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**Vascular calcification—a matter of damage limitation?**

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**Introduction**

Vascular calcification has now been recognized as an important determinant of cardiovascular mortality in patients on dialysis. Recent cell biological studies, using phenotypically modulated, human vascular smooth muscle cells (VSMCs) *in vitro*, have highlighted the importance of vascular damage, leading to vesicle release, combined with loss of function of inhibitory proteins, as the major events in the calcification process. VSMC calcification is a regulated process, therefore the potential exists to inhibit progression or more significantly, induce regression. Identification of damage-inducing agents and calcification inhibitors is now quite advanced. The next challenge will be in determining ways to limit damage and induce expression and/or efficacy of inhibitors. Although new therapeutics have shown the potential to act on these pathways, there is still much to be learnt about how the complex ‘uraemic’ milieu appears to favour vascular calcification at the expense of bone mineralization.

**Vascular calcification—two sites with different consequences**

Vascular calcification or ‘hardening of the arteries’ has long been recognized as a complication of ageing and disease, yet until recently, little attention was paid to its clinical consequences. However, with the realization that vascular calcification is a time-dependent and widespread complication of patients on dialysis, and most likely contributes to their high cardiovascular mortality, much attention has now been focused on its aetiology, consequences and mechanisms [1,2].

Vascular calcification occurs at two anatomical sites in the vessel wall, the media and the intima. Dialysis patients have an increased prevalence of both forms, however, it is the extreme medial calcification that is
most pronounced [1]. Calcification per se predicts an increased risk of cardiovascular death/events but each anatomical site is associated with a different pathology as well as clinical consequences and outcomes [2,3].

Intimal calcification, as measured by computed tomography, predicts atherosclerotic load in the general population [2,4]. Calcification occurs in the midst of an inflammatory lesion in association with VSMCs, macrophages and lipid [3]. The role of vascular calcification, if any, in causing plaque rupture is controversial. Changes in the mechanical stability of the plaque or an increased local inflammatory response to hydroxyapatite crystals might be potential destabilizing mechanisms leading to rupture [5,6].

Extensive medial calcification of skin arterioles, known as calciphylaxis, is a rare but devastating complication of dialysis that leads to gangrene. Medial calcification of larger arteries is associated with increased arterial stiffness causing increased pulse wave velocity, an increase in cardiac load and decrease in perfusion [1,2]. This may be further exacerbated in dialysis patients by calcification of heart valves and even the myocardium, potentially predisposing to sudden cardiac death [7]. Importantly, a recent prospective study showed that atorvastatin, a lipid-lowering drug known to reduce the risk of myocardial infarction and death in coronary artery disease, had no statistically significant effect in reducing sudden cardiac death [7].

Matrix Gla protein (MGP) was identified as the first potent endogenous inhibitor of VSMC calcification. Mice lacking the MGP gene develop extensive medial calcification of their arteries and die shortly after birth [9]. Subsequent studies have shown that pyrophosphate and osteopontin are also important inhibitors of soft tissue mineralization produced by VSMCs [10,11]. Fetuin-A is of particular interest because it is produced only in the liver and under normal circumstances, it is highly abundant in the circulation. Mice lacking the fetuin-A gene develop extensive soft tissue calcifications [11].

The cell biology of vascular calcification

VSMCs in vitro and in vivo produce vesicles with similar properties to matrix vesicles in bone. In addition, they express a number of bone-regulating proteins such as MGP, osteopontin, alkaline phosphatase, bone sialoprotein and osteocalcin and the osteo/chondrocytic transcription factors Runx2 and Sox9 [12,13]. However, it remains unclear whether expression of osteo/chondrocytic proteins by VSMCs is an adaptive response that acts to regulate mineralization or whether it indicates that VSMCs orchestrate the mineralization process. What is clear is that central to the calcification process is VSMC phenotypic change.

Importantly, in contrast to normal bone formation, VSMC vesicle release appears to be a consequence of damage, particularly calcium overload. In vivo, VSMC vesicle release occurs in association with aneurysms, hypertension, atherosclerosis and vitamin D toxicity. Vesicles are derived from both apoptotic and viable VSMCs. In the healthy vessel wall, these vesicles may not necessarily cause calcification and may be eventually ‘mopped up’ by phagocytosis. However, if damage is overwhelming and phagocytosis is limited, then calcification is more likely [14]. Importantly, in vitro studies have shown that under ‘normal’ conditions VSMC vesicles are loaded with mineralization inhibitors including MGP and fetuin-A and it is only under experimental conditions where supply or function of these inhibitors is impaired that these vesicles have mineralizing properties [15,16]. Therefore, loss of inhibitors is a major factor in inducing calcification and it is clear that the production and function of both fetuin-A and MGP are likely to be impaired in dialysis patients. Indeed, circulating fetuin-A levels are reduced, and this is potentially a function of the increased inflammation present in these patients, while poor diet (lack of vitamin K) and warfarin treatment are likely to reduce MGP function by interfering with post-translational modifications [17,18].

