.1 for Macintosh; SPSS Inc, Chicago). Analyses over time were performed with either a repeated-measures analysis of variance or a Student’s paired $t$ test. For specific time points and for areas under the curve, comparisons between groups were performed with a Student’s independent $t$ test. A $P$ value < 0.05 was considered statistically significant in all analyses.

RESULTS

All 12 subjects completed the protocol. No adverse events occurred during the study.

The dietary depletion and warfarin phase

At the end of the 11-d dietary depletion and warfarin phase, subjects in both groups had mild, subclinical vitamin K deficiency, as suggested by the increases in PIVKA-II, vitamin K$_{1}$-2,3-epoxide, and percentage undercarboxylated osteocalcin ($P = 0.05, 0.016, and 0.001$, respectively) (Figure 1) However, plasma phylloquinone, total osteocalcin, urinary Gla/d, and urinary Gla·g urinary creatinine $^{-1}$·d$^{-1}$ did not change significantly from days 1 through 11. This subclinical vitamin K deficiency was no different in the two groups of subjects. Analyses on days 1, 5, and 11 showed no significant differences between the group destined to receive lipid emulsion and that destined to receive the saline solution with respect to PIVKA-II, phylloquinone, vitamin K$_{1}$-2,3-epoxide, and percentage undercarboxylated osteocalcin (Table 1).

FIGURE 1. Changes during the dietary depletion and warfarin phase. Vitamin K$_{1}$-2,3-epoxide, PIVKA-II, and percentage undercarboxylated osteocalcin increased during the dietary depletion and warfarin phase ($P = 0.016, 0.05,$ and $0.001$ for vitamin K$_{1}$-2,3-epoxide, PIVKA-II, and percentage undercarboxylated osteocalcin, respectively, with one-way repeated-measures ANOVA). $\bar{x} \pm$ SEM.
Similarly, no significant differences were seen in these indexes between groups during the course of the entire 11-d dietary deprivation and warfarin phase after analysis with a repeated-measures analysis of variance. A sequential analysis of urinary Gla/d and urinary Gla·g urinary creatinine⁻¹·d⁻¹ also revealed comparability between groups throughout this entire period. Furthermore, all subjects had detectable concentrations of warfarin by day 10, and by day 11, before the infusions began, no significant differences were seen between groups in terms of concentrations of S-warfarin, the active enantiomer (20).

The infusion and postinfusion period

The infusions affected several vitamin K–related indexes. Plasma concentrations of phylloquinone increased markedly during and 12 h after the infusions (\( P = 0.001 \)). Vitamin K₁²,3-epoxide decreased in the two groups from the time the infusions were completed to 24 h later (\( P = 0.002 \)) (Figure 2). PIVKA-II decreased significantly when comparisons were made between a preinfusion value on day 11 and a postinfusion value 36 h after the start of the infusion (\( P = 0.005 \)). On the other hand, a trend toward a decrease in the percentage of undercarboxylated osteocalcin over the same time period was not significant (\( P = 0.18 \)); the lack of a significant decrease was due to one subject whose percentage undercarboxylated osteocalcin increased substantially with the infusion. In addition, urinary Gla/d and urinary Gla·g urinary creatinine⁻¹·d⁻¹ did not change during or after the infusions.

The most notable findings in our study were those seen in comparisons between groups during and after the infusions. The areas under the curves were not significantly different for phylloquinone and vitamin K₁²,3-epoxide in either group: 116 ± 13 and 102 ± 20 nmol·h/L for the saline and lipid groups for phylloquinone, respectively, and 38.6 ± 7.5 and 31.3 ± 9.0 nmol·h/L for the saline and lipid groups for vitamin K₁²,3-epoxide, respectively. In addition, absolute concentrations of PIVKA-II and percentage change from baseline of PIVKA-II behaved in a parallel fashion in the groups in response to the infusions, with no significant changes evident between saline- and lipid-treated subjects. In summary, both solutions partially—and no differently—reversed subclinical vitamin K deficiency.

DISCUSSION

Our study confirms that large quantities of phylloquinone are present in the intravenous lipid emulsion Intralipid and shows that a single 10% 500-mL bottle of this product can partially reverse the effects of phylloquinone dietary deprivation and the antagonistic effects of minidose warfarin on vitamin K. This reversal is no different from that observed with the equimolar infusion of phylloquinone in a simple saline vehicle.

Our study consisted of the induction of subclinical vitamin K deficiency with dietary phylloquinone deprivation, an exacerbation of this deficiency with warfarin administration, and a partial reversal of this deficiency with either a phylloquinone saline solution or a lipid emulsion. The eventual decrease in plasma vitamin K₁²,3-epoxide concentrations and concurrent decrease in PIVKA-II shows that the phylloquinone in the lipid emulsion was sufficiently utilized by the host’s metabolism to override the antagonistic effects of warfarin on the enzymes 2,3-epoxide reductase and quinone reductase, to enhance the synthesis of the quinone and hydroquinone forms of vitamin K, and, ultimately, to promote the carboxylation of vitamin K–dependent proteins (Figure 3). Our study shows that prothrombin precursors can be carboxylated by the phylloquinone present in an intravenous lipid emulsion. Our findings also show that intravenous lipid provides bioavailable phylloquinone to the liver, where prothrombin is synthesized. Whether the phylloquinone in the lipid emulsion is bioavailable to extraphepatic tissues is not entirely clarified by this study. A trend toward a diminished percentage of undercarboxylated osteocalcin was observed after infusion, but because of one subject whose percentage undercarboxylated osteocalcin increased, the trend was not significant. Nevertheless, these findings may have important implications given the growing body of evidence that points to important physiologic roles for nonhepatic, vitamin K–dependent proteins (21).

