

Vitamin K in vascular health – more than just a role in coagulation

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■ INTRODUCTION

The annual mortality in patients with chronic kidney disease (CKD) is markedly greater than that of the normal population, especially in young patients. Cardiovascular disease accounts for more than 50% of all deaths in end-stage renal disease (ESRD) patients (Foley, 1998; Goodman, 2000). As a consequence, revealing the pathomechanisms of cardiovascular disease in CKD patients is mandatory since this can help to find solutions which may lead to improved outcomes. Traditional risk factors for arteriosclerosis, i.e. the Framingham risk factors of diabetes mellitus, hypertension, dyslipidaemia, cannot explain fully the excessive cardiovascular risk in CKD patients. Accordingly, therapeutic strategies (e.g. statins), successfully applied in classical arteriosclerosis patients, have only a limited benefit for CKD patients (Wanner, 2005).

Numerous so-called non-traditional risk factors have been discovered that contribute to the pathogenesis of vascular disease in uraemia. Cardiovascular calcification is regarded as the hallmark of CKD (Ketteler, 2005). The process of vascular calcification (VC), formerly regarded as a passive precipitation, is now known as an actively regulated, cell-mediated process (Moe, 2008). Numerous local and systemic factors influence VC development, either acting as pro-calcification or anti-calcification agonists (Schoppet, 2008). Among the calcification inhibitors, matrix Gla protein (MGP) merits special attention. It is expressed by vascular smooth muscle cells (VSMCs)

within the arterial media. MGP needs to undergo post-translational gamma-glutamyl carboxylation of the 5 glutamate residues to achieve full biologic activity. This activation is completely dependent on the availability of vitamin K. Therefore, MGP metabolism exhibits similarities to the biology of the coagulation factors II, VII, IX, X and protein C and protein S, which also require vitamin K for activation. In cases of vitamin K deficiency, MGP is not activated (under-carboxylated MGP, ucMGP) in contrast to the fully carboxylated, bioactive cMGP. The importance of a careful distinction between these two MGP subtypes has already been shown, since only the ucMGP accumulates in areas of vascular calcification (Schurgers, 2005). This accumulation has been interpreted as a sign of insufficient defence against VC.

■ VITAMIN K

Vitamin K consists of the group of phyloquinones (vitamin K₁) and several menaquinones (vitamin K₂) as well as the synthetic forms, menadion (vitamin K₃) and esterified menadion (vitamin K₄). Vitamin K₁ is found in membranes of chloroplasts of green leafy vegetables. The main source of vitamin K₂ is fermented food such as cheese (both soft and hard cheeses) and a Japanese specialty called natto, which is a fermentation product from soy beans containing very high concentrations of vitamin K₂ (MK7). In addition, the mammalian intestinal flora is able to produce vitamin K₂, but the extent to

which this contributes to the daily intake is still unknown (Suttie, 1995).

The discovery of vitamin K goes back to the 1930s, with the observation that chickens on a fat-free diet developed haemorrhages (Dam H., 1934). Shortly thereafter, the clotting factor prothrombin was the first coagulation factor to be discovered as being decreased in animals with haemorrhagic syndrome. Administration of vitamin K was then shown to cure bleeding complications in patients with hepatic and biliary diseases (Suttie, 1978).

The individual denomination of the several isoforms of menaquinones is dependent on the number of isoprenoid residues in the side chain. In human food we find the isoforms MK-4 to MK-10, and here we find those having 7, 8 or 9 isoprenoid groups in the side chain are most common. Due to the increased hydrophobic properties of longer side chains, the various isoforms exert different half-lives. This results in a half-life time of MK-4 of 1 hour, and a half-life time of MK-7 of around 3 days. The kinetic of the half-life of MK-7 is biphasic; within the first 1.5h it lasts 6-8h, whereas in a second phase the half-life is around 50h, suggesting initial redistribution and tissue uptake followed by the incorporation of vitamin K₂ in lipoproteins and

release by the liver (Schurgers, 2000). This difference in bioavailability is important for the supply of vitamin K₂ towards extra-hepatic tissues such as the arterial vessel wall.

In mammals, vitamin K serves as a redox partner in cellular metabolism pathways of gamma-glutamyl carboxylations, and a recycling mechanism helps to reduce the daily requirement of vitamin K. The vitamin K cycle can be effectively inhibited by coumarins like warfarin, primarily known as potent coagulation inhibitors.

