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Vitamin K status and stability of oral anticoagulant therapy

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Abstract

Background

Vitamin K supplementation improves anticoagulant stability in patients using vitamin K antagonists.

Objectives

To determine whether there is an association between vitamin K status and stability of anticoagulant treatment.

Methods

We examined the relationship between vitamin K₁ and its metabolite, vitamin K 2,3-epoxide and stability of oral anticoagulant treatment in participants of a double-blind, placebo-controlled trial on the effect of vitamin K supplementation on anticoagulant stability. Vitamin K₁ and vitamin K 2,3-epoxide serum levels were determined in 91 patients receiving placebo and 91 patients receiving 100 µg vitamin K per day. The effect of these variables on time in therapeutic range (TTR) was assessed using linear regression analysis.

Results

In the placebo group each standard deviation (SD) increase in vitamin K₁ level was associated with a 3.4% higher TTR. Vitamin K 2,3-epoxide levels increased the TTR with 2.7% per SD, but this effect disappeared when vitamin K₁ and vitamin K 2,3-epoxide levels were combined in one model. In the group receiving the vitamin K supplement we found no effect of vitamin K status on the TTR.

Conclusion

These findings contribute to the evidence that an adequate intake of vitamin K can improve anticoagulant control.

Introduction

Vitamin K antagonists are indicated for the prevention of venous and arterial thrombosis.¹ Even though their effectiveness has been well established, many patients are denied the benefits of these drugs due to the difficulty in maintaining stable anticoagulation.² Vitamin K antagonists have a narrow therapeutic window and a high variability in anticoagulant response. Despite intensive monitoring, the intensity of anticoagulation, expressed as the International Normalized Ratio (INR), is within the target range only approximately 60% of the time.³

One of the causes of a fluctuating INR is a variable vitamin K intake. Vitamin K in its reduced form (vitamin K hydroquinone) is an essential cofactor for the posttranslational carboxylation of various proteins involved in blood coagulation, among which the procoagulant factors II, VII, IX and X. During the carboxylation reaction, the vitamin K hydroquinone is converted to its inactive metabolite, vitamin K epoxide, which is subsequently converted back to vitamin K hydroquinone by vitamin K epoxide reductase (VKOR). Vitamin K antagonists inhibit VKOR, thereby blocking the turnover of vitamin K epoxide resulting in depletion of the active vitamin K stores. This leads to the desired anticoagulant effect due to reduced production of fully carboxylated vitamin-K dependent clotting factors.⁴

Previous studies have suggested that patients with a low vitamin K intake have a less stable anticoagulation than patients with a higher intake.⁵⁻⁸ Supplementation with low doses of vitamin K has been shown to improve anticoagulant stability.^{9,10} This can be explained by the hypothesis that the INR is relatively resistant to a fluctuating vitamin K intake when average vitamin K intake is high. If this hypothesis were true one would also expect to find an association between vitamin K status and stability of anticoagulation.

We examined the relationship between serum levels of vitamin K₁ and its metabolite, vitamin K 2,3-epoxide and stability of oral anticoagulant treatment in participants of a clinical trial on the effect of vitamin K supplementation on anticoagulant stability.

Methods

We determined the vitamin K status in participants of a randomized double-blind placebo-controlled clinical trial on the effect of vitamin K supplementation on anticoagulant stability.¹⁰ In this trial 200 patients using the vitamin K antagonist phenprocoumon were randomized to receive either adjusted-dose phenprocoumon and 100 µg vitamin K once daily or adjusted dose phenprocoumon and a placebo.

Participants were recruited from the Leiden anticoagulation clinic in the Netherlands. They were between 18 and 80 years of age, had an indication for long-term anticoagulant therapy and had been using phenprocoumon for at least one year. Patients were enrolled in the study from December 2004 to January 2006. Treatment duration was 24 weeks. During this time patients were treated according to the guidelines of the anticoagulation clinic. During the study period the maximum interval between two visits was 4 weeks. The primary outcome was the percentage of time the INR was within the therapeutic range.¹⁰ This study was approved by the local Medical Ethics Committee and was registered as an International Standard Randomized Controlled Trial, number ISRCTN14473912.

