Vitamin K Dependent Proteins and the Role of Vitamin K2 in the Modulation of Vascular Calcification: A Review

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Abstract

Vascular calcification, a cause of cardiovascular morbidity and mortality, is an actively regulated process involving vitamin K dependent proteins (VKDPs) among others. Vitamin K is an essential micronutrient, present in plants and animal fermented products that plays an important role as a cofactor for the post-translational γ-carboxylation of glutamic acid residues in a number of proteins. These VKDPs require carboxylation to become biologically active, and they have been identified as having an active role in vascular cell migration, angiogenesis and vascular calcification. This paper will review the process of vascular calcification and delineate the role that vitamin K2 plays in the modulation of that process, through the activation of VKDPs. One such VKDP is Matrix Gla Protein (MGP), which when activated inhibits osteogenic factors, thereby inhibiting vascular and soft tissue calcification.

Keywords: Vitamin K; Vitamin K2; Menaquinone; Vascular Calcification; Matrix glutamate-Protein (MGP); Warfarin; Vitamin K antagonists.

Introduction

Vascular Calcification

Evidence that the process of vascular calcification is a regulated one and is present even in the early stages of disease has surfaced, drawing the attention of scientists and clinicians to study its regulation, in an effort to find possible inhibitors or substances that induce its regression. Vascular calcification is characterized by the deposition of calcium phosphate complexes mostly in the form of hydroxyapatite. It can present as medial calcification or intimal calcification. Medial calcification, or Monckeberg's medial sclerosis, is prevalent among diabetic patients and patients with renal or hyperparathyroid disease.[1,2] Intimal calcification occurs on the surface of atherosclerotic plaques,[3] while medial calcification is normally associated with the elastic lamina.[4]

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Several factors including hypertension, inflammation, oxidized low density lipoproteins (oxLDL), stress, hypercalcemia, hyperphosphatemia and a high calcium-phosphorous ion product (Ca × P) can influence and transform vascular smooth muscle cells (VSMC) into osteocyte-like cellular elements through the transcription of osteochondrogenic morphogens.[5,6] Notably, these inducing factors are increased among patient populations with a prevalent incidence of vascular calcification, namely patients with diabetes, renal dysfunction and atherosclerosis. The role that secreted osteochondrogenic factors (the polypeptides also responsible for bone morphogenesis during skeletal development and fracture repair) play in the pathophysiology of vascular calcification has become better appreciated.[7] On the molecular level, one of the factors that both initiates and contributes to the calcification process is a reduction in the expression of vitamin K dependent calcification-inhibiting proteins such as matrix gla-protein (MGP).[8] One such mechanism by which MGP plays its role is through the inhibition of such osteogenic factors. Therefore, the same mechanisms that govern skeletal calcification also cause vascular calcification, in the absence or inactivation of soft tissue calcification inhibitors such as MGP.[9]

Vitamin K and its Dependent Proteins

Vitamin K is a lipid-soluble vitamin that was first identified by Henrik Dam in 1929 for its anti-hemorrhagic activities.[10-13] It was later coined with the letter K for the Danish word Koagulation. It consists of a group of vitamins that may be further classified as vitamin K_1 (VK_1) orphylloquinone; vitamin K_2 (VK_2) ormenaquinone; and vitamin K_3 (VK_3) or menadione.[14] Vitamin K compounds share the same 2-methyl-1, 4-naphthoquinone backbone, yet differ by the substituent on the third carbon of the naphthoquinone ring.[15] VK_1's substituent is a phytyl chain on the 3' position, is mainly found in plants and is involved with photosynthesis.[14,16] VK_2 is formed mostly by bacteria. Humans may obtain VK_2 from fermented dietary sources, such as curd cheese and natto, a Japanese food, or convert high doses of VK_1 into VK_2 in their bodies.[17,18] Vitamin K_3 is a synthetic vitamin K with no organic moiety on the 3' position.[14] Vitamin K is mainly found in food in the unreduced quinine (K) form that is in turn reduced into hydroquinone (KH_2) in the body. Of importance, Vitamin K is present in significantly lower quantities as compared to the amount of γ-carboxylation that takes place, which suggests that this vitamin is recycled in the body through the vitamin K cycle, presented in Fig. 2.
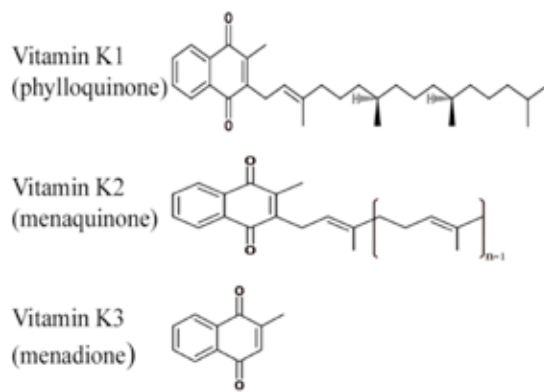
Figure 1: The chemical structures of vitamin K. Vitamin K_2 represents a group of molecules with a varying number of isoprene units. Each molecule can be named as mk-n, which represents the number of isoprene units it contains.
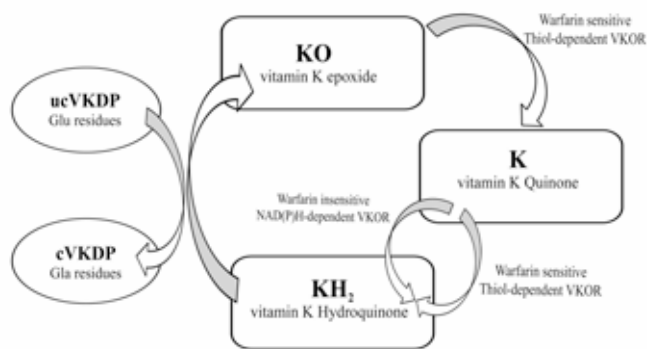

