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Original Article

Vitamin K Administration to Elderly Patients with Osteoporosis Induces No Hemostatic Activation, Even in Those with Suspected Vitamin K Deficiency

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Abstract. The administration of menaquinone-4 (MK-4), one of subclasses of vitamin K_2, significantly reduces bone loss in postmenopausal osteoporotic women. However, concerns have been raised about whether vitamin K administration alters the hemostatic balance by inducing a thrombotic tendency. We investigated here whether the administration of vitamin K in the form of MK-4 induced a thrombotic tendency in 29 elderly patients with osteoporosis (5 men, 24 women; age range 78.7±5.1 years). Patients were administered 45 mg/day (three times a day, 30 min after each meal) of MK-4 for 12 weeks. Blood samples were obtained from the patients at 0, 4 and 12 weeks after the start of MK-4 administration. A number of hemostatic parameters remained stable under the markedly increased plasma levels of MK-4. However, in patients with suspected vitamin K deficiency, whose plasma levels of vitamin K or factor VII were low, vitamin-K-dependent clotting factors such as factor VII and prothrombin were gradually increased after administration of MK-4. No changes in the sensitive molecular markers such as TAT and F1+2, which reflect the amount of thrombin generated in the blood stream, were observed, even in those patients with suspected vitamin K deficiency. These results indicate that MK-4 can be administered safely, with regard to maintaining the hemostatic balance, to osteoporotic patients receiving no anticoagulant therapy.

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Introduction

Vitamin K is involved in the biosynthesis of a limited number of blood coagulation factors (prothrombin, factors VII, IX and X), physiologic anticoagulants (protein C and protein S) and bone proteins (bone Gla protein, also known as osteocalcin, and matrix Gla protein). The vitamin-K-dependent step in the biosynthesis of these proteins is the conversion of glutamate residues into γ-carboxyglutamic acid (Gla). This reaction is called a γ-carboxylation, and vitamin K is an important cofactor in this reaction [1–3].

Vitamin K has long been considered essential for the synthesis of four clotting factors [4,5]. Another important role of vitamin K is its contribution to bone metabolism. Osteocalcin, which is the most abundant vitamin-K-dependent bone protein synthesized by osteoblasts, normally contains three closely arranged Gla residues that have the capacity to bind to calcium ions. The strong affinity of these Gla residues to hydroxyapatite is recognized to be a key property of osteocalcin [6,7]. Some clinical evidence exists for the relationship between vitamin K deficiency and osteoporosis: (1) the concentration of circulating vitamin K is reduced in patients with recent femoral fractures or prior vertebral compression fractures [8], (2) the concentration of circulating undercarboxylated osteocalcin reportedly increases with age, low bone mineral density and hip

fracture risk [9], and (3) vitamin K supplementation decreases bone loss and calcium excretion [10,11].

In a Japanese trial, the administration of 45 mg/day of menaquinone-4 (MK-4), one of the subclasses of vitamin K_2, induced a significant reduction in bone loss in postmenopausal osteoporotic women [10]. Vitamin K is now one of the most frequently administered drugs for the treatment of patients with osteoporosis in Japan [11–14]. However, concerns have been raised that vitamin K administration may alter the hemostatic balance by inducing a thrombotic tendency. Vitamin K administration might activate hemostasis because of the increased activity of vitamin-K-dependent coagulation factors, particularly in patients with vitamin K deficiency. We investigated here whether vitamin K administration induces a thrombotic tendency in patients with osteoporosis.

Materials and Methods

Patients

Twenty-nine osteoporotic patients (5 men, 24 women; age range 78.7±5.1 years; height 150±8 cm; body weight 41.3±6.5 kg) were enrolled in this study. The diagnosis of osteoporosis was made according to the criteria proposed by the Japanese Society of Bone Metabolism [15]. The lumbar bone mineral density (BMD) was classified into three grades: grade 0, normal lumbar BMD ($\geqslant$80% of young adult mean); grade 1, low lumbar BMD ($<$80% of young adult mean); grade 2, low lumbar BMD ($<$70% of young adult mean). Patients with low lumbar BMD ($<$70% of young adult mean) or with one or more nontraumatic vertebral fractures and lumbar BMD less than 80% of the young adult mean were diagnosed as having osteoporosis.