Eight to twelve weeks after starting the study medication, fasting blood samples were obtained by venipuncture. All specimens were kept in the dark. Blood samples were processed within 4 hours and aliquots of plasma and serum were stored at -80°C. Serum levels of vitamin K₁ and vitamin K 2,3-epoxide were measured by high-performance liquid chromatography (HPLC) using zinc postcolumn reduction and fluorescence detection as described earlier^{11;12} with some modifications. In brief, after deproteinization of the samples with ethanol and liquid extraction using hexane, solid phase extraction is performed using a 500 mg/3 ml Strata C18-M column (Phenomenex, USA). The columns are pretreated with 5 ml hexane-2-propanol (98:2) and 5 ml hexane before the addition of the sample (2 ml). The vitamin K₁ and vitamin K 2,3-epoxide are eluted in a total volume of 10 ml hexane and dried under N₂ (g) at 40 °C. The sample is taken up in ethanol and injected onto a Lichrospher®100-RP-18 (125-4mm; 5 µm) column (Merck, Darmstadt, Germany) and eluted at a flow of 1.5 ml/min using 1M sodiumacetate/acetic acid, 2M Zn Cl₂ in methanol (ratio

6:1000). A stainless steel column (50-2.1mm) containing zinc particles (Vitamin K₁ HPLC kit, Immundiagnostic, Bensheim, Germany) was placed between the HPLC column and the fluorimetric detector (model FP2020 Jasco, Tokyo, Japan). After the zinc postcolumn reduction the separated vitamin K₁ and vitamin K 2,3-epoxide were detected at Ex/Em of 248/418 nm.

We used linear regression analysis to assess the association between vitamin K₁ and vitamin K 2,3-epoxide levels and time in therapeutic range. Because both vitamin K₁ and vitamin K 2,3-epoxide levels showed a right-skewed distribution, we log-transformed the data. We then used the z-scores of the placebo group as coefficients in the model, so results show the change in time in therapeutic range per standard deviation (SD) change in serum vitamin K₁ or vitamin K 2,3-epoxide. Outliers were identified as being greater than the mean $\pm$ 3 SD of the logarithm. This resulted in the removal of 4 outlier values: 2 patients had a serum vitamin K₁ level higher than the mean +3 times the SD and 2 patients had a vitamin K 2,3-epoxide lower than the mean -3 times the SD. Values were removed rather than cases, because in those subjects with extreme values of vitamin K₁, vitamin K 2,3-epoxide levels were within the mean $\pm$ 2 SD and vice versa. Furthermore, the INR values of subjects with outliers were within the therapeutic range.

Age, sex, use of co-medication and use of medication interacting with the anticoagulant drug were considered potential confounders and were adjusted for in the analysis. Analyses were done separately for the placebo group and the vitamin K group. All statistical analyses were performed using SPSS 17 (SPSS Inc, Chicago, Ill, USA).

Results

In the 200 patients randomized for the trial, serum vitamin K₁ and vitamin K 2,3-epoxide serum levels were determined in 91 patients in the vitamin K group and 91 patients in the placebo group. For 18 individuals serum or assays were unavailable.

Table 1: Baseline characteristics of the study population. Data are median (IQR) or number (%).

	Placebo group (n = 91)	Vitamin K group (n = 91)
Age	66 (58-72)	65 (60-74)
Female sex	26 (29%)	20 (22%)
Duration of anticoagulant treatment (years)	4 (2-8)	4 (2-8)
Previous stability (%time in therapeutic range)	80 (73-84)	79 (72-84)
Indication		
Atrial fibrillation	38 (42%)	39 (43%)
Secondary prevention venous thrombosis	12 (13%)	15 (17%)
Mechanical heart valve	12 (13%)	9 (10%)
Other arterial	29 (32%)	28 (31%)
Therapeutic range		
Low (2.0-3.5)	51 (56%)	56 (62%)
High (2.5-4.0)	40 (44%)	35 (38%)
Number of co-medications	3 (2-5)	3 (1-4)
Interacting medication		
No	44 (48%)	55 (60%)
Yes	47 (52%)	36 (40%)

Patient characteristics are shown in Table 1. Age, sex, duration of anticoagulant treatment, previous stability and indication for anticoagulant treatment were similar in both groups. In the placebo group there were slightly more patients in the high target range. Patients in the placebo group used more drugs interacting with anticoagulants.

Table 2 gives an overview of the vitamin K₁ and vitamin K 2,3-epoxide levels in the placebo group and the group receiving vitamin K. Mean serum vitamin K₁ was 0.83 ng/mL in the placebo group and 1.02 ng/mL in the vitamin K group (difference 0.20 ng/mL, 95%CI: 0.05 to 0.34). Mean vitamin K 2,3-epoxide was 4.60 ng/mL in the placebo group and 7.00 in the vitamin K group (difference 2.40 ng/mL, 95% confidence interval [95%CI]: 0.68 to 4.12). In the placebo group, the SD of the logarithm of the vitamin K₁ level, used as the regression coefficient in the linear regression model, was 0.52. The SD of ln(vitamin K 2,3-epoxide) was 1.05.