Figure 2: Vitamin K carboxylation cycle. The oxidation of the hydroquinone form is coupled to the carboxylation of Vitamin K dependent proteins (VKDPs). Vitamin K can be reduced into its hydroquinone form through the action of two enzymes; one of these is sensitive to the action of vitamin K antagonists (VKA) such as warfarin, while the other is not. However, the reduction of vitamin K epoxide can only be achieved by an enzyme sensitive to VKA. This cycle is coupled to the carboxylation of glutamic acid residues in proteins. *VKOR: Vitamin K epoxide reductase; ucVKDP: under-carboxylated VKDP; cVKDP: carboxylated VKDP; Glu: glutamic acid; Gla: γ-carboxyglutamic acid.*

Vitamin K-dependent Proteins (VKDP)

The VKDPs include a number of clotting factors involved in the coagulation cascade (Factors II, VII, IX, X), circulating anti-coagulants (proteins C, S and Z), as well as proteins involved in bone and soft-tissue mineralization like osteocalcin (OC) and MGP respectively. Carboxylated osteocalcin, produced by osteoblasts and carboxylated by Vitamin K, binds to hydroxyapatite in the extracellular matrix of bone. Although its expression has been observed in calcifying vasculature, its biological significance in the progression of calcification remains unknown.[19] MGP is of particular interest for the role it plays in vascular calcification. Although it is found in normal vascular smooth cells, MGP is up-regulated during calcification. More recently, additional VKDP have been discovered including the Growth Arrest Specific Gene 6 (Gas-6), the Transmembrane Gla proteins (TMG3 and TMG4),

the Proline-Rich Gla proteins (PRGP1 and PRGP2), the Gla-Rich Protein (GRP), periostin and transhyretin. Besides MGP, Gas-6 is also involved in the vasculature, affecting the apoptosis of smooth muscle cells.[20] Its function is not limited merely to the vasculature, but it also plays important roles in the nervous system, and in platelet and bone metabolism.[21] GRPs, which concentrate in calcified regions, regulate calcium metabolism extracellularly. Angiogenesis and cell movement are regulated by periostin, a VKDP involved in inflammation, asthma and cancer.[21-24] Meanwhile, the roles of TMGs, found in different tissues and transhyretin play are currently being investigated.

The role that these VKD proteins play in the process of vascular calcification and the potential beneficial effect of Vitamin K_2 or menaquinone is being uncovered. In particular, higher levels of dp-ucMGP correlate with a higher risk of mortality in patients with aortic stenosis,[25] end-stage renal disease,[26] and chronic heart failure.[27] Similarly, correlations between MGP levels and degree of calcification are being drawn which may render MGP an early biomarker for disease. Furthermore, VKDP are being used as markers for vitamin K deficiency in various organs of the body. The levels of dephosphorylated, non-carboxylated (dp-ucMGP) are used as a marker for vitamin K deficiency in vasculature, non-carboxylatedosteocalcin (ucOC) for deficiency in bone.[28] Another such protein is des-carboxy-prothrombin (PIVKA-II), the under-carboxylated forms of prothrombin, a vitamin K-dependent clotting factor synthesized in the liver. PIVKA-II levels are used as markers of vitamin K deficiency in the liver,[29] as well as for peripheral vitamin K deficiency.[30] Table 1 summarizes the use of VKD proteins as biomarkers.

