Vitamin K and vascular calcification

Jennifer S Lees[1]

Patrick B Mark[1]

Miles D Witham[2]

1. Institute of Cardiovascular and Medical Sciences, BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, UK

2. AGE Research Group, NIHR Newcastle Biomedical Research Centre, Newcastle University and Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle, UK

Correspondence to: Professor Miles Witham, NIHR Newcastle Biomedical Research Centre, Biomedical Research Building, Campus for Ageing and Vitality, Newcastle upon Tyne NE4 5PL, UK. Tel: +44 (0)191 208 1317. Email: Miles.Witham@newcastle.ac.uk

Abstract

Purpose of review

Vascular calcification is a common and important cardiovascular risk factor in patients with chronic kidney disease (CKD). Recent advances in the understanding of the biology of vascular calcification implicate vitamin K dependent proteins as important regulators in this process. This review highlights recent key advances in vascular biology, epidemiology and clinical trials in this rapidly evolving field.

Recent findings

Vitamin K deficiency is associated with increasing severity of vascular calcification among patients with CKD, but the relationship with cardiovascular disease and mortality is inconsistent. Vitamin K may reduce calcification propensity by improving the activity of vitamin K-dependent calcification inhibitors or by down-regulating components of the innate immune system to reduce inflammation. However, recent randomized controlled trials in patients with diabetes, CKD, renal transplant and on hemodialysis have failed to demonstrate improvement in vascular calcification or stiffness after vitamin K treatment.

Summary

Current evidence does not support a clinically useful role for vitamin K supplementation to prevent or reverse vascular calcification in patients with CKD. Knowledge gaps remain, particularly whether higher doses of vitamin K, longer duration of supplementations or use a vitamin K as part of a package of measures to counteract vascular calcification might be effective.

Key words:

Vascular calcification; chronic kidney disease; vitamin K

Introduction

Vascular calcification is a major clinical issue in patients with chronic kidney disease (CKD). The prevalence and severity of vascular calcification increases with worsening CKD [1,2], being a particular challenge for patients with end stage kidney disease on dialysis. Vascular calcification increases the risk of cardiovascular events, both by increasing vascular stiffness but also via an independent increase in risk from vascular stiffness [3]. Finding ways to prevent or reverse vascular calcification is therefore a topic of great importance within nephrology and in other groups affected by vascular calcification [4].

Until recently, the biology underlying vascular calcification was poorly understood and calcification was assumed to be a passive process involving precipitation of calcium salts out of the blood into blood vessel walls. Over the last two decades however, our understanding of the biology of vascular calcification has been transformed and it is now clear that this is an active, regulated process akin to bone formation. As such a number of potential targets for pharmacological manipulation have been revealed by discovery science studies, and this has led to a slew of recent observational and clinical intervention studies.

Most prominent amongst these pharmacological targets have been vitamin K dependent proteins [5]. It is now clear that such proteins play key roles in the regulation of calcification, particularly inhibition of calcification within the vasculature [6-8]. Although vitamin K is best known as a cofactor for proteins in the coagulation cascade, it is now clear that it has a much wider range of biological roles. Its safety, widespread availability and low cost make it an attractive intervention for study. Vitamin K intake in older patients (who make up the majority of patients with CKD) is known to be low [9]; in addition, uremia is associated with a relative resistance to vitamin K (sometimes referred to as 'functional' vitamin K deficiency [10]; additional vitamin K is needed to overcome this resistance. Although serum vitamin K levels are a poor guide to whole body vitamin K status, measurement of biomarkers of vitamin K repletion (desphospho-uncarboxylated matrix Gla protein [dp-ucMGP] and related species)

in patients with CKD confirm that biological insufficiency of vitamin K is present in this group of patients [11].

This review will focus on three key areas - the most important advances in our understanding of vitamin K biology pertaining to vascular calcification, recent results from observational studies in this field, and results from a series of randomized controlled trials testing vitamin K in populations with CKD and with related cardiovascular conditions.

Advances in our understanding of Vitamin K and vascular calcification

Vascular smooth muscle cells (VSMC) are pivotal in the pathophysiology of intimal and medial vascular calcification. Contractile VSMC are found in the medial layer of the vessel wall and maintain blood vessel elasticity. Alterations to the extracellular environment – such as in hyperphosphatemia, uremia and diabetes – can lead to VSMC trans-differentiation to synthetic VSMC, exosome release [12] and apoptosis [13], resulting in calcium-phosphate deposition, medial vascular calcification and arterial stiffening. Figure 1 illustrates the interplay of these processes.