All patients were hospitalized because of their underlying disease: 12 for old cerebral infarction and/or hemorrhage, 7 for chronic obstructive lung diseases and 10 for chronic neurologic diseases. We included patients in the study because osteoporosis and poor nutritional status (including low body mass index (BMI); average BMI 18 kg/m^2) were considered clinical signs of suspected vitamin K deficiency. All patients gave informed consent prior to enrollment. The following patients were excluded from the study: those who had received antithrombotic agents (anticoagulant and/or antiplatelet agents) or vitamin K in the previous month, those with an infection who had or had not taken antibiotics, and those who had laboratory findings of an infection (elevation of CRP and WBC), liver dysfunction, obstruction of the bile duct, or were under intravenous hyperalimentation.

The patients were administered 45 mg/day (three times a day, 30 min after each meal) of MK-4, a subclass of vitamin K_2, for more than 12 weeks. Blood samples were obtained from the patients at 0 (just prior to MK-4 administration), 4 and 12 weeks after the start of MK-4 administration.

Assay Methods

Prothrombin time (PT) was measured according to the method of Quick et al. [16]. Plasma fibrinogen (Fbg) content was determined by Clauss's method [17]. Plasma levels of thrombin–antithrombin III complex (TAT), which reflects the amount of thrombin generated in the blood stream, were measured using an ELISA kit (Behringwerke, Germany) [18]. Plasma levels of prothrombin fragment 1+2 (F1+2), which reflects the amount of FXa generated in the blood stream, were measured using an ELISA kit (Behringwerke, Germany) [19]. Plasma antithrombin activity (AT) was measured by a chromogenic assay [20] and plasma activity of protein C (PC) and protein S (PS) was measured with a APTT clotting assay [21,22]. Factors VII (FVII) and II (prothrombin; FII) activities were measured by single-stage methods using plasma deficient in each factor. Protein induced by the absence of vitamin K (PIVKA)-II was measured by the latex particle agglutination method [23].

Plasma levels of vitamin K were determined by high-pressue liquid chromatography for the following three subclasses [24,25]: (1) phylloquinone (PK; vitamin K_1), which is exclusively of plant origin, the main sources of which are green vegetables and cow's milk, (2) MK-4, which is a member of the menaquinone family (MK; vitamin K_2) and is a component of the vitamin K drug administered to osteoporotic patients, and (3) MK-7, which is the main component of MK detected in fermented foods such as yogurt and natto (a popular Japanese food prepared from fermented soybeans) and is produced by the bacterial flora in the colon.

Blood Samples

Blood samples were obtained from the patients by antecubital venipuncture just before the midday meal. For the assays of FVII, FII, TAT and F1+2, the blood samples were anticoagulated with 3.8% sodium citrate (9/1, blood/anticoagulant volume) and centrifuged at 2000 *g* for 10 min at 4 °C immediately after venipuncture. Plasma was separated and stored at –70 °C until use.

For the assays of PK, MK-4 and MK-7, the blood samples were anticoagulated with heparin (15 u/ml final concentration of heparin) and centrifuged at 2000 *g* for 10 min at 4 °C immediately after venipuncture. Plasma was separated and stored at –70 °C until use. The samples were protected from the light throughout the above procedure.

Statistics

Data are presented as the median (25th percentile–75th percentile). Significant differences between values obtained from different sampling points were analyzed using the nonparametric Wilcoxon signed rank test. A value of $p < 0.05$ was considered statistically significant.

Results

Table 1 shows the changes in plasma levels of the three subclasses of vitamin K in all 29 patients. Plasma levels of MK-4, the component of the vitamin K drug, were significantly increased 4 weeks after administration began, and these elevated levels continued for 12 weeks. On the other hand, plasma levels of the other two subclasses, PK and MK-7, did not change during the 12-week administration period. Furthermore, of the 10 hemostatic parameters examined three times throughout the course of the study, in none were significant changes observed in any of the 29 patients (Table 2). Notably, levels of the vitamin-K-dependent proteins FVII, FII, PC and PS remained unchanged.