MatixGla protein (MGP)

Matrix Gla protein (MGP), an extracellular protein, is a VKD protein that plays a major role in the process of vascular calcification. Gla-containing proteins were detected in human and porcine calcified aortic valves while none were found in normal and stenotic tissues.[41] Subsequently, MGP gene knock-out mice were shown to develop soft-tissue calcification,[42] similar to that observed in humans suffering from Keutel syndrome, a disease resulting in defective MGP synthesis.[43] More recently, MGP has been shown to have an inhibitory role on the process of vascular calcification.[44]

The relationship between MGP and Vitamin K lies in the fact that inactive MGP requires vitamin K to carboxylate it for its activation.[45,46] Since MGP is a peripheral protein, VK_2 the most prevalent form of vitamin K in non-hepatic tissue – is the vitamin mostly available to carboxylate MGP. As calcification develops, MGP is up-regulated in VSMC possibly as part of a negative feed-back mechanism.[47] This up-regulation may cause a local deficiency in vitamin K_2 status leading to the production of inactive, non-carboxylated MGP (ucMGP). Phosphorylated MGP remains attached to the growing calcification crystals while dephosphorylated MGP (dp-MGP) is released into the circulation. Levels of dp-MGP in circulation can be measured and have been found to correlate with cardiovascular morbidity.

Table 1: VKDP used as markers for Vitamin K deficiency and disease states.

| Organ	Marker protein	Disease	Reference(s)
Vasculature	MGP	Atheromatous plaque	Shanahan, 1994.[31]
		Diabetes	Thomson, 2010.[32]
		Carotid stenosis	Pop, 2011.[33]
	Dp-ucMGP	Coronary Heart Failure (CHF)	Ueland, 2011.[27]
		Higher mortality in patients with aortic stenosis	Ueland, 2010.[25]
	Dp-cMGP	CHF	Ueland, 2011.[27]
		Higher mortality in hemodialysis patients	Schlieper, 2011.[26]
	Uc-MGP	Aortic stenosis and calcification	Cranenburg, 2008.[34]
		Diabetes	Parker, 2010.[35]
		Hypertension	Rennenberg, 2010.[36]
Bone	Osteoclacin	Glycemic Index	Guntur, 2012.[37]
	ucOC	Insulin metabolism	Bulló, 2012.[38]
		Bone fractures	Herrmann, 2002.[39]
Liver	PIVKA-II	Vitamin K deficiency	Schurgers, 2007.[40]
			Westenfeld, 2012.[29]
Vasculature	PIVKA-II	Vitamin K deficiency	Holden, 2010.[30]
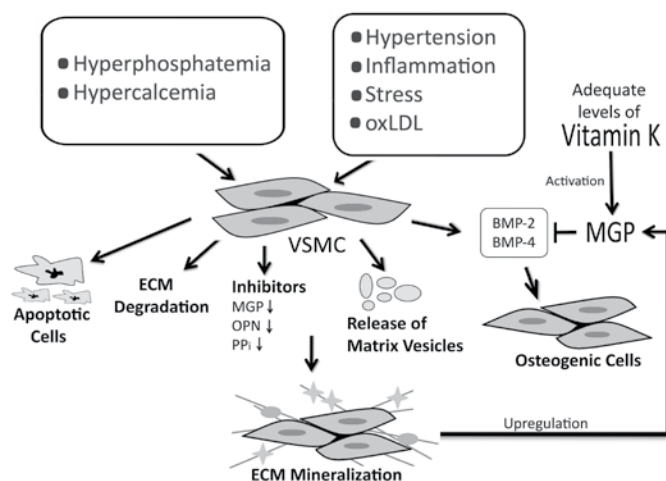
Figure 3: The process of vascular calcification. Mineralization of the ECM induces an increase in the expression of MGP as a negative feedback mechanism. The up-regulation of MGP causes a relative deficiency in vitamin K which, if not replenished, will lead to inactive MGP and vascular calcification. A decrease in the expression of calcification inhibitors such as OPN, Osteopontin; PPi, pyrophosphate; BMP-2 and BMP-4, Bone Morphogenetic Protein-2 and 4, resepctively, may also result in vascular calcification.