Both vitamin K₁ and vitamin K₂ catalyse the gamma-glutamyl carboxylation of all vitamin K-dependent proteins, including coagulation factors, osteocalcin, and MGP. Buitenhuis *et al.* (1989) showed that K₂ vitamins have a lower Km for the enzyme gamma-glutamyl carboxylase, demonstrating a preference for K₂ vitamins as cofactor. Additionally, while vitamin K₁ predominantly accumulates in the liver, it is most important in the catalysation of the γ -glutamyl carboxylation of coagulation factors. However, vitamin K₂ has a more wide-spread tissue distribution and is thus also involved in the carboxylation of osteocalcin and MGP. Osteocalcin (OC), also called bone Gla-protein (BGP), is exclusively

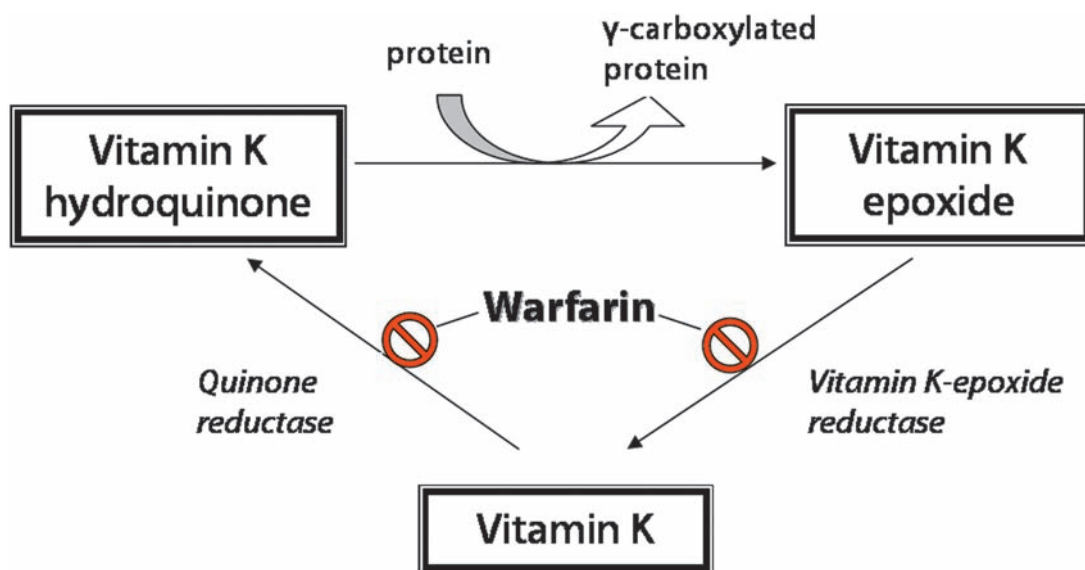


Figure 1
The Vitamin-K-Cycle (from Stafford, 2005)

synthesised by osteoblasts and odontoblasts and is a regulator of bone formation. MGP is involved in the inhibition of osseous and extraosseous calcification, e.g. in bone, cartilage and also in vascular calcification.

The recommended daily allowance (RDA) of total vitamin K is 1 µg/kg/d; the estimated daily intake in a Western diet ranges from 60 µg to 200 µg, of which vitamin K₁ constitutes the major part. However, only 10% of K₁ is absorbed from food because of the tight binding to the chloroplast membranes. There are no exact data on the amount of vitamin K₂ intake, but it is assumed that around 10 percent of the total vitamin K intake is vitamin K₂ (Schurgers, 2007). The mean daily dose is sufficient to maintain a normal coagulation status, but is it unclear whether the dose is sufficient to enable extrahepatic protein carboxylation. Determination of the ratio of extrahepatic carboxylated *versus* under-carboxylated vitamin K-dependent proteins may help us assess whether vitamin K intake is sufficient. Osteocalcin may serve as an indicator for extrahepatic protein carboxylation. With a Western diet, the carboxylation grade of serum osteocalcin has been shown to be around 70% (having 30% inactive osteocalcin species), indicating suboptimal vitamin K₂ concentrations in extrahepatic organs (Schurgers, 2005; Luukinen, 2000). Interestingly, the percentage of circulating under-carboxylated MGP in the healthy population is around 40%, suggesting also a local vitamin K-deficiency in the vessel wall (Schurgers, LJ unpublished data). The question of whether vitamin K₂ substitution is able to raise the amount of activated, carboxylated MGP is currently the subject of research.

