Pleiotropic actions of vitamin K: protector of bone health and beyond?

Masao Kaneki, M.D., Ph.D.,a,b,* Takayuki Hosoi, M.D., Ph.D.,c Yasuyoshi Ouchi, M.D., Ph.D.,d and Hajime Orimo, M.D., Ph.D.e

a Department of Anesthesia and Critical Care, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts, USA
b Shriners Hospital for Children, Boston, Massachusetts, USA
c Department of Advanced Medicine, National Center for Geriatrics and Gerontology, Aichi, Japan
d Department of Geriatric Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
e University of Health Science, Yamanashi, Japan

Manuscript received December 6, 2005; accepted May 4, 2006.

Abstract

Vitamin K is a nutrient that was originally identified as an essential factor for blood coagulation. Recently, vitamin K has emerged as a potential protector against osteoporosis, atherosclerosis, and hepatocarcinoma. Accumulated evidence indicates that subclinical non-hemostatic vitamin K deficiency in extrahepatic tissues, particularly in bone and possibly in vasculature, exists widely in the otherwise healthy adult population. Vitamins K1 and K2 have been shown to exert protective effects against osteoporosis, although it is important that the beneficial effects will be further confirmed by large-scale, randomized, clinical trials. Increasing evidence implicates a role for vitamin K in calcification of arteries and atherogenesis. Moreover, the therapeutic potential of vitamin K2 as an antihepatoma drug has recently been highlighted. Most of the new biological functions of vitamin K in bone, vasculature, and hepatoma cells are considered attributable to promotion of γ-carboxylation of glutamic acid residues in vitamin K–dependent proteins, which is shared by vitamins K1 and K2. In contrast, vitamin K2–specific, γ-carboxylation–unrelated functions have also been demonstrated. Thus, biological differences between vitamins K1 and K2 and potential involvement of γ-carboxylation–independent actions in the new roles of vitamin K remain open issues. Molecular bases of coagulation-unrelated pleiotropic actions of vitamin K and its implications in human health deserve further investigations. © 2006 Elsevier Inc. All rights reserved.

Keywords: Menaquinone; Phylloquinone; Subclinical vitamin K deficiency; Undercarboxylated osteocalcin; Osteoporosis; Hepatocellular carcinoma; Atherosclerosis

Introduction

Vitamin K was originally identified as a fat-soluble nutrient required for coagulation and then discovered to be an essential cofactor for post-translational modification of glutamic acid (Glu) residues to γ-carboxyglutamic acid (Gla) residues of vitamin K–dependent hepatic blood-coagulating proteins including prothrombin and factors II, VII, IX, and X [1]. Hence, vitamin K deficiency results in a bleeding tendency due to malfunction of vitamin K–dependent clotting factors. In particular, neonates are susceptible to vitamin K–deficiency bleeding, and therefore prophylactic vitamin K supplementation has been successfully employed in neonates [2,3]. Recommended dietary intake of vitamin K has been determined based on γ-carboxylation status of coagulation factors.

Recently, however, coagulation-unrelated functions of vitamin K have attracted scientific attention [4–6]. These pleiotropic actions of vitamin K include potential protective effects against osteoporosis, hepatocarcinoma, and atherosclerosis. In contrast to newborn babies, in the absence of aggravating factors such as chronic gastrointestinal disorders or parental feeding in critically ill patients, vitamin K deficiency in terms of blood coagulation, referred to as “classic (clinical)” vitamin K deficiency, is rare. Nonetheless, a growing body of evidence indicates that “subclinical” vitamin K deficiency in extrahepatic tissues, particularly in
bone, is not uncommon in the adult population. Classic (clinical) vitamin K deficiency causes hemorrhage. In contrast, subclinical vitamin K deficiency is related to pleiotropic actions in bone, possibly in the vasculature, and locally in hepatoma cells. It has been proposed to contribute to osteoporosis, aortic calcification, atherosclerosis, and hepatoma development (Table 1).