Vitamin K is an essential co-factor for carboxylation (activation) of the vitamin K dependent proteins (VKDP) by the gamma glutamyl carboxylase (GGCX) enzyme. Extra-hepatic VKDP (including osteocalcin, Gas-6 and Gla-rich protein) regulate vascular calcification, but MGP is considered the most potent inhibitor. The negatively-charged Gla domains in active MGP bind calcium, directly preventing the growth and deposition of calcium crystals. MGP directly inhibits bone morphogenetic proteins (BMP), which in turn regulates the trans-differentiation of VSMC from contractile to synthetic phenotypes [14,15].

Vitamin K deficiency is more directly associated with inflammation: vitamin K deficiency is observed in various inflammatory diseases including CKD, peripheral arterial disease, diabetes mellitus, inflammatory bowel disease and even cognitive dysfunction [16-20]. In animal models, vitamin K

supplementation can directly reduce inflammation [21,22] by manipulating activity of the NLRP3-inflammasome, a critical modulator of the innate immune system [23*,24]. Inflammation is itself an important and direct driver of vascular calcification, through endothelial-to-mesenchymal transition and enhanced BMP signaling: a process which ultimately results in mineralization of endothelial cells [25].

Although much research has concentrated on the effects of vitamin K-dependent proteins as mediators of the biological effects of vitamin K on vascular calcification, recent findings are a reminder that vitamin K biology is complex and that not all vitamin K dependent pathways inhibit vascular calcification. Administration of the MK-4 vitamin K2 subtype to rodents increased lipid levels in one recent study [26], potentially accelerating atherosclerosis and its associated calcification. In another recent study, MK-4 administration accelerated calcification of aortic valve cells in a high-phosphate environment (hence similar to that found in advanced CKD); this effect was mediated by vitamin K's actions as a nuclear steroid receptor ligand at the pregnane X receptor (the murine counterpart of the steroid and xenobiotic sensing nuclear receptor in humans) [27*]. Such findings suggest a balance of pro and anti-calcification activities driven by vitamin K, and the subtype of vitamin K may thus be of importance in which side of the balance is favored.

Recent observational studies pertaining to vitamin K and vascular calcification

There is limited direct epidemiological evidence that dietary vitamin K intake reduces risk of future vascular calcification. However, there are three main strands of enquiry which support this concept. Firstly, there are studies that suggest that higher dietary intake of vitamin K may be associated with lower risk of future cardiovascular events in groups who are otherwise at higher risk of cardiovascular disease. Secondly, there are observational data supporting a relationship between biochemical markers of vitamin K deficiency and cardiovascular events. Thirdly, there are a number of

observational cross-sectional studies suggesting that either vitamin K intake or biochemical evidence of vitamin K deficiency are correlated with clinical measures of vascular calcification.

Dietary intake and risk of future cardiovascular disease events

Although there is a fairly widespread belief that dietary intake of food rich in vitamin K (e.g., green vegetables such as kale, spinach, lettuce) is 'healthy' and might be associated with lower risk of future cardiovascular events, there are fairly limited data to support this notion [28]. Large observational studies suggest that increased intake of green vegetables may be associated with lower risk of cardiovascular or 'chronic disease' [29,30], but other studies have shown no specific reduction of risk of cardiovascular events with increased dietary intake of green vegetables although there may be fewer cardiovascular events specifically with cruciferous vegetables intake (likely to be high in vitamin K) [31]. These studies are limited by quantifying vegetable consumption rather than assessing vitamin K intake combined with being undertaken in populations at low risk of vascular calcification mediated vascular events. The strongest new evidence that there may be a protective effect of vitamin K intake comes from the Hordaland Health Study of 2986 participants aged 46-49 in Norway. A careful dietary assessment was conducted to estimate vitamin K intake. Over 11 years follow up higher intake of vitamin K2 was associated with lower risk of coronary heart disease, while there was no association between intake of vitamin K1 and coronary heart disease events [32**].

Evidence associating biochemical markers of vitamin K intake and CVD outcomes

There are a number of observational studies in patients at high risk of cardiovascular disease which tend to suggest that biochemical evidence of vitamin K deficiency is associated with future cardiovascular events or increased mortality in patients with established cardiovascular disease or at elevated cardiovascular risk. The majority of these studies demonstrate that patients with highest