Besides vitamin-K dependent γ-carboxylation, MGP can also be activated through its phosphorylation on 3 serine residues (residues 3, 6 and 9).[48] Once activated, MGP is attracted to hydroxyapatite crystals through the negatively charged phosphate group hence forming a coat on the surface of the crystals. This results in the inhibition of crystal growth by preventing the aggregation of the crystals.[49,50] Another proposed mechanism of action of MGP is

through the binding and inhibition of bone morphogenetic proteins 2 and 4 (BMP-2, BMP-4), members of the transformation growth factor beta (TGF-β) superfamily.[25,45,51,52] BMP-2 promotes the process of calcification by inducing apoptosis,[53] and the trans-differentiation of VSMC into osteoblast-like cells,[54] both of which increase vascular calcification. Additionally, through its binding to vitronectin, a protein that reduces cell apoptosis, MGP influences cell differentiation.[55,56] Therefore, when MGP is inactive or absent from tissues, the action of BMP becomes pronounced, causing extensive calcification by stimulating VSMC to express osteogenicmorphogens. Thereby, VSMC transdifferentiate into osteocyte-like cells and the surrounding ECM becomes mineralized. Indeed, the levels circulating undercarboxylated MGP can be used as an indicator for vascular calcification, whereby an inverse relationship exists between serum ucMGP and the degree of arterial calcification.[34]

Other effects of Vitamin K₂ on vascular calcification

Of the three forms of vitamin K, VK₂ has been shown to exhibit profound effects on reducing vascular calcification. It was found to decrease arterial calcification on cultured bovine aortic smooth muscle cells treated with inorganic phosphate. This effect was amplified when the treatment was combined with bisphosphonates.[57] In another study, VK₂ reduced the advancement of atherosclerosis in hypercholesteremic rabbits.[58] The ability of VK₂ to improve lipid profile by increasing HDL levels and decreasing total cholesterol levels, and therefore affect the process of vascular calcification and plaque formation in atheroma was demonstrated in the Rotterdam study.[59] Dietary VK₂ intake was associated with a lower occurrence

of aortic calcification and coronary heart disease in those patients.[59] Similarly, a study on post-menopausal women found a lower incidence of coronary calcification with increased consumption of VK_2 ormenaquinones, particularly MK-4.[60] In recognition of the effect of VK_2 on reducing the risk of coronary heart disease, the International Life Sciences Institute (ILSI Europe), recently recommended taking VK_2, in addition toVK_1, into consideration when calculating the daily recommended value of vitamin K.[61]

Vascular calcification is associated with a number of diseases. People suffering from familial hypercholesteremia are at high risk of developing aortic calcification.[62-64] Two other patient populations are especially vulnerable to vascular calcification; patients with renal disease and diabetics. In patients suffering from end-stage renal disease (ESRD), the presence of calcification in their arteries correlates with higher risk of mortality.[65] In this patient population, calcification is probably induced by high calcium and phosphate circulating levels perhaps due to impaired kidney function, or by vitamin K deficiency.[26,66] A study on hemodialysis patients subjected to varying doses of mk-7 intake resulted in a decrease in serum dp-ucMGP, ucOC and PIVKA-II levels significantly in the group treated with 360 microgram/day, but not at lower doses.[29] In order to sufficiently carboxylate peripheral VKDPs, VK_2 is needed at doses near to/or higher than the currently accepted RDA value. The administration of MK-7 reduced ucMGP and dp-ucOC levels significantly at doses near RDA but not significantly at lower doses.[67]

Diabetic patients suffer from calcification in the tunica media mainly in the thoracic aorta, coronary arteries and tibial arteries.[68,69] The possibility of calcification in these patients is four times higher than in non-diabetics.[70] The incidence of advanced glycation end-products (AGE), which correlates with the occurrence of coronary artery calcification (CAC), may be induced by the elevated blood glucose levels.[71,72] Alternatively, calcification in these patients may be triggered by vascular inflammation or the formation of foam cells from macrophages that in turn contribute to the calcification process.[73] MGP levels were found to be higher in diabetic patients,[32] with higher ucMGP levels indicating higher risks of mitral arterial calcification a trend that was contrary to that observed in non-diabetics.[35] This indicates that vitamin K dependent proteins may play a role in the observed incidence of calcification in diabetics, an effect which may be counteracted with sufficient vitamin K treatment.