Vitamin K exists in two forms in nature: vitamin K1 (phylloquinone) and vitamin K2 (menaquinones). Vitamin K1 is produced by plants and algae and is widely distributed in green and leafy vegetables; vitamin K2 is of microbial origin and is contained in meats, eggs, curd, cheese, and fermented soybeans. Menaquinones comprise a family of molecules distinguished from phylloquinone by unsaturated side chains of isoprenoid units varying in length from 1 to 14 repeats (Fig. 1). With regard to hemostasis, actions of vitamins K1 and K2 have been considered quite similar or essentially the same. In contrast, several lines of evidence suggest that vitamins K1 and K2 may also have distinct roles in the pleiotropic actions. Vitamin K2 may also have \(\gamma\)-carboxylation–independent functions. In this review, we provide an overview of recent studies on the emerging new roles for vitamin K in bone, vasculature, and hepatoma cells and attempt to clarify questions to be answered for future research.

### Subclinical vitamin K deficiency in bone

Among vitamin K–dependent proteins in bone, osteocalcin (OC, also termed bone Gla protein), matrix Gla protein (MGP), and protein S, \(\gamma\)-carboxylation of OC has been extensively studied. In healthy adults, a very small portion of blood clotting factors is undercarboxylated. In contrast, a substantial portion of circulating OC is undercarboxylated [7,8]. Thus, circulating undercarboxylated OC (ucOC) is a more sensitive measurement of vitamin K status than are the conventional blood coagulation tests [9].

The cutoff value of ucOC for subclinical vitamin K deficiency has not been established, although Shiraki et al. [10] proposed an ucOC level of 4.0 ng/mL. They found that postmenopausal women with a serum ucOC level \(\geq 4.0\) ng/mL displayed lower serum vitamin K concentrations, higher bone resorption markers, deoxypyridinoline, and increased vertebral fracture incidence. To facilitate the discussion on the guideline of subclinical vitamin K deficiency, however, ucOC values measured by distinct assay systems will need to be standardized, because there are substantial variations in ucOC values among immunoassays.

A pathogenic role for ucOC in osteoporosis is poorly understood. OC knockout mice exhibited increased bone formation and resistance to ovariectomy-induced bone loss [11], suggesting that, although OC seems to be a regulator of bone formation, decreased function of OC due to impaired \(\gamma\)-carboxylation may not be necessarily associated with osteopenia. In contrast, MGP knockout mice displayed short stature, osteopenia and fractures, and accelerated calcification of arteries and cartilage [12]. These findings sug-

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**Table 1**

<table>
<thead>
<tr>
<th>Vitamin K Deficiency</th>
<th>Classic (clinical)</th>
<th>Subclinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\gamma)-Carboxylation of coagulation factors</td>
<td>Impaired</td>
<td>Normal</td>
</tr>
<tr>
<td>Bleeding tendency</td>
<td>(+)</td>
<td>(−)</td>
</tr>
<tr>
<td>Marker</td>
<td>Blood clotting factors (e.g., prothrombin time)</td>
<td>Undercarboxylated osteocalcin</td>
</tr>
<tr>
<td>Infants</td>
<td>Relatively common</td>
<td>Unknown</td>
</tr>
<tr>
<td>Adults</td>
<td>Rare</td>
<td>Relatively common</td>
</tr>
<tr>
<td>Vitamin K target tissue</td>
<td>Liver</td>
<td>Bone (possibly vasculature, hepatoma cells)</td>
</tr>
<tr>
<td>Actions (roles) of K(_1) versus K(_2)</td>
<td>Quite similar</td>
<td>Different (?)</td>
</tr>
<tr>
<td>Mechanisms of vitamin K action</td>
<td>(\gamma)-Carboxylation dependent</td>
<td>(\gamma)-Carboxylation dependent and independent (?)</td>
</tr>
<tr>
<td>Category of vitamin K function</td>
<td>Classic</td>
<td>Pleiotropic actions</td>
</tr>
</tbody>
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Fig. 1. Chemical structure of vitamins K1 and K2.
suggest that γ-carboxylation of MGP may have an important role in bone formation and mineralization.

