REVIEW

Warfarin and Vascular Calcification

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ABSTRACT

The vitamin K antagonist, warfarin, is the most commonly prescribed oral anticoagulant. Use of warfarin is associated with an increase in systemic calcification, including in the coronary and peripheral vasculature. This increase in vascular calcification is due to inhibition of the enzyme matrix gamma-carboxyglutamate Gla protein (MGP). MGP is a vitamin K-dependent protein that ordinarily prevents systemic calcification by scavenging calcium phosphate in the tissues. Warfarin-induced systemic calcification can result in adverse clinical effects. In this review article, we highlight some of the key translational and clinical studies that associate warfarin with vascular calcification.

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KEYWORDS: Atherosclerosis; Matrix gamma-carboxyglutamate Gla protein; Vascular calcification; Warfarin

Vascular calcification plays a key role in the pathophysiology of coronary artery disease, ischemic stroke, and peripheral arterial disease. Commonly recognized risk factors for vascular calcification include hypertension, diabetes, increasing age, chronic kidney disease, cigarette smoking, and systemic inflammation. Treatment with vitamin K antagonists such as warfarin is associated with vascular calcification, even when other risk factors are controlled.[1]

Systemic calcification has adverse clinical implications. Developments in basic science have uncovered mechanisms responsible for increased systemic calcification.

MECHANISMS OF VASCULAR CALCIFICATION

Inflammation, hyperlipidemia, and other factors cause vascular smooth muscle cells to lose their contractile ability and differentiate into osteoblast-type cells.[2] These factors lead to genetic changes, with upregulation of transcription factors such as the bone morphogenetic proteins, osterix, and core binding factor-α1.

To prevent tissue calcification, there are numerous protective inhibitory factors, such as osteopontin and osteoprotegerin.[2] One of these protective factors is matrix gamma-carboxyglutamate Gla protein (MGP), a highly insoluble protein synthesized by vascular smooth muscle cells. MGP is an 84-amino acid protein in the Gla family that has been linked to vascular calcification.[3] MGP functions via 5 unusual gamma-carboxyglutamate residues that bind calcium phosphate, preventing calcification at the cellular level. Like other Gla proteins, such as the procoagulant factors II, VII, IX, and X, and anticoagulant factors protein C and S, MGP must undergo posttranslation modification into its active form. The posttranslation carboxylation reaction requires vitamin K as a cofactor, and in the setting of vitamin K deficiency, MGP cannot be converted to its active form (**Figure 1**).

Much of the initial work in the biology of MGP was conducted in a mouse knockout model.[3] The MGP allele was disrupted by embryonic gene targeting to produce mice that were heterozygous for a null function MGP gene. When these heterozygous mice were crossed, they produced homozygous MGP-deficient mice. These mice appeared to be normal at birth, but every homozygote mutant developed extensive arterial calcification and died within 2 months, compared with an average life span of about 2 years for typical laboratory mice. On autopsy, these mice died of aortic rupture, with widespread calcification of the carotid

arteries, aorta, celiac axis, renal arteries, and iliac arteries. This calcification occurs in the internal elastic lamina of the coronary arteries as well as in the elastic fibers and collagen fibrils in the media of the aortic wall. Further work has shown that MGP administration prevents calcification in these in vitro models.[4] A high ratio of uncarboxylated:carboxylated MGP correlates with vitamin K defiency,[5] and supplementation of vitamin K in humans decreases the ratio of uncarboxylated:carboxylated MGP.[6]

WARFARIN AND VASCULAR CALCIFICATION

When the calcification inhibition function of MGP was discovered to be vitamin K dependent, it triggered research into whether treatment with vitamin K antagonists could result in uncontrolled vascular calcification. In a mouse model, warfarin rapidly calcified the elastic lamellae in arteries and heart valves.[7] This was later shown in rats to occur in both a time- and dose-dependent fashion. Rats treated with warfarin at higher doses and for longer periods of time developed increasing calcification in multiple tissues.[8] Warfarin-treated mice also developed coronary artery disease with a vulnerable plaque phenotype.[9] Lending support to the theory that vitamin K status is vital to the function of MGP, Schurgers et al[10] administered vitamin K in a rat model that prevented and even reversed vascular calcification.

The same relationship between vitamin K status, warfarin, and vascular calcification has been found in humans. In otherwise healthy women, poor vitamin K status

as measured by MGP assays was linked with higher rates of vascular calcification.[11] Observational studies have shown an association between warfarin treatment in coronary[1] and femoral arteries,[12] likely as a result of decreased vitamin K activity. **Figure 2** demonstrates calcification in the coronary arteries and bronchi of a 59-year-old woman with atrial fibrillation who had been treated with warfarin for nearly 30 years.

One emerging technique for monitoring small-vessel calcification in women is mammography. Because screening mammograms are obtained as often as every year, they can be used to track the development of vascular calcification. Tantisattamo et al[13] compared mammograms between women who were treated with warfarin vs age and diabetes status-matched controls. In this population, they found no difference between the 2 groups prior to warfarin therapy, but after treatment with warfarin, the treated group developed calcified vessels at a rate 50% greater than controls. For patients who were treated for more than 5 years with warfarin, the prevalence of vascular calcification increased to nearly 75%.

## CLINICAL MANIFESTATIONS OF WARFARIN-INDUCED SYSTEMIC CALCIFICATION

Patients with chronic kidney disease develop vascular calcification due to a number of MGP-independent pathways, including impaired calcium-phosphate handling and hypertension. In the chronic kidney disease population, vitamin K deficiency has been linked to an increase in

CLINICAL SIGNIFICANCE

- Matrix gamma-carboxyglutamate Gla protein (MGP) prevents vascular calcification.

