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PERSPECTIVES IN RENAL MEDICINE

Pathophysiological mechanisms of vascular calcification in end-stage renal disease

MATTHEW R. DAVIES and KEITH A. HRUSKA

Renal Division, Barnes-Jewish Hospital, St. Louis, Missouri, USA

Pathophysiological mechanisms of vascular calcification in end-stage renal disease. Vascular calcification has been clearly defined as a risk factor for cardiovascular mortality in the general population and is highly prevalent in end-stage renal disease (ESRD), where it is associated with a number of markers of increased mortality such as left ventricular hypertrophy. The pattern of calcification in ESRD is characterized by mineral deposition in the tunica media, in contrast to non-ESRD populations, where calcification of atheromatous plaque predominates. This difference may have important clinical implications. The pathophysiological mechanisms underlying both types of vascular calcification remain to be clarified; however, current evidence suggests that they are active processes rather than passive mineral precipitation, and the presence in the vasculature of cells expressing an osteoblastic phenotype may be of central importance. In ESRD, the presence of secondary and tertiary hyperparathyroidism, disordered calcium and phosphate homeostasis, and the use of vitamin D- and calcium-based treatments in its therapy may all contribute to vascular calcification. These issues and the impact on other current and future therapies have great importance for clinical nephrology, and a better understanding of vascular calcification through a focused research effort is essential.

Registry data from both the United States and Northern Europe have consistently demonstrated an excess mortality in patients with end-stage renal disease (ESRD) [1, 2], despite measures to optimize dialysis treatment. Approximately 50% of all deaths are attributed to cardiovascular disease (CVD) [3], and this remains one of the major outstanding issues in clinical nephrology. An influential article by Lindner et al concluded that accelerated atherosclerosis was the major cause of this excess cardiovascular mortality [4]. Given the high incidence of established cardiovascular risk factors in the chronic renal failure (CRF) population, this is an attractive hypothesis; however, subsequent reports have challenged its validity [5–8]. While there is undoubtedly an excess of cardiovascular mortality, it remains to be shown conclusively that this is caused by accelerated atherosclerosis per se, and other factors have been implicated [9, 10]. One such factor may be vascular calcification (VC). VC is clearly important in the general population, where coronary artery calcification correlates with and predicts mortality [11]. In CRF, histologic [6] and radiographic [12] evidence of VC is much more striking than in the general population, and it has been shown to have an important adverse influence on a number of surrogate markers for CVD mortality such as left ventricular hypertrophy (LVH) [13]. Several explanations for this marked calcification in CRF are proposed, including the presence of dysregulation of calcium/phosphate metabolism implicit in renal osteodystrophy (ROD), as well as the iatrogenic influence of its treatment with calcium-containing phosphate binders and with vitamin D analogues. In addition, new therapies emerging for the treatment of various aspects of CRF, such as bone morphogenetic protein 7 (BMP-7) for renal fibrosis [14], may theoretically exacerbate VC [15] and may, in turn, have a significant negative impact on mortality. At present, understanding of the processes and implications of VC is incomplete, and there are few data that specifically relate to VC in the context of CRF. Here, we present an interpretation of the current state of understanding of VC from the viewpoint of CRF, speculate on putative pathophysiological mechanisms, and discuss its impact on current and future management of CRF.

ARTERIOPATHY IN CHRONIC RENAL FAILURE

Vascular calcification has been recognized for over 150 years [16]. For most of that time, it has been regarded as a passive process associated with atherosclerosis or normal aging, resulting from the accumulation of debris from cell necrosis. It has not been thought to be clinically important and, as a result, received little scientific attention. However, in the last decade, evidence has emerged to suggest that VC is an active and regulated process

Key words: medial vascular calcification, osteoblast-like cell, renal osteodystrophy, hyperparathyroidism, left ventricular hypertrophy, mineral deposition, atheromatous plaque.

with important clinical consequences [11, 13, 15] and that it may be particularly important in CRF. Two patterns of calcification are recognized. First, in the context of atherosclerotic plaque formation, calcification occurs in association with lipid-laden foam cells at the base of the plaque in proportion to the severity of the lesion [17]. This process is focal, and adjacent regions of the vessel wall may be remarkably normal. The second form of calcification is sometimes referred to as Mönckeberg's sclerosis and is characterized by diffuse mineral deposition throughout the vascular tree [18], occurring predominantly in the media of the vessel, particularly at the internal elastic lamina, and with no relationship to atheromatous plaque formation. This form of calcification is characteristic of aging, but is also a striking feature of the vascular disease seen in diabetes mellitus (DM) [18] and CRF [6, 19]. It is important to note that the vast majority of patients with VC, whether related to atherosclerosis or not, have normal calcium and phosphate metabolism.

Vascular calcification is common in CRF, often from a young age [12]. Medial calcification may be particularly striking compared with other clinical situations and correlates to duration of hypertension and duration of renal disease [6]. It is also often associated with intimal proliferation [19], which in turn correlates with hyperparathyroidism and hyperphosphatemia [20], both common in ESRD. It primarily affects the aorta, but plain x-rays often show a characteristic tramline appearance of calcification extending to the peripheral small vessels, a finding confirmed histologically [19].