Epidemiologic evidence of a link between vitamin K and osteoporosis

Accumulating epidemiologic evidence suggests that subclinical vitamin K deficiency contributes to age-related bone loss and osteoporotic fractures. First, an increased circulating level of ucOC is associated with an increased fracture incidence [13–15] and low bone mineral density (BMD) [16]. The Epidémiologie de l’ostéoporose (EPIDOS) prospective cohort study followed 7598 healthy elderly women for 22 mo and demonstrated that circulating levels of ucOC, but not of total OC, predicted hip fracture risk [15]. Second, cross-sectional studies have shown that low circulating levels of vitamin K (phyllloquinone and menaquinones) were associated with low BMD and bone fractures [17–22]. Third, prospective cohort studies have reported that vitamin K1 (phyllloquinone) intake is also correlated with fracture risk and BMD [23–25].

In contrast to phyllloquinone, a relation between menaquinone intake and bone mass or fracture has not yet been investigated. In Western populations, phyllloquinone constitutes a major part of vitamin K intake. However, previous studies have suggested that menaquinones may be more efficiently absorbed than phyllloquinone [26] and therefore argued that phyllloquinone and menaquinones may contribute to nutriment to a comparable extent even in Western populations, in which phyllloquinone intake is much greater than that of menaquinones [27]. Moreover, particularly in Japan, intake of natto, a Japanese fermented soybean food containing a large amount of menaquinone-7 (∼10 μg/g), has a great effect on circulating vitamin K status [28]. Collectively, therefore, relative contributions of phyllloquinone versus those of menaquinones to pleiotropic actions of vitamin K remain an open issue.

Osteoporosis is characterized by decreased bone mass and deranged microarchitecture, which contribute to bone fragility. In many previous studies, a significant correlation between bone fracture and various indices of vitamin K has been found [19–21,23–25]. However, with regard to its relation to BMD, controversial results have been reported. In some studies, phyllloquinone intake was correlated with fracture risk, but not with BMD [24], whereas phyllloquinone intake was significantly associated with BMD in other studies [25]. These apparent discrepancies may be explained by differences in methodology in measurements and target population, as discussed by the investigators [25]. Moreover, although Delmas et al. [16] showed a significant inverse correlation between circulating ucOC and BMD, they also demonstrated that circulating ucOC predicted fracture risk independent of BMD [15]. It has been suggested, therefore, that subclinical vitamin K deficiency may contribute to increased fracture incidence through BMD-dependent and -independent mechanisms, the latter of which is assigned to decreased bone quality [29]. Further studies are required to clarify this issue.

Treatment of involutional osteoporosis with vitamin K

A 48-wk, double-blind study found that menaquinone-4 (45 mg/d, n = 272) significantly improved metacarpal BMD compared with vitamin D3 (0.75 μg/d, n = 274), as judged by the microdensitometric method, in patients with involutional (primary) osteoporosis, i.e., postmenopausal and senile osteoporosis [30]. A 24-wk, randomized, open-label study [31] compared the effects of the combination of menaquinone-4 (45 mg/d) plus calcium supplementation (150 mg/d, n = 120) with those of calcium alone (n = 121) in patients with involutional osteoporosis and found that menaquinone-4 prevented age-related decrease in lumbar BMD and decreased fracture incidence, with a simultaneous decrease in ucOC and increase in total OC [31]. A double-blind, placebo-controlled study that enrolled 80 subjects found that menaquinone-4 (90 mg/d) increased metacarpal BMD in patients with involutional osteoporosis. These findings of beneficial effects of menaquinone-4 in involutional osteoporosis are consistent with results of other randomized clinical studies performed in Asia, particularly in Japan, by several independent groups [32–37]. Thus, these relatively small, randomized, intervention studies have consistently demonstrated the effectiveness of menaquinone-4 in involutional osteoporosis. However, it is important that the efficacy of menaquinone-4 be further confirmed by larger-scale, randomized, clinical trials. A 2003 report from the World Health Organization classified the efficacy of menaquinone-4 on BMD and vertebral fracture into the evidence B level, i.e., positive evidence from smaller, non-definitive, randomized, controlled trials [38], whereas the efficacy of estrogens and some bisphosphonates (alendronate and risedronate) was categorized into the evidence A level, i.e., positive evidence from at least one adequately powered, randomized, controlled trial.