- MGP is a vitamin K-dependent protein and is inhibited by warfarin.

- Due to its interaction with MGP, warfarin increases systemic calcification.

- Warfarin is associated with impaired bone health.
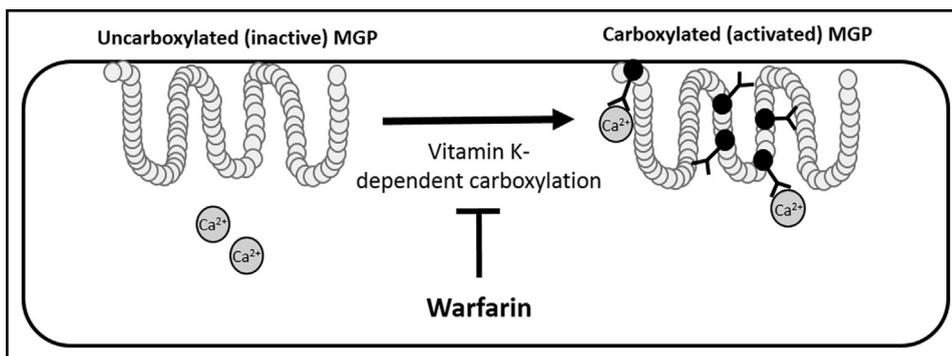
Figure 1 Diagram illustrating the interaction of matrix gamma-carboxyglutamate Gla protein (MGP) and warfarin. MGP has 5 gamma-carboxyglutamate residues, which must be carboxylated by a vitamin K-dependent process into its active form. The activated form of MGP is then able to scavenge calcium phosphate in blood vessels and tissues to prevent calcification. The activated gamma-carboxyglutamate residues are represented by the 5 dark black circles on the right. Warfarin interferes with the activity of MGP by inhibiting the vitamin K-dependent carboxylation reaction, eventually leading to systemic calcification.
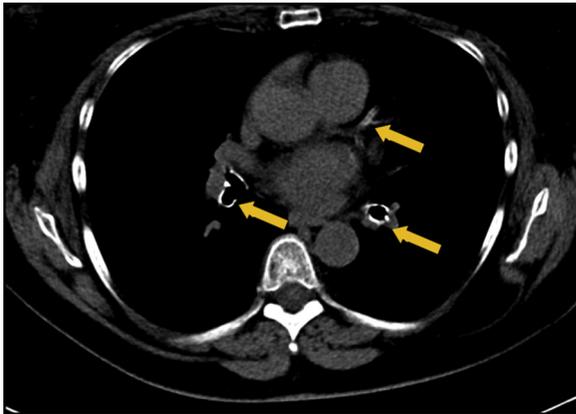
Figure 2 Computed tomography scan of the chest in a 59-year-old woman on warfarin. This patient had a long-standing history of pulmonary embolism and no other vascular risk factors and had been treated with warfarin for 30 years. This scan shows significant calcification of the left anterior descending artery and the left and right bronchi.

vascular calcification.[14] In one study of warfarin-treated hemodialysis patients, warfarin use had a hazard ratio of 1.97 for overall mortality, even when confounding variables were controlled.[15] The observation that low vitamin K levels are associated with excessive mortality in hemodialysis patients led to a pilot trial of vitamin K treatment in hemodialysis patients without atrial fibrillation and not on treatment with a vitamin K antagonist.[16] This open-label trial showed that vitamin K administration can increase activated serum MGP levels and provides the basis for interventional trials aimed at supplementing high-risk patients with vitamin K, with the goal of preventing cardiovascular events.

Treatment with warfarin has been linked to calcification in the tracheobronchial tree. Although tracheobronchial calcification can occur as a result of numerous disorders, there are case series showing tracheobronchial calcification in patients treated with warfarin.[17] These patients lacked traditional risk factors for systemic calcification, and warfarin was the leading suspect. Tracheobronchial calcification and dyspnea can also occur in the Keutel syndrome, a genetic syndrome that results from mutations deleterious to the function of the MGP protein.[18] Via the same mechanism, patients with warfarin-induced tracheobronchial calcification could develop chronic dyspnea from airway dysfunction. This association further supports the theory that vitamin K-dependent MGP activity is required to prevent systemic calcification.

In addition to the association of warfarin's effects on systemic calcification, warfarin use has also been associated with impaired bone health. This effect is likely mediated through warfarin's effects on the pro-osteoblastic protein osteocalcin. Osteocalcin, a gamma-carboxyglutamate Gla protein that requires vitamin K-dependent carboxylation for

activation, is impaired by warfarin administration.[19] In a rat model, warfarin administration decreased bone mineralization and turnover in as little as 6 weeks, a finding that was not replicated in rats treated with dabigatran.[20] In some human studies, long-term warfarin use has resulted in decreased bone mineral density, which has led for some investigators to recommend calcium and vitamin D supplementation for all patients on warfarin.[21,22] Due to increasing evidence that vitamin K metabolism may play an important role in bone health, there is now an ongoing clinical trial to study the effect of supplementation of vitamin K in patients with osteoporosis.[23]

CONCLUSIONS

In conclusion, warfarin causes an increase in systemic calcification through its effects on MGP. This calcification can result in arterial and tracheobronchial calcification. In addition, warfarin use has been associated with impaired bone health. Further studies on the long-term vascular and systemic effects of warfarin need to be performed.

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