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Rapidly Progressive Severe Vascular Calcification Sparing the Kidney Allograft Following Warfarin Initiation

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Abstract

We describe a case of rapid onset of vascular calcification coincident with the initiation of warfarin therapy in a kidney transplant patient. Calcification developed within the media of the blood vessel wall, with relative intimal sparing. Medium and small sized arteries were affected, but the aorta was mostly free of calcifications, suggesting a differential response to warfarin between the intima and media as well as between different vascular beds. In addition, unlike the highly calcified native kidney's vessels, the kidney allograft was not calcified, suggesting local, genetically determined, mechanisms in preventing vascular calcification. Distal subcutaneous necrosis ultimately lead to the patient's demise.

Index words

vascular calcification; warfarin; hemodialysis; MGP

Vascular calcification is a major contributor to the morbidity and mortality of end-stage renal disease (ESRD) patients. Whereas atherosclerosis typically involves the vessel intima, ESRD patients are particularly susceptible to a form of vascular calcification involving the media and elastic lamina of large and medium sized arteries. Recently, vitamin K dependent proteins have gained recognition as important components in this process.¹ MGP (matrix Gla protein) acts as a potent vascular calcification inhibitor, and Gas6 (growth arrest-specific 6) controls vascular smooth muscle cell death and calcification. Both are produced by vascular smooth muscle cells, and undergo post-translational carboxylation in order to become biologically active, a process requiring sufficient levels of reduced vitamin K, as well as several important enzymes, including vitamin K epoxide reductase and gamma-glutamylcarboxylase.

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Warfarin, widely used as an anticoagulant, interferes with the post-translational modification of vitamin K dependent proteins, rendering them inactive. Although its anticoagulant effect has gained widespread therapeutic use, less is known about its potential side effect on vitamin K dependent proteins within the vasculature. In animal models, warfarin administration, albeit at supraphysiological doses, induces widespread medial vascular calcification². Its clinical use has been associated with the development of calciphylaxis³, a form of metastatic small vessel calcification, aortic valve and coronary calcification⁴, and stroke⁵.

We describe a case where the administration of warfarin was temporally associated with extensive and rapid vascular calcification. The presence of a transplanted organ provided the opportunity to evaluate how different genetic backgrounds respond to the same metabolic milieu.

Case report

A 50 year-old white male, who had received a cadaveric kidney transplant from an 18 year-old accident victim in June 1988 for progressive reflux nephropathy, developed a febrile illness in December, 2006. His post transplant course had been notable for diabetes and skin cancers, but had otherwise enjoyed excellent health, with a baseline creatinine of 1.5 mg/dl (132.6 μ mol/L). He was admitted to the Intensive Care Unit for a sepsis-like illness, with no pathogen ever identified. His course was complicated by kidney failure, requiring the initiation of hemodialysis. In addition, he developed atrial fibrillation, and warfarin was initiated for anticoagulation. Imaging studies from this time showed minimal vessel calcification involving his coronaries and splenic artery, but otherwise normal blood vessels (Figure 1a, 1d).

He was discharged to a rehabilitation center in January 2007, and maintained on 2.5 mg daily of warfarin. His international normalized ratios fluctuated in the 2–4 range. Phosphate binders were not required. Paricalcitol, 2 μ g with each treatment, was used briefly during the first few months of dialysis, but was discontinued in March. Serum phosphate levels ranged between 3.6 – 4.8 mg/dL (1.16 – 1.55 mmol/L), serum calcium between 8.9 – 9.3 mg/dL (2.23 – 2.33 mmol/L) and PTH between 126 – 275 pg/mL (126 – 275 ng/L).

In June 2007, he developed left foot cellulitis that progressed to gangrenous involvement of the first and second toes. Angiography showed extensive calcification in all major vessels of his legs. Warfarin was discontinued in August. Despite attempts at conservative management, he required a left below the knee amputation in September, 2007. Later that month, he developed large areas of necrosis along his penis and perineum, and additional lesions on both hands, both legs, and eventually his left ear.

Follow up radiographic imaging showed extensive vascular calcifications, likely the cause of his skin necrosis (Figure 1b, 1c). Testing for proteins C and S showed normal function. Management included broad-spectrum antibiotics, a trial of intravenous bisphosphonates, vitamin K supplementation, and local wound care. After a prolonged hospital stay complicated by pain, narcotic overdose, sigmoid colon bleeding, and hypotension, the patient elected to cease aggressive measures and passed away in November 2007. Autopsy was performed.

Histology of multiple blood vessels, including all major organs, and the femoral, iliac, temporal, splenic, and renal arteries, showed extensive medial wall and elastic laminae calcification (data not shown). His aorta was relatively spared from medial calcification, and had minimal intimal atherosclerotic plaques (Figure 1c, 1e). Von Kossa staining of representative histology sections from his native kidney and transplanted kidney are

provided in Figure 2a and 2b. Blood vessels within the native kidney had extensive medial calcification, whereas the 18 year old transplanted organ had no vessel calcification. Extra-vascular calcifications were present in the subcutaneous fat, but not within other native organs such as the liver or spleen.

To further elucidate potential mechanisms for such rapid calcification, staining for carboxylated (cMGP) and uncarboxylated MGP (ucMGP) was performed (figure 2c–f). The calcified femoral artery had equal staining for both the uncarboxylated and carboxylated forms (compare 2e, cMGP to 2f, ucMGP), whereas the uncalcified aorta stained predominantly for cMGP (compare 2c, cMGP to 2d, ucMGP). While both the native and transplant kidneys stained for cMGP and ucMGP, there was no perceptible difference in staining between the two forms of MGP and between the kidneys (data not shown).