With respect to degree of effectiveness, small clinical studies have shown that menaquinone-4 (45 mg/d) decreases the relative risk of vertebral fracture to an extent comparable to that of bisphosphonate (etidronate), hormone replacement, and calcitonin in postmenopausal osteoporosis [33,37], although larger clinical trials will be required for conclusive clarification of the efficacy of menaquinone-4 compared with other antosteoporotic drugs. Although menaquinone-4 significantly preserved BMD compared with the no-treatment group, the effects of other treatments on BMD seemed to be more pronounced than that of menaquinone-4 [37]. With regard to the effects on bone turnover, menaquinone-4 has been shown to decrease bone resorption by osteoclasts and to promote bone formation by osteoblasts [31,39,40]. In comparison with other antosteoporotic agents, such as estrogen and bisphosphonates, pro-
motion of bone formation by menaquinone-4 is more prominent than the anti–bone resorption action [33,41]. Based on in vitro studies, direct actions on cell survival/apoptosis, differentiation, and functions of osteoclasts, osteoblasts and their precursors have been proposed to mediate the salutary effects of menaquinone-4 [42,43].

In contrast to a relatively large dose of menaquinone-4 (45 mg/d), a much lower dose of phylloquinone (1 mg/d) has been demonstrated to exert protective effects on BMD in postmenopausal women [44], although the effects of phylloquinone on fracture incidence has not been investigated. Vermeer et al. [44] compared Caucasian postmenopausal women who received a placebo (n = 600), a supplement containing minerals (500 mg of calcium, 10 mg of zinc, 150 mg of magnesium) plus vitamin D (8 μg, n = 46), or minerals plus vitamin D with additional phylloquinone (1 mg/d, n = 56) and followed up BMD for 3 y. The double-blind intervention study found that supplementation with minerals, vitamin D, and phylloquinone significantly attenuated femoral neck bone loss compared with placebo and with minerals plus vitamin D [44]. In contrast, minerals plus vitamin D alone did not exhibit beneficial effects on bone mass.

Vitamin K has a wide safety range [5,40], although no sufficient data are available to define an upper tolerable level for vitamin K. No adverse side effects of vitamin K2 have been reported thus far, whereas menaquinone-4 (45 mg/d) has been used in patients with osteoporosis in Japan, Korea, Thailand, and Taiwan on a large scale since 1995. Animal and clinical studies have indicated that vitamin K administration does not result in a hypercoagulable state [31,45–47], although use of vitamin K is contraindicated in patients on anticoagulant (e.g., warfarin) therapy. Overall, the safety of vitamin K, up to 45 mg/d of menaquinone-4, has been well established in the adult population, except in pregnant women.

Menaquinone-4 versus phylloquinone

The beneficial effects of phylloquinone and menaquinone-4 are consistent. Nevertheless, the difference in doses of vitamin K used in the studies, namely 45 mg/d of menaquinone-4 versus 1 mg/d of phylloquinone, raises new questions: (1) What dose of vitamin K supplement is needed to revert subclinical vitamin K deficiency in bone? (2) Can the protective effects of menaquinone-4 (45 mg/d) be accounted for by reversal of subclinical vitamin K deficiency alone? (3) Do as-yet-undetermined γ-carboxylation–independent mechanisms also contribute to the protective effects of vitamin K, especially menaquinone-4? Considering that dietary vitamin K intake is an order of 100–400 μg/d, 45 mg/d of menaquinone-4 appears to be far beyond the level required for reversal of subclinical vitamin K deficiency. Further, administration of phylloquinone (1 mg/d) significantly decreased ucOC [44]. Therefore, 1 mg/d of vitamin K supplement may be sufficient to correct subclinical vitamin K deficiency in bone. Nonetheless, evidence arguing against this presumption also exists. In a randomized, open-label, clinical trial [48], patients with osteoporosis were administered with different doses of menaquinone-4 (15, 45, 90, and 135 mg/d) or vitamin D3 (0.75 μg/d). As expected, all doses of menaquinone-4 treatment significantly increased urinary excretion of Gla residue, a surrogate marker of total vitamin K–dependent γ-carboxylation as compared with vitamin D3. However, urinary Gla excretion was greater in patients who received higher doses of menaquinone-4 (45, 90, and 135 mg/d) than in those who received 15 mg/d of menaquinone-4 (urinary Gla [nanomoles per milligram of creatinine] : vitamin D [n = 38], 54 ± 4 [mean ± SEM]; 15 mg of menaquinone-4, [n = 41], 62 ± 3; 45 mg of menaquinone-4, [n = 40], 71 ± 4; 90 mg of menaquinone-4 [n = 41], 71 ± 4; 135 mg of menaquinone-4, [n = 34], 74 ± 5; P < 0.01, vitamin D versus 15, 45, 90, or 135 mg of menaquinone-4; P < 0.05, 15 mg versus 45 or 90 mg of menaquinone-4; P < 0.01, 15 versus 135 mg of menaquinone-4). Undercarboxylated OC was not examined in the study. These observations suggest that oral administration of 15 mg/d of menaquinone-4 may be insufficient to achieve maximal generation of vitamin K–dependent Gla residues in patients with involutional osteoporosis.