Table 2: Serum levels of vitamin K1 and vitamin 2,3-epoxide

	Vitamin K1 (ng/mL)		ln(Vitamin K1)		Vitamin K 2,3-epoxide (ng/mL)		ln(Vitamin K 2,3-epoxide)	
	Mean $\pm$ SD	Median (IQR)	Mean $\pm$ SD		Mean $\pm$ SD	Median (IQR)	Mean $\pm$ SD	
Group receiving placebo	0.83 $\pm$ 0.53	0.70 (0.55-0.97)	-0.33 $\pm$ 0.52		4.60 $\pm$ 4.40	3.84 (1.27 - 5.95)	1.07 $\pm$ 1.05	
Group receiving vitamin K	1.02 $\pm$ 0.44	0.92 (0.72-1.17)	-0.06 $\pm$ 0.40		7.00 $\pm$ 6.96	5.13 (2.64 - 9.62)	1.50 $\pm$ 1.06	

Table 3: Vitamin K status and time in therapeutic range. The table shows the results of linear regression, with vitamin K levels as independent variables and time in therapeutic range (TTR) as the dependent variable. The independent variables are expressed as z-scores of the logarithm of the serum levels. The constant is equivalent to a z-score =0, which is the TTR for the mean of the logarithm of the vitamin K level; the coefficient shows the change in TTR (%) per unit change in vitamin K level, one unit being one standard deviation of the logarithm of the level. * Adjusted for age, sex, number of co-medications and use of interacting medication.

Time in Therapeutic range (%)				
Determinant	Constant	B	Adjusted B*	vitamin K1 and vitamin 2,3-epoxide combined
Group receiving placebo				
Vitamin K1 level (Z-score ln(vitamin K))	85.2	3.4 (0.3 to 6.6)	3.2 (-0.4 to 6.5)	3.1 (-0.9 to 7.0)
Vitamin K 2,3-epoxide level (Z-score ln(vitamin KO))	85.2	2.7 (-0.5 to 5.8)	2.4 (-1.0 to 5.7)	0.6 (-3.4 to 4.7)
Group receiving vitamin K				
Vitamin K1 level (Z-score ln(vitamin K))	89.6	1.0 (-2.9 to 4.8)	0.7 (-3.0 to 4.5)	-0.9 (-5.1 to 3.4)
Vitamin K 2,3-epoxide level (Z-score ln(vitamin KO))	89.5	1.3 (-1.9 to 4.4)	1.2 (-1.9 to 4.3)	1.8 (-1.5 to 5.1)

Table 4a: Vitamin K status and time below therapeutic range.

Determinant	Time below Therapeutic range (%)			vitamin K1 and vitamin 2,3-epoxide combined
	Constant	B	Adjusted B*	
Group receiving placebo				
Vitamin K1 level (Z-score ln(vitamin K))	2.8	0.1 (-1.2 to 1.5)	0.3 (-1.1 to 1.7)	-0.5 (-2.1 to 1.2)
Vitamin K 2,3 epoxide level (Z-score ln(vitamin KO))	2.7	0.7 (-0.6 to 2.1)	0.9 (-0.4 to 2.3)	1.1 (-0.6 to 2.8)
Group receiving vitamin K				
Vitamin K1 level (Z-score ln(vitamin K))	2.3	-0.4 (-1.8 to 0.9)	-0.4 (-1.8 to 1.0)	-0.1 (-1.6 to 1.3)
Vitamin K 2,3 epoxide level (Z-score ln(vitamin KO))	1.9	0.1 (-0.9 to 1.2)	0.2 (-0.9 to 1.3)	0.1 (-1.0 to 1.3)

Table 4b: Vitamin K status and time above therapeutic range

Determinant	Time above Therapeutic range (%)			vitamin K1 and vitamin 2,3-epoxide combined
	Constant	B	Adjusted B*	
Group receiving placebo				
Vitamin K1 level (Z-score ln(vitamin K))	12.0	-3.6 (-6.5 to -0.6)	-3.5 (-6.6 to -0.5)	-2.6 (-6.3 to 1.1)
Vitamin K 2,3 epoxide level (Z-score ln(vitamin KO))	12.0	-3.4 (-6.4 to -0.4)	-3.3 (-6.4 to -0.2)	-1.7 (-5.5 to 2.0)
Group receiving vitamin K				
Vitamin K1 level (Z-score ln(vitamin K))	8.2	-0.5 (-3.9 to 2.8)	-0.3 (-3.6 to 2.9)	1.0 (-2.7 to 4.8)
Vitamin K 2,3 epoxide level (Z-score ln(vitamin KO))	8.6	-1.4 (-4.1 to 1.3)	-1.4 (-4.1 to 1.3)	-1.9 (-4.8 to 1.0)