To investigate whether sequence variants in vitamin K dependent protein genes might explain the differential vascular response between the native and transplant kidneys, partial candidate gene sequencing was pursued. No coding sequence mutations were identified in MGP, Gas6, or vitamin K epoxide reductase complex subunit 1 (encoded by the *VKORC1* gene). A sequence variant in the promoter region of MGP was identified (g.-138T>C, ie, 138 nucleotides upstream of the transcription start site in the genomic DNA). Due to the fragmented nature of extracted formalin-fixed DNA, our ability to screen for multiple candidate genes was limited.

Discussion

The role of vitamin K dependent proteins in preventing vascular calcification is being increasingly recognized. Previous studies have shown an association with warfarin use and vascular calcification.^{6, 7} Our case report extends this relationship, suggesting that warfarin use precipitated extensive vascular calcification in a genetically susceptible individual.

Within several months after initiation of warfarin, our patient developed extensive vascular calcification. (Table 1 summarizes similar presentations)⁸ The histology of the calcified vessels in our patient was similar to that seen in animal models of warfarin induced calcification², primarily involving the elastic laminae of the vessel media, with relative intimal sparing. Although medial calcification can be seen in long standing diabetics and in chronic dialysis patients, the rapidity of our patient's calcification is not in keeping with these etiologies. In addition, immunohistochemical staining showed a relative deficiency of the biologically active form of MGP in some calcified vessels. The patient's uncalcified aorta predominantly stained for the biologically active carboxylated MGP, whereas the calcified vessels had a much lower ratio of carboxylated to uncarboxylated MGP. Animal models have shown that MGP must be present within the vessel wall to prevent calcification⁹. The relative deficiency of carboxylated MGP in the calcified vessels, when compared to the aorta, potentially explains the differential calcification amongst tissue beds.

In addition, the difference in calcification between the native kidney and the kidney transplant highlights the role of genetic factors. Both native and transplant vascular beds were exposed equally to dysregulated serum phosphate and parathyroid hormone levels, uremic toxins, the availability of nutrients such as vitamin K, the effects of diabetes, and warfarin. Yet, the transplant remained protected from disease, while the native kidney developed intense vascular calcification. The patient had been on hemodialysis for 18 months prior to his transplant in 1988, and it is also possible that uremic damage from that time predisposed the native vessels to calcification. However, given a normal kidney function over the intervening 18 years, it is less likely that native vascular smooth muscle cells will have sustained and perpetuated an injured, calcification-prone phenotype.

Sequencing of the genes that code for the two most important vascular vitamin K dependent proteins, MGP and Gas6, as well as vitamin K epoxide reductase, was pursued without revealing coding region mutations. MGP promoter analysis showed a T>C sequence variant (resulting in a CC allele at the -138 position in the genomic DNA), associated with reduced promoter activity in transient transfection assays in vitro.^{10, 11} The prevalence of this variant was 4–5% in a Northern Europe population.¹¹ However, linkage analysis of this variant failed to show an association with hard cardiovascular outcomes (myocardial infarction) in that population. In addition, staining for total MGP was not different between the native and transplant kidney. Differences in circulating MGP species have been linked to cardiovascular calcification¹². It is not known if this variant results in lower circulating levels of MGP, or if it affects its carboxylation. We were unable to assess MGP levels as banked serum was not available.

It is likely that the combination of kidney failure, superimposed on a genetic predisposition to warfarin, prevented the local activation of vitamin K dependent proteins in the vessel wall, leading to vascular calcification. Given the widespread use of warfarin, further research into whether the vitamin K dependent proteins protect the vasculature from calcification is warranted.

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Figure 1.

Axial and coronal sections from abdomen and pelvis computed tomography scans taken 9 months apart (December 2006 and September 2007). A, D. Images from December 2006 show minimal vessel calcification in the pelvis and abdomen. B, C, E. Images from September 2007 show extensive and widespread calcification, involving the splenic artery, iliac and femoral vessels, as well as smaller vessels supplying the penis and superficial structures in the lower extremities. The aorta is spared from calcification.

Figure 2.

A, B. Von Kossa stain of kidney tissue, 20× magnification. A. Transplant kidney; arrow denotes the lumen of an artery. No vascular calcification is seen. B. Native kidney; arrow denotes the lumen of an artery. Vascular calcification affected all vessels examined. C–F, immunohistochemical staining for carboxylated MGP (C, E) or undercarboxylated MGP (D, F) of aorta or femoral artery. Arrow denotes the lumen of the vessel. C, D: Aorta 20× magnification. E, F: Femoral artery 10× magnification.

Table 1

Spectrum of vascular calcification/dysfunction disorders

	Warfarin skin necrosis	Calcific uremic arteriopathy (Calciphylaxis)	Present case
Clinical manifestations	Commonly in extremities, presents with acute skin ulcers and necrosis; within 3–8 days of start of warfarin therapy	Commonly in soft tissue/thick adipose tissue; presents with red-purple plaques which progress to non-healing ulcers; most common in hemodialysis patients	Central and peripheral ischemic disease, presenting with non-healing ulcers, cellulitis, dry gangrene
Pathologic findings	Skin and subcutaneous tissue necrosis without vascular calcification	Intra-arterial fibrin thrombi, calcification in small and medium dermal vessels; calcium deposition in the subcutaneous fat	Large, medium and small artery medial calcification with flow limitation; concomitant calciphylaxis in dermal vessels
Coagulation studies	Prolonged PT	Normal	Normal
Genetic abnormalities	Protein C; protein S, antithrombin III deficiency, factor V Leiden mutations	Unknown	Unknown

Abbreviation: PT, prothrombin time.

Based on Nazarian et al.⁸