Recently, increasing evidence has suggested that menaquinones, but not phylloquinone, have γ-carboxylation–independent functions. γ-Carboxylation–independent actions have been proposed to contribute to the protective effects of menaquinone-4 on bone health, although direct in vivo evidence is lacking. Menaquinones function as ligands of “orphan” nuclear receptors, steroid and xenobiotic receptor, and pregnant X receptor, whose ligands and/or functions remain to be determined [49]. Phylloquinone exhibits one order of magnitude lower affinity to these steroid receptors relative to menaquinones, although the effectiveness of phylloquinone and menaquinones on γ-carboxylation in vitro is quite similar [50].

Another possibility is the antioxidant property of vitamin K [51]. Menaquinones have been proposed to protect neuronal cells from apoptosis by decreasing oxidative stress [52]. Phylloquinone and menaquinone-4 inhibited oxidative stress–induced cell death in oligodendrocyte precursors with EC50 (50% effective concentration) values of 30 and 2 nM, respectively, suggesting that menaquinone-4 is 15-fold more potent than phylloquinone as an antioxidant [52]. An earlier study reported that most phylloquinone is distributed in micromes in mammalian cells, where γ-carboxylation is catalyzed, but that menaquinones are preferentially localized to mitochondria [53]. In bacteria, menaquinones play a critical role in electron transfer in mitochondria and redox signaling, as does ubiquinone [54]. Moreover, a recent study has demonstrated that menaquinone-4 binds to 17β-hydroxysteroid dehydrogenase-4 and modulates estrogen metabolism [55]. These findings suggest that menaquinones, but
Vitamin K and atherosclerosis

In addition to bone, the role of vitamin K in atherosclerosis has been an issue of investigation for a couple of decades. Atherosclerotic plaque contains a vitamin K–dependent protein, MGP. Gene disruption of MGP results in extensive aortic and coronary calcification and osteopenia [12]. Warfarin, an antagonist of vitamin K–dependent γ-carboxylation, induces calcification in arteries and heart valves of rats [61, 62]. Oral anticoagulant treatment with coumarin was associated with increased aortic valve calcification in humans [63]. MGP binds to bone morphological protein-2 (BMP-2) and inhibits BMP-2–induced osteoblast differentiation and calcification in cultured cells. Undercarboxylated MGP does not retain the capability of binding to BMP-2. Therefore, it has been proposed that impaired γ-carboxylation of MGP increases vascular calcification by allowing BMP-2 to induce mineralization [64]. Recently, undercarboxylated MGP was detected in the intima of human atherosclerotic arteries and in media of Mönckeberg atherosclerotic lesions with calcification, but not in non-atherosclerotic arteries [65], thus supporting a role for MGP in atherosclerotic development and vascular calcification.