Table 3 shows the associations between vitamin K status and time in therapeutic range, for the placebo group and the vitamin K group separately. In the placebo group, time in therapeutic range increased 3.4% for each SD increase in serum vitamin K₁ (95%CI: 0.3 to 6.6%). An effect in the same direction was observed for serum vitamin K 2,3-epoxide: one SD increase resulted in a rise of the time in range of 2.7% (95%CI: -0.5 to 5.8%). Adjustment for age, sex and use of co-medication did not change the results. When we combined vitamin K₁ and vitamin 2,3-epoxide in one model, the effect of vitamin K₁ remained similar and the effect of vitamin 2,3-epoxide disappeared. In the group receiving vitamin K supplementation there was no association between vitamin K status and the time in therapeutic range.

The increase of the time in therapeutic range was attributable to a decrease in time above the therapeutic range rather than below the therapeutic range, i.e. high serum vitamin K₁ levels prevented overanticoagulation (Tables 4a and 4b).

Discussion

We found that time in therapeutic range increases by approximately 3% for each standard deviation increase in vitamin K levels. This demonstrates that patients with a high vitamin K status have a more stable anticoagulation than individuals with a low vitamin K status. This relationship was found both for levels of vitamin K₁ as vitamin K 2,3-epoxide. In the group receiving the vitamin K supplement we found no association between vitamin K status and time in therapeutic range, suggesting that instability caused by dietary vitamin K changes in patients was effectively reduced by the vitamin K supplement.

Our findings are in agreement with earlier studies that focused on vitamin K intake. Patients with low vitamin K intake were shown to have a less stable anticoagulant control⁷ and a higher risk of subtherapeutic anticoagulation.⁸ Changes in dietary vitamin K intake influence the INR^{5,13,14} and do so more when vitamin K intake is low.^{5,8} In addition, starting a low dose vitamin K supplement of 25 µg/day results in subtherapeutic INR

values in individuals with a low plasma vitamin K only.⁶ These studies imply that patients are more susceptible to changes in vitamin K intake when vitamin K status is low and suggest that supplementation of vitamin K may be beneficial, especially in vitamin K depleted patients. Two placebo-controlled trials have been performed on the effect of vitamin K supplementation on quality of anticoagulant treatment. Sconce *et al.* studied 70 atrial fibrillation patients with unstable anticoagulant control and randomized them to receive 150 µg of vitamin K or a placebo for six months.⁹ Vitamin K supplementation resulted in a decrease in the SD of the INR values and an increase of the percentage time in therapeutic range from 78% in the placebo group to 87% in the vitamin K group. The second trial, which was the basis for the study presented here, was conducted in 200 unselected patients who had been using the vitamin K antagonist phenprocoumon for over a year.¹⁰ Vitamin K supplementation resulted in a small increase of time in therapeutic range from 85% in the placebo group to 89% in the vitamin K group. The larger effect in the trial by Sconce may have been caused because in this study unstable patients were selected, who may have had a lower vitamin K status and benefit more from supplementation. Indeed, the mean plasma vitamin K concentrations at baseline were 0.60 ng/mL in the vitamin K group and 0.69 ng/mL in the placebo group of the trial by Sconce, which is considerably lower than the level of 0.87 ng/mL in the placebo group of our study. Unfortunately we did not measure serum vitamin K at baseline, so we could not perform a subgroup analysis on the effect of vitamin K supplementation conditioned on vitamin K status.

A possible limitation of this study is that we determined the vitamin K status in a single day's sample. Even though we used fasting blood samples, circulating vitamin K concentrations respond to daily changes in intake.¹⁵ This may have resulted in misclassification of the exposure variables (vitamin K₁ and vitamin K 2,3-epoxide levels). Because this misclassification is just as likely to have occurred in individuals with stable anticoagulation as in individuals with unstable anticoagulation, it is likely that this caused at most an attenuation of the observed effects, in which case the true association between vitamin K levels and stability would be stronger than observed here.

Changes in dietary vitamin K intake can be a significant factor in the stability of anticoagulant treatment. Our results contribute to the evidence that patients using vitamin K antagonists should not restrict their vitamin K intake, which is frequently advised. On the contrary, a vitamin K intake in the high normal levels should be advised to increase stability of anticoagulant treatment. In addition, these findings support the proposal of using a vitamin K supplement to improve quality of anticoagulant therapy.

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