levels of serum dp-ucMGP indicating relative vitamin K deficiency are at highest risk [33]. The relationship between high dp-ucMGP and mortality has been demonstrated in renal transplant recipients [34] and in otherwise stable cohorts following myocardial infarction and/or coronary revascularization or with stable vascular disease [35, 36]. Serum phylloquinone concentration as a marker of vitamin K status was associated with an increased risk of all-cause mortality, but not of cardiovascular disease in 3891 participants in the combined Multi-Ethnic Study of Atherosclerosis and Framingham Offspring Study [37*]. The relationship between dp-ucMGP and future cardiovascular events is somewhat inconsistent which may reflect the nature of the cohorts studied; it is possible that this relationship is more consistent in patients at higher cardiovascular risk. In support of this hypothesis, dp-ucMGP was not significantly associated with CVD risk overall in 1061 participants aged 70-79 free of cardiovascular disease over 12 years follow up [38] but dp-ucMGP levels were associated with increased risk of cardiovascular events in 518 patients with type 2 diabetes mellitus followed up over 11 years [39]. Similarly, in a cohort of 188 hemodialysis patients, who are at increased risk of both cardiovascular events and mortality, patients with low levels of desphospho-carboxylated matrix Gla protein (dp-cMGP; another marker of vitamin K deficiency) had an increased risk for all-cause and cardiovascular mortality. In this study, dialysis patients had profoundly elevated dp-ucMGP at 6.5-fold higher plasma levels compared to age matched healthy controls [40]. Finally, using Mendelian randomisation from data generated using large cohort studies in over 100,000 individuals with coronary heart disease, genetic risk scores associated with higher dp-ucMGP levels appeared to causally link to increased risk of cardiovascular events although a similar relation for phylloquinone and lower coronary heart disease risk was not seen [41].

Observational data specifically associating either vitamin K status with measures of vascular calcification

The inconsistency between populations studied and methods for assessing vascular calcification makes it difficult to draw direct comparisons between studies of vitamin K status and vascular calcification. Nonetheless, there a number of cross-sectional studies which suggest that vitamin K deficiency is associated with vascular calcification particularly in patients with CKD. Schlieper *et al* showed lower levels of dp-ucMGP in hemodialysis patients was associated with more extensive calcifications using a composite score based on imaging of the vessels of the arm and/or calcification of the aortic and mitral valves on ultrasound [40]. Similarly, in a cohort of 107 patients at various stages of CKD (37% were on dialysis), dp-ucMGP levels rose with advancing CKD stage with serum dp-ucMGP associated with increasingly severe calcification of the aortic valve on multi slice computed tomography scans [42]. Similarly, elevated dp-ucMGP has been associated with increasing calcification of the peripheral vasculature in patients with type 2 diabetes [43]. In a study combining histology of the inferior epigastric artery sampled at the time of kidney transplantation, and coronary artery calcium scoring, plasma levels of dp-ucMGP was a predictor of increased calcification both by coronary calcium scoring and by degree of medial calcification on histology [44].

This relationship between vitamin K deficiency and measures of calcification may be mediated by or specific to the presence of CKD or dependent on the methods used to assess vitamin K status, as in a cohort of healthy patients age 60-80 years, there was no correlation between plasma ucMGP and coronary artery calcification on computed tomography [45].

Evidence from studies of anticoagulants and other drug interactions

Further evidence of the importance of vitamin K status to vascular calcification and disease can be seen amongst patients treated with vitamin K antagonists (VKA) such as warfarin. VKA induce a state of iatrogenic vitamin K deficiency and are known to increase the risk of calciphylaxis [46], a condition characterized by aggressive calcification of the small blood vessels supplying the soft tissues. In one trial among older participants, the risk of cardiovascular disease in those treated with VKA was

substantially reduced in those who followed a Mediterranean diet (rich in vitamin K) compared with a control diet (hazard ratio 1.71 (95% CI 0.83-3.52) versus 4.22 (95% CI 1.92-9.30) [47]. In patients treated with anticoagulation for atrial fibrillation, the risk of adverse cardiovascular events is lower amongst patients treated with direct oral anticoagulants (DOAC) compared with VKA [48*]. Hyperphosphatemia is another important driver of calcification, which results from disrupted mineral and bone homeostasis and is common in advanced CKD and dialysis. The evidence for treating hyperphosphatemia in CKD is weak, but international guidelines recommend lowering serum phosphate towards normal [49]. However, this may not be a benign intervention: in dialysis patients, sevelamer carbonate - a commonly-used phosphate binder - is associated with vitamin K (MK-4) deficiency, aortic calcification and fracture risk [50**], possibly due to disruption of the gut microbiome [51].

Recent intervention studies using vitamin K to prevent or reverse vascular calcification

A number of randomized controlled trials testing the effect of vitamin K supplementation in patients with CKD have been published over the last 12 months. The largest trial [52*] was a randomized controlled trial examining the effect of 12 months of vitamin K2 (MK-7 subtype) 400 mcg once daily versus placebo in a population of 159 patients with CKD stage 3B or 4. No difference was seen in vascular stiffness as measured by carotid-femoral pulse wave velocity and no difference was seen in vascular calcification measured on plain film abdominal radiography using a semiquantitative scoring system. This was despite significant reductions in levels of dp-ucMGP and significant changes in osteocalcin, another vitamin K dependent protein.

