

New Perspectives for the Nutritional Value of Vitamin K in Human Health

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Abstract

Vitamin K is an essential micronutrient in the post-translational modification of specific glutamic acid residues (Glu) into γ -carboxyglutamic acid residues (Gla) in target proteins known as vitamin K-dependent proteins (VKDPs). In healthy conditions of sufficient vitamin K status, a vitamin K recycling system maintains sufficient vitamin K levels for proper γ -carboxylation of VKDPs, and vitamin K antagonists (VKAs) widely used as anticoagulants inhibit vitamin K recycling. Besides its well-known function in the maintenance of normal coagulation, vitamin K has been reported to have other diverse physiological functions with impact in human health. In extra-hepatic tissues vitamin K deficiency results in impairment of VKDPs γ -carboxylation with important implications in bone and cardiovascular health. Although most of the vitamin K effects have been associated with regulation of mineralization in connective tissues through the action of matrix Gla protein (MGP) and osteocalcin (OC), the discovery of Gla-rich protein (GRP) opens new perspectives on the potential therapeutic range of vitamin K.

Keywords: Vitamin K; γ -carboxyglutamic acid; Menaquinones; Phylloquinone

Introduction

Vitamin K is a lipid-soluble vitamin originally discovered by Dam as a necessary factor for normal haemostasis [1]. For many years considered as required only for the synthesis of blood clotting factors in the liver, the continuous discovery of extra-hepatic Gla proteins clearly showed that vitamin K functions lies beyond its function in blood coagulation, and is involved in multiple biological processes such as in bone and in the vasculature [2-7]. Vitamin K is an essential micronutrient acting as cofactor for the post-translational modification of vitamin K-dependent proteins (VKDPs), where specific glutamic acid (Glu) residues can be modified to calcium binding γ -carboxyglutamic acid (Gla) residues, through the action of γ -glutamyl carboxylase (GGCX) enzyme [6-8]. The use of vitamin K antagonists (VKAs) such as warfarin, known to influence the carboxylation of Glu residues of the coagulation factors in liver, has been shown to also impair the γ -carboxylation of extra-hepatic VKDPs, resulting in unwanted pathological side-effects in tissues such as bone and blood vessels [6,7,9-12]. Recently, additional functions of vitamin K, such as anti-inflammatory, transcriptional regulator of osteoblastic genes, and inhibition of tumor progression, have been proposed to be mediated by a direct vitamin K effect rather than through VKDPs action [13,14]. However, the function of VKDPs associated to these emerging functions of vitamin K has been poorly explored. Vitamin K status has been inversely correlated with circulating inflammatory markers and proposed to exert its anti-inflammatory function by suppressing nuclear factor κ B (NF- κ B) signal transduction [15-17]. Furthermore it has been suggested to have a protective effect against oxidative stress through blocking of reactive oxygen species generation [18].

Vitamin K forms and recycling

Naturally occurring vitamin K compounds with classical cofactor activity required for γ -carboxylation reaction, comprise the phylloquinone (vitamin K1), and a series of menaquinones (MKs) (vitamin K2), which share a common 2-methyl-1,4-naphthoquinone ring structure (also known as menadiolone (MD) or vitamin K3) and an isoprenoid side chain, that differs in length and degree of saturation, depending on the organism by which they are synthesized [18,19]. Phylloquinone contains a phytyl side chain, which has only one unsaturated bond, and is found in plants and cyanobacteria [18,19]. Menaquinones are predominantly produced by bacteria and composed by a side chain with repeating isoprene residues, each containing an unsaturated bond [18-20]. In human diet their main sources are fermented foods represented by cheese and curd in Western diets, and the traditional Japanese food made from soybeans, natto. Depending on the number of prenyl repeats, menaquinones are subcategorized as MK-n, with n corresponding to the number of isoprenoid units, generally ranging from 4 to 13 [18-20]. MK-4 and MK-7 are commonly used as vitamin K2 supplements. These molecular forms of vitamin K have different cofactor activities and behave differently in processes such as absorption, transport, cellular uptake, tissue distribution and turnover [18,19]. While K1 is a major type (>90%) of dietary vitamin K, its concentrations in animal tissues is remarkably low compared with those of menaquinones, especially MK-4, which is the major form (>90%) of vitamin K in tissues. It is known that mammals have the ability to convert dietary K1 into MK-4, which is then stored in specific tissues [21]. This conversion was recently shown to be via side chain removal/addition mechanism with MD as the intermediate molecule, and specifically regulated at tissue level [21].