Despite the fact that none of the 29 patients had a detectable PIVKA-II level, which is one of the most reliable markers of vitamin K deficiency, we suspected that 5 were vitamin K deficient because their plasma levels of all three vitamin K subclasses were very low (MK-4 < 0.1, PK < 0.45 and MK-7 < 0.4 ng/ml). In fact, two hemostatic parameters were significantly increased among these 5 patients: plasma activity of FVII and FII gradually increased during the 12-week administration period (FVII: 92 (91–112)%, 103 (99–115)%, 126 (109–133)%, FII: 100 (93–115)%, 103 (95–109)%, 116 (100–117)% before, 4 weeks after and 12 weeks after the administration of vitamin K, respectively; presented as median and 25th–75th percentile). However, the other vitamin-K-dependent proteins such as PC and PS remained unchanged, as were plasma levels of TAT and F1+2 (data not shown), the latter being a sensitive marker of hemostatic activation.

Among the four vitamin-K-dependent coagulation factors, FVII has the shortest half-life ($T_{1/2}$ 5–6 h) in vivo [26]. We regard plasma activity of FVII as another sensitive marker of vitamin K deficiency, and 5 of the 29 patients were seen to have less than 80% plasma activity of FVII (these 5 patients were not the same as those who had very low levels of the three vitamin K subclasses). A gradual increase in both of the vitamin-K-dependent coagulation factors (FVII and FII), but particularly FVII (FVII: 76 (65–79)%, 90 (74–102)%, 95 (76–114)%, FII: 96 (91–104)%, 99 (96–111)%, 101 (96–124)%, before, 4 weeks after and 12 weeks after the administration of vitamin K, respectively; presented as median and 25th–

Table 1. Changes in plasma levels of vitamin K before and during the oral administration of vitamin K

	Pre	4W	12W	Pre vs 4W	Pre vs 12W	4W vs 12W
MK-4 (ng/ml)	0.1 (0.1–0.1)	85.1 (52.7–143.0)	81.7 (57.8–139.8)	<0.001	<0.001	NS
PK (ng/ml)	0.8 (0.4–1.2)	0.6 (0.4–0.9)	0.5 (0.3–0.8)	NS	NS	NS
MK-7 (ng/ml)	0.4 (0.4–0.8)	0.4 (0.4–0.5)	0.4 (0.4–0.5)	NS	NS	NS

Data are presented as median (25th percentile–75th percentile).
Patients were administered 45 mg/day of MK-4 for 12 weeks (W).

Table 2. Changes in hemostatic parameters before and during the oral administration of vitamin K

	Normal value	Pre	4W	12W	Pre vs 4W	Pre vs 12W	4W vs 12W
PT	10.0–12.0 (s)	11.4 (11.1–11.9)	11.3 (11.0–11.7)	11.3 (10.9–11.7)	NS	NS	NS
APTT	25.0–35.0 (s)	30.0 (27.7–30.9)	29.8 (27.4–31.1)	30.0 (29.4–32.1)	NS	NS	NS
Fbg	170–410 (mg/dl)	350 (288–464)	358 (302–413)	378 (315–435)	NS	NS	NS
F VII	70–130 (%)	92 (86–122)	100 (90–117)	107 (92–128)	NS	NS	NS
F II	70–130 (%)	105 (96–116)	100 (94–111)	110 (95–119)	NS	NS	NS
AT III	70–130 (%)	105 (97–117)	104 (98–120)	112 (99–121)	NS	NS	NS
PC	70–130 (%)	121 (102–129)	116 (91–127)	116 (96–132)	NS	NS	NS
PS	70–130 (%)	74 (70–85)	76 (68–83)	77 (71–86)	NS	NS	NS
F1+2	0.4–1.2 (nM)	1.1 (0.7–1.4)	1.1 (0.7–1.4)	1.1 (0.7–1.4)	NS	NS	NS
TAT	<3.8 (ng/ml)	2.1 (1.2–4.2)	1.8 (1.0–4.0)	1.5 (1.0–2.1)	NS	NS	NS

Data are presented as median (25th percentile–75th percentile).
This table represents all 29 patients.