Our study also raises the issue of whether patients who receive long-term total parenteral nutrition with lipid emulsions require vitamin K supplementation to the extent currently prescribed. Our study was undertaken in a healthy, not diseased, population and only a partial reversal of vitamin K deficiency was observed. It therefore remains to be determined whether our findings are applicable in other clinical settings.

The observed antagonism between the lipid emulsion and minidose warfarin is important in view of the widespread use of the latter. Warfarin is now frequently prescribed at a dose of 1 mg/d to prevent thromboses in patients with central venous access devices and as a prophylactic measure for deep vein thrombosis in patients in the postoperative setting. Although randomized, prospective trials confirm the efficacy of these approaches (5, 6), the precise quantity and type of lipid delivered is not stated in these studies. Our study raises concern that warfarin prophylaxis may not be as effective in patients who receive lipids.

To our knowledge, only one other study has examined the bioavailability of phylloquinone in lipid emulsions. Goulet et al (4) examined 25 pediatric patients to assess the effects of lipid

| TABLE 1 |
| Phylloquinone, vitamin K₁²,3-epoxide, PIVKA-II, percentage undercarboxylated osteocalcin, and warfarin during the diet depletion and warfarin phase in the saline and lipid groups¹ | | | | |
| --- | --- | --- | --- | --- | --- |
|  | Day 1 | Day 5 | Day 11 |
| Saline | Lipid | Saline | Lipid | Saline | Lipid |
| Phylloquinone (nmol/L) | 0.72 ± 0.09 | 1.63 ± 1.64 | 0.40 ± 0.06 | 0.54 ± 0.1 | 0.64 ± 0.12 | 0.75 ± 0.3 |
| Vitamin K₁²,3-epoxide (nmol/L) | 0.05 ± 0.02 | 0.13 ± 0.07 | 0.02 ± 0.01 | 0.03 ± 0.07 | 0.24 ± 0.05 | 0.30 ± 0.12 |
| PIVKA-II (μg/L) | 1.03 ± 0.24 | 1.43 ± 0.41 | 1.42 ± 0.19 | 1.73 ± 0.36 | 51.15 ± 39 | 23.31 ± 16 |
| Undercarboxylated osteocalcin (%) | 15.89 ± 1.01 | 16.33 ± 3.2 | 13.90 ± 2.02 | 15.01 ± 2.5 | 29.32 ± 2.4 | 28.82 ± 3.2 |
| Warfarin (μg/L) | — | — | — | — | 0.228 ± 0.02 | 0.175 ± 0.03 |

¹ X ± SEM. No differences were seen between the saline and lipid groups for phylloquinone, vitamin K₁²,3-epoxide, PIVKA-II, percentage undercarboxylated osteocalcin, or warfarin before the start of the infusions.
emulsions on PIVKA-II concentrations. These investigators found that PIVKA-II concentrations remained normal in hospitalized patients who were followed from 4 to 29 wk. Although these investigators’ conclusions agree with our own, their study has several confounding factors that make it impossible to conclude that it was solely the phylloquinone in lipid emulsions that maintained normal concentrations of PIVKA-II. These confounders include the administration of a dose of intramuscular vitamin K before the initiation of lipids, no apparent restriction of phylloquinone from dietary sources, and the lack of a control group. Our study is therefore the first to examine critically the question of bioavailability.

Some final qualifications regarding our observations are warranted. Although we showed that the infusion of a lipid emulsion can reverse the effects of subclinical vitamin K deficiency, we did not show that all proteins we studied are affected. Urinary Gla concentrations were not affected significantly in either the dietary depletion and warfarin phase or the repletion phase. Earlier studies from our laboratory showed decreases in urinary Gla with a diet of 10 μg phylloquinone/d after 11 d (22) and after 15 d with a diet of 100 μg/d (23). Perhaps if the dietary depletion and warfarin phase had been between 11 and 15 d and the follow-up extended longer than 24 h after repletion, we would have seen changes in this protein that reflected the bioavailability of the infused phylloquinone. As mentioned previously, the percentage undercarboxylated osteocalcin did not decrease significantly after the infusions. A significant decline would have been observed had it not been for one subject who had a marked increase in percentage undercarboxylated osteocalcin 36 h after the start of his infusion. The percentage change of PIVKA-II declined in this subject, however, reflecting the fact that the kinetics for the gamma carboxylation of various proteins differs.

Second, the mean vitamin K₇,2,3-epoxide concentration climbed after the start of the infusions. This increase in vitamin K₇,2,3-epoxide might appear paradoxical but can be explained by the subjects’ ingestion of warfarin through day 11. The ongoing antagonism of vitamin K by warfarin most likely drove the rise in vitamin K₇,2,3-epoxide because of the latter’s inhibition of the enzyme vitamin K epoxide reductase.

Third, we studied only Intralipid brand lipid emulsion. Although we had chosen this product because of its generous phylloquinone content (1), it is not necessarily true that other lipid emulsions, including those used for drug delivery, also contain bioavailable phylloquinone. These products are made from different vegetable oils (1), and such compositional differences may possibly affect the bioavailability of phylloquinone. The results of our study, which used the soybean oil–based product Intralipid, do not allow us to speculate on the bioavailability of vitamin K from other products.

Finally, our study examined only healthy, well-nourished individuals and not a group of ill patients. We do not yet know whether the conclusions we have reached are applicable to the latter group.

In summary, we showed that the vitamin K in Intralipid lipid emulsion is bioavailable and can promote the carboxylation of certain vitamin K–dependent proteins in the body. We also showed that the phylloquinone in Intralipid lipid emulsion can partially reverse the effects of minidose warfarin. These results indicate that patients who receive clinically relevant doses of Intralipid lipid emulsion are receiving significant amounts of bioavailable phylloquinone.
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REFERENCES