While VK_2 is being studied for its role in the modulation of calcification, VK_1does not seem to have a significant effect on vascular calcification, as shown in several studies.[59,60,74] This may be partially attributed to the VK_2 being present in higher concentrations than VK_1 in LDL molecules, which target extra-hepatic organs, and is thereby more potent in carboxylating peripheral VKDPs.[36] This may explain the observed anti-calcification effects of VK_2 as opposed to VK_1.[75] VK_1 has been shown to reduce coronary calcification at high intake levels probably through the conversion of VK_1 into VK_2 in the human body. Besides carboxylating VKDPS, VK_2 may exhibit its effect by regulating gene transcription of several proteins involved in calcification. It was found to reduce the levels of osteopontin (OPN) mRNA, while increasing the expression of MGP. These trends were opposite to the ones observed when treatment with inorganic phosphate, a calcification inducer, was used.[57]

Vitamin K antagonists (VKA), such as warfarin and their derivatives, are administered as anticoagulants to many patients. They inhibit the recycling of vitamin K in the epoxide cycle thereby reducing its availability to carboxylate coagulation factors in the liver; however, they have a similar effect on non-hepatic vitamin-K dependent proteins. VKAs were found to cause calcification in human femoral arteries,[76] mitral valves,[77] aortic valves,[77] and rat carotid artery and aorta.[49] Coronary calcification was also observed in arterial fibrillation patients with low-cardiovascular risk, as a cause of VKA treatment.[78] The administration of VKA was associated with higher MGP levels in the vasculature of rats, particularly in the calcified regions. However, a 3 fold decrease in circulating serum MGP was recorded in the treated rats, perhaps due to more MGP becoming attached to the growing crystals.[50] In human subjects, a study by Rennenberg and colleagues suggested higher levels of dephosphorylated, non-carboxylated MGP (dp-ucMGP) in patients using Coumadin for over ten years.[76] Elevated dp-ucMGP levels may indicate peripheral vitamin K deficiency and may serve as a biomarker for vascular calcification. In support of this, normal subjects treated with VKA had elevated dp-ucMGP,[25,79] while those on vitamin K supplementation had decreased levels.[79]
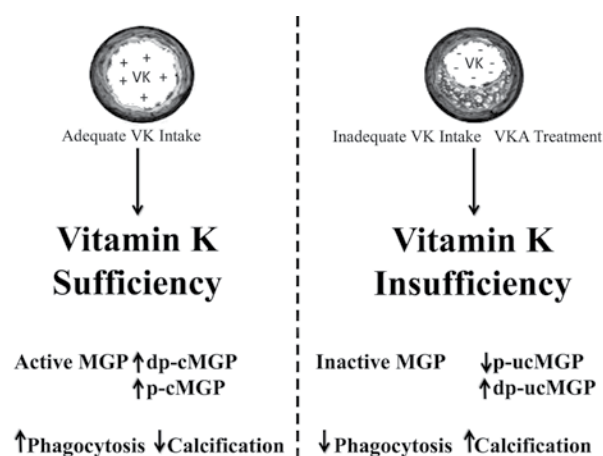

Figure 4: The effect of vitamin K on MGP and vascular calcification. Different species of MGP are affected by the presence of vitamin K. These include Dp-cMGP, non-phosphorylated carboxylated MGP; p-cMGP, phosphorylated MGP; p-ucMGP, phosphorylated-non-carboxylated MGP; dp-ucMGP, non-phosphorylated non-carboxylated MGP.

Some concerns regarding the use of VK_2 in treatment have been raised, including the possibility of achieving a hypercoagulable state. In fact, a study on hypercholesterolemic rabbits showed that such a state was not reached even when high doses were administered for ten weeks.[58] In human subjects not receiving oral anti-coagulation treatment, no effects of the use of such doses on coagulation was found using a highly sensitive chloramphenicol acetyltransferase

(CAT) assay.[67] Nevertheless, the administration of VK_2, especially the longer chain mk-7, should be closely monitored in patients receiving VKA treatment since mk-7 is a 3-4 times more potent inhibitor of VKA than VK_1.[40,80]

Conclusion

Vitamin K_2 has promising potential to be used as treatment or prevention for the development of vascular calcification especially in at risk patient groups with high incidence of calcification or vitamin K deficiency. Through its peripheral distribution in low density lipoproteins, VK_2 exercises its effect by activating various VKDPs including the soft-tissue calcification inhibitor MGP. Clinical trials looking into the effect of VK_2 on vascular calcification and its beneficial cardiovascular effects will help elucidate its potential role as a therapeutic strategy.

Acknowledgements

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