75th percentile), and a slight increase in the plasma activity of ATIII (ATIII: 104 (99–106)%, 105 (103–118)%, 117 (113–118)%, before, 4 weeks after and 12 weeks after the administration of vitamin K, respectively; presented as median and 25th–75th percentile) after vitamin K administration was found among these 5 patients. Plasma levels of TAT or F1+2, however, remained unchanged throughout the administration period (data not shown), despite the apparent increase in FVII activity.

Discussion

Vitamin K is essential for the synthesis of four coagulation factors, two anticoagulant proteins and at least two bone-related proteins (osteocalcin and matrix Gla protein) [1–3]. It is now one of the most frequently administered drugs for osteoporotic patients in Japan [11–14]. However, the possibility exists that vitamin K administration for osteoporotic patients might induce a hypercoagulable state, and, in fact, a report supports this notion. Sakata et al. [27] reported that the intake of natto, which is a popular Japanese food prepared from fermented soybean and extremely rich in MK-7 (860 μg/100g), induced a hypercoagulable state in 8 healthy subjects, as shown by increased levels of TAT, F1+2 and FVIIa, although this surprising finding should be confirmed in a larger sample population.

On the other hand, Ronden et al. [28] reported that extremely high doses of vitamin K (250 mg/day/kg body weight) affected neither the blood coagulation characteristics nor the blood platelet aggregation rate in an experimental model in rats, but they investigated only a limited number of hemostatic parameters (plasma prothrombin activity, thrombin potential and platelet aggregation) before and after 10 days of treatment. Therefore, the present report is the first clinical study to report the effects of MK-4 administration on the hemostatic balance in osteoporotic patients, by carefully measuring sensitive molecular markers of hemostatic activation after a relatively long period of MK-4 administration.

Given our results, it is reasonable to conclude that the hemostatic balance remains stable under markedly increased plasma levels of MK-4 induced by the administration of MK-4 (Tables 1, 2). Plasma levels of TAT, one of the sensitive markers of hemostatic activation, were somewhat decreased after the start of MK-4 administration, but not at a significant level (Table 2). Vitamin K is responsible for the carboxylation of the FII (as well as FVII, IX and X), and a specific aid to the diagnosis of vitamin K deficiency is measurement of the decarboxylated forms of FII (PIVKA-II). Since PIVKA-II in plasma, a marker of definite vitamin K deficiency, was not detected in any patient, it appeared from these results that none of the patients was definitely vitamin K deficient. Therefore, to compare the hemostatic parameters of osteoporotic patients who possibly had vitamin K deficiency, we determined patients with suspected vitamin K deficiency by their plasma concentrations of three subclasses of vitamin K or FVII activity. Although PIVKA-II was not detected in the patients who had low FVII activity or no detectable vitamin K levels, this may be due to the relatively low sensitivity of PIVKA-II as a marker of vitamin K deficiency. We diagnosed these patients as having suspected vitamin-K-deficiency. In fact, vitamin K dependent coagulation factors were significantly increased after the administration of vitamin K. Judging from the results for the molecular markers TAT and F1+2, no hemostatic activation was observed in either of the groups of 5 patients suspected of having vitamin K deficiency.

We propose the following theoretical basis for the notion of no hemostatic activation after vitamin K administration, which partially agrees with the results of Ronden et al. [28]: (1) the upregulation of coagulation factor synthesis by vitamin K is unlikely in patients without vitamin K deficiency (shown in Table 2), because vitamin K acts neither at the level of transcription nor at that of translation, (2) overcarboxylation of Gla proteins has never been reported.

This is the first study to investigate whether oral vitamin K administration, given in the form of MK-4 to elderly patients with osteoporosis, adversely affects hemostatic balance. Our findings indicate that MK-4 can be administered safely to osteoporotic patients receiving no anticoagulant therapy.

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