The γ -carboxylation of VKDPs, that comprises the conversion of Glu to Gla residues catalysed by the GGCX enzyme, requires the presence of a reduced form of vitamin K (vitamin K hydroquinone, KH₂), carbon dioxide and oxygen, and the continuous recycling of vitamin K 2,3-epoxide (KO) to its quinone (K) and hydroquinone

(KH2) forms, in successive reactions catalysed by vitamin K reductases [8,22,23]. The two enzymes known to be involved in this vitamin K cycle are vitamin K epoxide reductase (VKOR) and vitamin K reductase (VKR), in a process known as the vitamin K cycle [8,22]. Each vitamin K molecule can thus be recycled several thousand times with this vitamin K cycle, which is the reason why minute vitamin K amounts are sufficient to cover its daily diet requirements [24]. However, the present dietary reference values for vitamin K (90 µg/day for women and 120 µg/day for men) [10] are based on proper functioning of the blood coagulation factors to maintain normal haemostasis, and not on the γ -carboxylation status of other VKDPs such as matrix Gla protein (MGP), Gla-rich protein (GRP), and osteocalcin (OC), known to be of vital importance in bone and/or vascular health [2-7,18,24]. Interestingly, extra-hepatic Gla proteins have been shown to be present as incompletely γ -carboxylated forms in the majority of healthy adults [4,25], and thus the biological activity of these proteins could be considered sub-optimal. Since VKOR is a dithiol dependent enzyme known to be inhibited by 4-hydroxycoumarin anticoagulant drugs, such as warfarin, acenocoumarol, and phenprocoumon, the widely use of these oral anticoagulants acting as vitamin K antagonists (VKAs), has also been linked to unwanted side effects in several extra-hepatic tissues with adverse clinical outcomes [4,7-9,11,12]. Remarkably, despite the long use of VKA the exact mechanism of inhibition of VKOR remains to be elucidated [26].

Vitamin K-antagonists as indicators of vitamin K importance in health

The extra hepatic effects of VKAs were first suggested when it was found that women receiving VKAs treatment between the 6th and 12th week of pregnancy gave birth to children with severe bone abnormalities [27]. Since then, many in vitro and in vivo experiments have shown that VKAs induce vitamin K deficiency, which has been unequivocally related to increased mineralization of several tissues, particularly in the vascular tree and skeletal elements. Rats treated with warfarin presented extensive vascular calcification which could be inhibited by simultaneous treatments with vitamin K [28-30]. Furthermore, vitamin K was shown to induce a regression of pre-formed warfarin-induced vascular calcifications, with restoration of arterial distensibility [31]. The ApoE knockout mice model of atherosclerosis treated with warfarin displayed increased atherosclerotic plaque calcification and plaque vulnerability [32]. In humans, VKA use was associated with coronary artery plaque calcification in patients with suspected coronary artery disease (CAD), where calcification of coronary plaques significantly increased with prolonged VKA use [32,33]. Also, increased calcification of aortic valves was observed in patients receiving preoperative VKAs relative to non-treated patients [34,35]. Chronic kidney disease (CKD) patients, a population with a high prevalence of cardiovascular mortality and vascular calcifications, have been associated with subclinical vitamin K deficiency [35,36]. In a CKD rat model, warfarin treatments increased vascular calcification while high dietary vitamin K1 increased vitamin K tissue concentrations and attenuated vascular calcification [37]. Calciphylaxis is an often fatal complication of end-stage renal disease characterized by subcutaneous small arterioles calcification. Interestingly, approximately 50% of stage 5 CKD patients who develop calciphylaxis were on VKA therapy, which was proposed as one of the risk factors in the absence of severe disorders of calcium metabolism [9,38]. In the population-based Rotterdam study vitamin K2 intake was found inversely related to all-cause mortality and severe aortic

calcification [39]. Vitamin K supplementation has been demonstrated as able to reduce bone turnover and improving bone strength [40,41], and higher levels of vitamin K intake were associated with decreased risk of hip fracture [42-44]. Higher doses of vitamin K2 have been administered for osteoporosis treatment in Japan for several years [45]. Decreased bone mineralization and turnover was reported in rats treated with warfarin [46], and long-term warfarin therapy in humans has been associated with decreased bone mineral density [47,48]. Subclinical vitamin K levels have also been associated with an increased risk of osteoarthritis development [49,50].