Pharmacologic doses of menaquinone-4 have consistently ameliorated vitamin D–induced aortic calcification in rats [66]. Supplementation with menaquinone-4, but not with phylloquinone, prevented warfarin-induced aortic calcification in rats [50]. Utilization of menaquinone-4 is more efficient than that of phylloquinone in the aorta, although phylloquinone and menaquinone-4 were utilized equally in the liver [50]. However, it is important to note that phylloquinone can be converted into menaquinone-4 in rodents [67], and this conversion is tissue specific [68]. Our preliminary results showed that oral phylloquinone administration results in increased serum concentration of menaquinone-4 and phylloquinone in postmenopausal women (M. Kaneki, T. Hosoi, Y. Ouchi, and H. Orimo, unpublished observations). Therefore, menaquinone-4 converted from phylloquinone might mediate the protective effects of prolonged supplementation of phylloquinone in vasculature in humans. As with osteoporosis, it is unknown whether the efficiency of pharmacologic doses of menaquinone-4, which is far beyond the level of dietary intake, in the vasculature of animals can be accounted for by reversal of subclinical vitamin K deficiency or attributed to as yet undetermined pharmacologic effects of menaquinone-4.

The Rotterdam Study, a prospective, population-based cohort study comprising 7983 individuals, demonstrated that dietary intake of menaquinones, but not of phylloquinone, was associated with aortic calcification and coronary heart disease after adjustment for age, gender, body mass index, smoking, diabetes, education, and dietary factors [69]. Compared with the lower tertile, the upper tertile of menaquinone intake exhibited a lower incidence of coronary heart disease mortality (relative risk, 0.43; 95% confidence interval, 0.24–0.77) and severe aortic calcification (odds ratio of 0.48; 95% confidence interval, 0.32–0.71). A 3-y, double-blind, placebo-controlled, intervention study analyzing 108 postmenopausal women demonstrated that supplementation of vitamin K1 (1 mg/d) with vitamin D (8 µg/d) and minerals (calcium, zinc, and magnesium) significantly preserved the elastic properties of the carotid artery, although vitamin D plus minerals alone did not exhibit beneficial effects [70].
Vitamin K as a potential inhibitor of hepatocarcinoma development

Des-γ-carboxyprothrombin (PIVKA-II) has been established as a marker in the diagnosis and prognosis of hepatocellular carcinoma (HCC) [71]. Prothrombin is a vitamin K–dependent plasma coagulation factor that is synthesized in the liver. Vitamin K–dependent γ-carboxylation of 10 glutamic acid residues in the precursor of prothrombin is necessary for the coagulation activity. PIVKA-II is an abnormal prothrombin that is not fully carboxylated. PIVKA-II is expressed not only in cancer tissue but also in the surrounding non-cancer tissue of HCC [72]. Vitamin K content was decreased in HCC tissues compared with non-tumorous parts of the liver [73]. However, molecular bases underlying increased PIVKA-II in HCC remain elusive.

An 8-y, randomized, but not placebo-controlled, study analyzing 40 cases found that the risk ratio in postmenopausal women with viral liver cirrhosis treated with menaquinone-4 (45 mg/d) was 0.20 (95% confidence interval 0.04–0.91), compared with those who did not receive it [74]. Moreover, a recent 3-y, randomized, non-placebo-controlled study that analyzed 61 patients showed that the hazard ratio of HCC recurrence in patients treated with menaquinone-4 (45 mg/d) was 0.27 (95% confidence interval 0.12–0.60) compared with those who did not receive it. Menaquinone-4 decreased serum PIVKA-II in patients with HCC [75]. Menaquinone-4 inhibited growth and invasion of HCC cells in vitro [76,77] and decreased tumor growth and body weight loss in a mouse model of human HCC [76]. Of interest, a recent study has demonstrated that PIVKA-II binds to Met, a receptor for hepatocyte growth factor, and activates the Janus kinase-1 signal transducers and activators of the transcription 3 (STAT3) signaling pathway [78]. The investigators proposed that PIVKA-II may have a pathogenic role as an autologous growth factor for HCC. These findings warrant a large-scale, placebo-controlled, randomized, clinical trial to determine the efficacy of menaquinone-4 against HCC.

Conclusions

An increasing body of work indicates that subclinical vitamin K deficiency may be associated with osteoporosis and possibly with hepatocarcinoma and atherosclerosis. The efficacy of vitamin K in the prevention and/or treatment of these diseases deserves large-scale intervention studies. Molecular mechanisms underlying the emerging new roles for vitamin K await further investigations.

Acknowledgments

The authors apologize to colleagues whose work has not been cited in this review due to space limitation.

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