Tissue mineralization and its regulating proteins

In a highly regulated process during cartilage differentiation, hypertrophic chondrocytes release matrix vesicles, small membrane-bound bodies equipped with sophisticated mechanisms to create a micro-environment permissible for the nucleation of calcium/phosphate crystals. The bone matrix is subsequently mineralized by the co-ordinated interaction of several mineralization-regulating proteins. However, all extracellular fluids are supersaturated with respect to calcium and phosphate, yet, under normal circumstances, mineralization only occurs in bone. This suggests that there must be powerful inhibitory processes to prevent ectopic mineralization in soft tissues and indeed a number of endogenous and circulating proteins have now been identified that act to restrict VSMC calcification.

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Causes and consequences of vascular damage in dialysis

If, as the accumulating cell biological data suggests, loss of inhibitors coupled with vascular damage, induce calcification by promoting VSMC vesicle release and phenotypic change, then the identification of damage-inducing agents is critical. Time on dialysis is a strong predictor of vascular calcification [19]. Therefore, it is likely that vascular damage in these patients is mediated by physiological changes associated with their disease process, but it may also be exacerbated by certain treatment regimens. Disturbances in mineral metabolism with increased serum calcium and/or phosphorous concentrations are ubiquitous in the dialysis population and correlate with increased cardiovascular mortality [20]. In vitro experiments have shown that calcium and phosphate, independently and synergistically induce VSMC calcification, with increased extracellular calcium significantly increasing VSMC vesicle release [15]. Moreover, vesicles released in the presence of elevated calcium and phosphate, are more likely to be 'mineralization competent' and nucleate preformed crystalline apatite that can rapidly proliferate on a permissive vascular matrix [15].

However, it is unlikely that a mineral imbalance is the only agent causing VSMC damage in dialysis patients. Traditional factors such as lipids and hyper-tension are likely to be detrimental, as are advanced glycation endproducts and other factors that induce oxidative stress. Our knowledge is still limited and we do not understand how other specific factors such as parathyroid hormone, inflammatory mediators and vitamin D impinge on calcification although limited in vitro studies suggest that they are also likely to have an impact by directly affecting VSMC phenotype [16]. A better understanding of the role of these factors is clearly an area for further investigation.

How to limit vascular damage in dialysis patients and help the inhibitors

A picture is emerging to show that vascular disease in dialysis patients is 'different' and therefore, attention should be focused on identifying specific risk factors in this patient group and normalizing them. For example, experimental evidence suggests that circulating calcium and phosphate levels should be maintained at as near normal levels as possible rather than accepting higher levels as ‘normal’ for this group. Patients who come to dialysis with pre-existing vascular calcification are the most at risk, as calcification is likely to rapidly progress in association with a mineral imbalance [21]. Evidence is accumulating to show that the new non-calcium-based phosphate binding drugs will not only maintain mineral balance, but may also act to limit progression of vascular calcification [22]. Another challenge will be to adapt therapies to increase expression of inhibitors with MGP and fetuin-A as obvious targets. Experimental data showing that MGP gene expression is regulated by calcium, potentially via the calcium sensing receptor, offer the tantalizing possibility that calcimimetics may up-regulate its expression, but this remains to be tested [23].

Recognition that vascular calcification is a regulated process has raised an important question of whether it may be possible to induce regression. Although there is an anecdotal evidence suggesting that calcification can regress, there are still no quantitative studies that clearly show regression in adult patients. Potential mechanisms for regression do exist and animal studies support two possibilities. One is the dissolution of deposited mineral by macrophages which share many properties with osteoclasts [24] and the other involves acidification of the local environment by expression of the enzyme carbonic anhydrase [25]. Although, to date there is little evidence in man that medial calcification is associated with an inflammatory cell infiltrate, further exploration of these mechanisms in human studies are warranted.

What we do not know

There is still a lot we do not know about vascular calcification but clearly, in vitro studies have provided us with the mechanistic clues required if we are to logically pursue important clinical questions (Figure 1). However, there are still many unanswered questions.

Fig. 1. Stages and effectors of VSMC calcification. Calcification in the vessel wall follows the same sequence of events as described during bone formation (left panels). However in the vessel wall calcification is clearly a pathological event involving VSMC damage and phenotypic change (left panels). Some of the factors involved in the induction of damage and phenotypic regulation are shown (right panels). The possibility of regression of calcification and potential factors that may be involved are also indicated.
that need to be pursued with in vitro studies, in animal models and in patients. Most importantly, we need to develop a greater understanding of the bone-vascular axis and the mechanisms leading to differential mineralization of these tissues in ageing and disease. More specifically, we need to investigate experimentally the much-discussed concept of ‘calcium load’ in dialysis patients and determine how this may act physiologically to promote calcification in the ‘wrong’ tissues.

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References


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