Vitamin K mechanism of action

It is presently accepted that the most plausible mechanism underlying dysregulated mineralization in soft tissues associated to vitamin K is the impairment of γ -carboxylation in VKDPs that have been known to have a role as regulators of mineralization. Insufficient γ -carboxylation either by dietary vitamin K deficiency or impairment of vitamin K recycling leading to the exhaustion of vitamin K storage, results in the production of inactive undercarboxylated VKDP forms. OC and MGP have been widely associated with mineralized tissues, regulation of mineralization processes, and vascular ectopic calcification, and considered the two VKD target proteins involved in bone and vascular tissues health, respectively [2-7,9,51]. The accumulation of their undercarboxylated (uc) protein forms has been implicated with loss of functionality and pathological mineralization. OC is a small secreted protein with 46-50 amino acids containing 3 Gla residues in most species, and highly expressed and accumulated in bone [52]. Initial in vitro studies have shown that OC binds to hydroxyapatite (HA) through its Gla residues inhibiting HA formation [53]. OC knockout mice evidenced that OC acts as a negative regulator of bone formation without altering bone resorption or mineralization, with a role in stimulating bone mineral maturation [54,55]. OC is presently considered a marker for bone formation [56] and circulating uncarboxylated OC has been proposed as a sensitive marker for vitamin K deficiency [57,58]. MGP contains 4-5 Gla residues and is synthesized by chondrocytes, vascular smooth muscle cells (VSMCs), endothelial cells, and fibroblasts [51]. It is presently considered one of the most powerful vascular calcification inhibitor known to date, and it is undercarboxylated form has been proposed as a biomarker for cardiovascular calcification and for vitamin K status [51,59-61]. Knockout mice for MGP die within 8 weeks of birth due to massive vascular mineralization of the main arteries, a phenotype similar to that obtain with warfarin treatments [62]. Interestingly, the Keutel syndrome in humans, which is characterized by loss-of-function mutations in the MGP gene results in non-lethal abnormal soft tissues calcification, which suggest additional or compensatory mechanisms of mineralization inhibition in humans. The discovery of an additional VKDP, Gla-rich protein (GRP), also expressed and accumulated in skeletal and vascular tissues [63-65], that we have recently shown to function as a calcification inhibitor in the cardiovascular [66] and articular systems [67], should open a new window of knowledge in the area of pathological calcification of connective tissues and increase the range of vitamin K action. GRP was initially identified in sturgeon calcified cartilage and characterized by the presence of an unprecedented 16 Gla residues within its 74 amino acid total protein sequence, and an impressive degree of evolutionary conservation [63]. The metal binding properties of Gla residues within the VKDP family have been associated with binding of calcium ions or calcium crystals, either through Ca²⁺ coordination in the Ca²⁺ dependent binding of coagulation factors to anionic phospholipids membrane surfaces [68],

or through binding to HA crystals, the major mineral component present in mineralized extracellular matrix, and associated either with physiological (eg., in bone) or pathological processes in soft tissues [69]. Fully γ -carboxylated GRP in human can include 15 Gla residues, which confers to this protein outstanding calcium and mineral binding capacity [25,63,64]. Undercarboxylation of GRP has been recently associated with several pathological calcification related diseases, as calcific aortic valve disease [66], osteoarthritis [67] and certain cancers [25], while only γ -carboxylated GRP has shown to display anti-mineralization capacity [66,67]. Interestingly, we have recently shown that GRP is involved in the crosstalk between inflammation and calcification of articular tissues in osteoarthritis, acting as an anti-inflammatory agent [67]. Since calcification and inflammation are common and interconnected events in calcification-related chronic inflammatory diseases, the importance of GRP and vitamin K might acquire a new dimension in healthy and pathological states. Since the focus of VKAs treatments in ectopic calcification has been mainly restricted to the function of MGP and OC, the effects on GRP functionality and the consequences in the mineralization levels are currently unknown. However, it reinforces the notion that special care should be given to the widely used VKAs agents whose side effects are certainly still not completely unraveled.

Conclusions

Although innumerable studies have shown a protective role of vitamin K against pathological mineralization by improving the function of VKDPs acting as calcification regulators, and no toxicity is known for higher vitamin K dosages, the unequivocal beneficial effect on vitamin K supplementation in human dietary is still debatable. Nevertheless, vitamin K is a potential therapeutic target for highly prevalent diseases involving pathological mineralization such as cardiovascular diseases and osteoarthritis. In addition, the knowledge of the association of inflammatory processes to the etiology of these diseases and the increased attention to novel biological functions of vitamin K, namely its antioxidant and anti-inflammatory role, should point for a more global evaluation of its role in human health and its beneficial use as a nutritional supplement. Furthermore, despite the detrimental side effects of VKAs therapy, additional studies on long-term vascular, bone and systemic effects of warfarin need to be performed, in order to understand whether its proven benefits in patients at risk of thromboembolic disease prevails over its negative impact in connective tissues. In this area there is a growing scientific knowledge underscoring a need for anticoagulants that do not interfere with the vitamin K-cycle.

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