■ THE ROLE OF MGP IN CARDIOVASCULAR CALCIFICATION

MGP is an approximately 10 kd protein which is expressed and secreted by osteoclasts, chondrocytes and VSMCs. It was first described in 1983 and the importance was elucidated by a knock-out mouse model which was initially designed to investigate the role of MGP in bone formation. As a result, bone mineralisation was disturbed in this model, but more strikingly, these animals all died at the age of around 8 weeks due to rupture of a severely calcified aorta

(Luo, 1997). A loss-of-function mutation within the MGP gene in humans – the Keutel syndrome – is characterised by abnormal cartilage calcification, brachytelephalangism, neural hearing loss and peripheral pulmonary stenosis (Keutel, 1971). Interestingly, older patients develop accelerated calcification of the aorta and of the arteries in the heart and brain (Meier, 2001). MGP exhibits its anti-calcification actions only if expressed locally in the vessel wall. Systemic (hepatic) overexpression does not inhibit the evolution of the disastrous phenotype of the MGP-null mice (Murshed, 2004).

■ How does MGP prevent vascular calcification?

MGP was initially put forward as a strong candidate of calcification inhibition due to its 5 glutamate (Gla) residues, which identifies it as one of the so called Gla-proteins. One of the characteristics of Gla-containing proteins (including coagulation factors) is their inhibitory effect on calcium precipitation and crystallisation (Vermeer, 1990). Another mechanism of calcification inhibitory action put forward is the bone morphogenetic protein-2 (BMP-2) antagonism of MGP (Zebboudj, 2001). MGP is found in so-called matrix vesicles (MV) where it helps to counterbalance potentially harmful high intracellular/intravesicular calcium concentrations (Shanahan, 2005) by mechanisms which are not fully understood at the molecular level. The development of conformation-specific antibodies against MGP enabled the detection of under-carboxylated MGP at sites of vascular calcification, whereas carboxylated MGP was found in intact vessel walls. This suggests that vitamin K may play a role in diminishing or inhibiting the development of vascular calcification (Schurgers, 2005). The dependency of gamma-glutamyl carboxylation of MGP on vitamin K leads directly to the question of potential side-effects of oral anticoagulation treatment with vitamin K antagonists (coumarins). This is a crucial question, since vascular calcification and diseases such as atrial fibrillation or thromboembolic events requiring OAC may be coincident, especially in the elderly. Indeed, coumarins are not neutral in terms of vascular disease, as shown in several animal experiments: Administration of coumarins in rats caused the development of vascular calcification which was strictly located within the tunica media (Price, 1998), i.e. the major site of MGP expression. In turn, the additional administration of

vitamin K₂ inhibited the formation of calcification in coumarin-induced VC in rats (Schurgers, 2007). This discovery leads us directly to the question of whether vitamin K₂ administration may be useful in inhibiting vascular calcification in human diseases.

■ DOES VITAMIN K DEFICIENCY PLAY A ROLE IN HUMAN DISEASE?

Since suboptimal vitamin K intake may be associated with impaired function of OC and MGP, bone and vascular diseases may be a consequence. Trials addressing the vitamin K status in postmenopausal women suffering from osteoporosis revealed that vitamin K levels are lower in affected patients than healthy controls (Binkley, 1995; Hart, 1985; Hodges 1991). *Vice-versa*, in a study performed in over 72,000 women, the risk of hip fractures was significantly lower in patients consuming higher levels of vitamin K (Feskanich, 1999). In two double-blind, placebo-controlled clinical intervention trials with vitamin K, it was demonstrated that over a three-year period the bone loss was reduced by 35% with vitamin K₁ (Braam, 2003), as opposed to a complete reduction in loss of bone strength with vitamin K₂ (Knapen, 2007). In 2005, the so-called Rotterdam trial, with more than 4800 subjects, showed that a low vitamin K₂ intake is associated with a higher incidence of severe aortic calcification and mortality in the older population (Geleijnse, 2004). This implies an insufficient nutritional supply of vitamin K₂ at least in parts of the population. In a clinical intervention trial among 200 postmenopausal women, the supplementation of vitamin K over three years demonstrated a significant benefit for the vascular properties as compared to placebo. (Braam, 2004). Nevertheless, the link between lower vitamin K₂ intake, via an increase of undercarboxylated MGP, to accelerated vascular calcification has not exactly been proven so far.