A further recent trial [53,54] conducted in 90 kidney transplant recipients again found no benefit of vitamin K supplementation on vascular stiffness or measures or vascular calcification. This trial compared 5mg of menadiol three times per week with placebo given for 12 months. The primary outcome was vascular stiffness (aortic distensibility) measured by MRI; neither this nor key secondary

outcomes of coronary artery calcium score or carotid-femoral pulse wave velocity were improved relative to placebo by vitamin K.

Recent trials in other patient groups at high risk of cardiovascular disease and of vascular calcification have also not shown a benefit of vitamin K supplementation. Patients with type 2 diabetes mellitus receiving six months of 360mcg daily of vitamin K2 showed no difference in the rate of change in whole-body vascular calcification compared to placebo [55]; interestingly, a previous analysis of this same trial population [56] showed that calcification activity measured by ^{18}F-NaF positron emission tomography increased more in the vitamin K group than in the placebo group over six months, although baseline calcification mass was higher in the vitamin K group.

Coumadin derivatives such as warfarin have long been known to work by antagonizing the effects of vitamin K. Furthermore, the use of warfarin in patients with CKD has long been known to be associated with calciphylaxis. These observations underpinned the VALKYRIE randomized controlled trial [57*] in 132 patients on hemodialysis maintained on vitamin K antagonists. The trial randomized participants to continuation of vitamin K antagonists or substitution with rivaroxaban with or without vitamin K2 2000mcg three times a week for 18 months. Despite improvements in dp-ucMGP levels in both the rivaroxaban and rivaroxaban plus vitamin K2 arms, no differences were noted in vascular stiffness, coronary artery calcification or aortic calcification scores compared to the control group at 18 months. This contrasts with results from a trial of 80 patients with atrial fibrillation reported from Japan [58]; short-term substitution of warfarin with rivaroxaban produced a small but statistically significant improvement in brachial-ankle pulse wave velocity at 3 months (1.4% improvement in the rivaroxaban group; 3.5% worsening in warfarin group). Vascular calcification was not measured in this trial.

Conclusion

Despite a body of observational data suggesting that vitamin K status is associated with both vascular stiffness and cardiovascular events, systematic reviews over the last 12-18 months have not demonstrated a consistent benefit of vitamin K supplementation on vascular stiffness or vascular calcification, either in patients with CKD or in broader groups of patients [33,52,59]. It is possible that higher doses (giving more complete suppression of dp-ucMGP levels) [60], or longer duration of therapy (years or decades) [61] may be required to demonstrate effects. Although further clinical trials are in progress [62-64], the planned duration of therapy is 12-18 months; similar to trials already conducted and thus unlikely to answer the question of whether long-term vitamin K supplementation is efficacious. Numbers recruited to trials to date are too low to draw conclusions about the effect of vitamin K supplementation on cardiovascular events, and trials now in progress have not been designed with these endpoints as a key focus.

A number of other treatments are now under study to prevent or reverse vascular calcification [65*,66*], which look more promising than vitamin K. Alternatively, vitamin K supplementation alone may not be a sufficient intervention and the effect of combining vitamin K with other interventions may provide strategies for testing in future clinical trials.

Key Points

- Vascular calcification is present in excess in people with CKD and represents one mechanism underpinning the excess cardiovascular mortality in this patient group

- Vitamin K is an essential co-factor for carboxylation (activation) of vitamin K dependent proteins which inhibit vascular calcification, although this is a complex process whereby not all vitamin K dependent proteins inhibit calcification

- Observational cohort studies suggest that increased intake of foods rich in vitamin K such as cruciferous vegetables may be associated with a reduced risk of future cardiovascular events

- To date randomized controlled trials of vitamin K supplementation in patients with advanced CKD have not demonstrated beneficial effect on vascular calcification or vascular stiffness

- Further clinical trials of vitamin K supplementation are warranted to assess optimal dose, formulation and in which patient groups vascular calcification may be modifiable such as those with proven vitamin K deficiency

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Figure 1. Central role of carboxylated matrix Gla protein in preventing vascular calcification

Legend: BMP: Bone morphogenetic protein. GGCX: gamma glutamyl carboxylase. cMGP: carboxylated matrix Gla protein. ucMGP: uncarboxylated matrix Gla protein. VSMC: Vascular smooth muscle cell.