While low vitamin K intake is associated with an increased risk for bone and vascular disease, the blocking of the vitamin K metabolism with vitamin K antagonists affects skeletal and vascular health as well. In a study involving males receiving warfarin, the risk for fractures was significantly increased in individuals on long-term warfarin therapy (Gage, 2006). In a small pilot study, Schurgers *et al.* demonstrated

a two-fold increase in aortic valve calcification in patients on coumarin (Schurgers, 2004). Koos and colleagues were able to demonstrate quantitatively, with multi-slide CT-scan, that patients under anticoagulant therapy with vitamin K antagonists exerted a higher incidence of coronary and valvular calcium scores, although uc/cMGP levels are lacking (Koos, 2006).

That lower MGP levels are associated with coronary calcification was demonstrated by Brandenburg and colleagues (Brandenburg, 2006). It was shown that patients with a low coronary calcification score had significantly higher ucMGP levels compared to groups with medium and high levels in the calcification score. Moreover, in multivariate analyses, ucMGP was a powerful predictor of coronary artery calcification. The incidence of calciphylaxis, a severe complication with vascular participation in CKD, is increased in patients receiving the vitamin K antagonist warfarin (Mehta RL 1990). UcMGP is decreased within the serum in patients with chronic renal disease (Hermans, 2007). The discordance between lower ucMGP and increased vascular calcification may be explained by the accumulation of ucMGP within the calcified vessel wall, and thus, lower serum levels (Hermans, 2007). Here, the authors were only able to assume suboptimal vitamin K serum concentrations in these patients. Shortly thereafter, Pilkey and colleagues confirmed data on subclinical vitamin K deficiency, and thus, the increased under-carboxylated osteocalcin levels in dialysis patients (Pilkey, 2007). This finding is consistent with previous data of vitamin K deficiency in dialysis patients (Kohlmeier, 1997; Malyszko, 2002). Due to its lipophilic properties and incorporation into lipoproteins, vitamin K is not supposed to be dialysed. Thus, low serum levels have to be regarded as being caused by a diminished vitamin K intake within the specialised diet of HD patients, although the exact amount of vitamin K intake in this group has not yet been assessed. This suggests that dietary vitamin K supplementation may be necessary in dialysis patients.

An essential question for the nephrologist emerges: will vitamin K supplementation help our patients? Taken together, the results of the available literature indicate that the answer to this question may be yes. In an interventional study performed in 190 women, the incidence of fractures was significantly reduced in patients receiving vitamin K₂ (Shiraki, 2000). In another study from Japan, dialysis patients received

vitamin K₂ over a 12-month period. Here, the amount of carboxylated osteocalcin (GlaOC) rose significantly within 1 month of vitamin K intake (Nakashima, 2004). Nevertheless, the lack of data on carboxylated MGP in these patients means we will have to wait for the results of our currently ongoing study into this question. In animal studies, the therapeutic administration of vitamin K₂ resulted in reduced vascular calcification (own observations and Schurgers, 2007) but confirmation in humans is lacking. Concerning the safety of vitamin K₂, there are no side-effects reported that may be related to vitamin K₂ intake; in particular, no thromboembolic events or shunt dysfunctions were reported (Nakashima, 2003; Shiraki 2000). One should keep in mind that vitamin K substitution has been performed in neonates in Asia and Europe for years and this is still being recommended with no side-effects being observed (Leaf, AA 2007; Sichert-Hellert, 2006). The optimal dose of vitamin K₂ remains to be tested and depends on the half-life of the chosen form of vitamin K₂. Here, isoforms with longer side chains, e.g. MK-7, may be the best choice. Schurgers and colleagues demonstrated that a daily dose of 146 µg significantly increased serum levels of vitamin K₂ after 14d (Schurgers, 2007).

Taken together, vitamin K may be a promising tool to influence a novel modifiable risk factor that exerts special relevance in CKD patients. Vitamin K can be regarded as a safe drug, although the efficacy and therapeutic benefit as well as the daily dosage are still unclear. Furthermore, we have to assume that the prescription of vitamin K antagonists may additionally be counterproductive in these patients. The introduction of non-coumarin-based oral anticoagulants may be an important development in patients at risk of VC.

Conflict of interest statement. None declared.

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