The clinical significance of these changes derives from biophysical alterations in the vasculature. Calcification around atherosclerotic lesions increases plaque fragility, which in turn makes that part of the vessel wall more vulnerable to shear stresses, precipitating acute plaque rupture and thrombosis [17]. This is likely to explain the correlation between coronary artery calcification and mortality [11], and complications after angioplasty [21] and bypass surgery [22]. The confluent nature of the mineralization of medial calcification influences morbidity and mortality differently, primarily by stiffening the vessel wall and reducing vascular compliance [23]. The hemodynamic consequences of this include an increase in systolic blood pressure in association with a fall in diastolic pressure, giving a widened pulse pressure, increased pulse-wave velocity, and increased left ventricular afterload. In turn, these changes have been shown to lead to both LVH and altered coronary perfusion [13]. Human studies [13, 23] and animal models [24, 25] have demonstrated the association between these vascular and cardiac changes in CRF, and recently reported data from the U.S. Renal Data System specifically confirm that pulse pressure is widened in the CRF population compared with the general population (abstract; Klassen, *J Am Soc Nephrol* 11:280A, 2000). LVH is an independent risk factor for cardiac mortality in the ESRD population, and while undoubtedly multifactorial in origin in this patient group (influenced among other factors by chronic anemia, hypertension, fluid overload, iron overload, and the direct effects of parathyroid hormone on cardiac fibrosis), the striking VC that is often present is likely to play an important role in its etiology. Recent data have clearly demonstrated that VC is correlated to increased stiffness of large arteries and LVH in the renal population [13] independent of age and hypertension.

In addition to these considerations, a form of VC that may be specific to renal failure has been described [26–28]. Calciphylaxis, or uremic arteriolopathy, manifests as a painful necrotizing rash that spreads rapidly from the periphery to the trunk and is associated with a high mortality. Histologically, it is characterized by medial calcification of cutaneous and subcutaneous arteries and arterioles, with associated occlusive intimal proliferation. Little is known about its etiology, but it has been associated with poorly controlled ROD and a longer duration of ESRD [28], although the importance of hyperparathyroidism has been questioned [29]. Whether calciphylaxis is a separate process to medial VC of large vessels or simply a more severe form of the same process in which the intimal fibrosis is sufficient to occlude critically small vessels is not clear.

PATHOGENESIS OF VASCULAR CALCIFICATION: A CENTRAL ROLE FOR AN OSTEOBLAST-LIKE CELL?

A number of lines of evidence suggest that VC is an active process [30]. First, clinical and histological descriptions have noted structures resembling bone and even bone marrow in calcified vessels [31]. Second, the particular form of calcium phosphate crystal found in vessel walls is hydroxyapatite, the form found in bone [15]. Third, matrix vesicles, which are the focus of initiation for calcification in normal mineralizing endochondral bone, have been described in calcifying vessel walls [32, 33]. Fourth, immunohistochemistry and in situ hybridization have demonstrated a variety of characteristic bone-related proteins in association with calcified atheromatous plaque, including collagen I [34], the major collagen of bone extracellular matrix (ECM), and a number of noncollagenous bone matrix proteins, including BMP-2a [15], osteopontin (OPN) [35–39], osteocalcin [30], matrix GLA-protein (MGP) [37], and alkaline phosphatase [reviewed in 30, 31, 40]. While the presence of these proteins could be interpreted as a response to calcification rather than its driving mechanism, the presence of osteocalcin, which is regarded as pathognomonic of osteoblastic terminal differentiation, suggests a role for a cell expressing an osteoblastic phenotype as a central component in its etiology, a hypothesis supported by in vitro data.

Table 1. Factors influencing calcification of vascular smooth muscle cells and pericytes in vitro

Factors increasing calcification	Factors decreasing calcification
cAMP [46]	Autocrine/paracrine PTHrP [55]
Inorganic phosphate [41, 49]	Osteopontin [42]
Alkaline phosphatase [41]	Collagen IV [45]
Corticosteroids [50]	
Vitamin D_3 [51]	
Estrogens [52]	
Oxidized lipids [48]	
TGF-β1 [48]	
Advanced glycation end products [53]	
Collagen I [45]	
? 'Intracrine' PTHrP [54]	

Abbreviations are: cAMP, cyclic adenosine monophosphate; TGF-β1, transforming growth factor-β1; PTHrP, parathyroid hormone-related peptide.

Demer et al [15], and subsequently others [41, 42], have derived primary vascular smooth muscle cell (VSMC) cultures from explants of medial tissue of both normal and diseased vessels, which adopt a calcifying phenotype in vitro and express the wide array of bone-related proteins mentioned previously in this article [15]. Of particular significance is the observation that these calcifying VSMCs, termed calcifying vascular cells or CVCs, follow the critical sequence and timing of expression of these proteins as it occurs in normal mineralizing bone [43, 44]. As cells differentiate to mature osteoblasts in bone, the ability to proliferate is lost, while at the same time synthesis of appropriate matrix components begins, predominantly collagen I. In turn, accumulating matrix signals to the cell to initiate secretion of proteins relating to mineralization, first alkaline phosphatase and OPN, later osteocalcin and MGP. The synthesis of an appropriate type of matrix critically influences calcification [45]. Collagen I and fibronectin enhance the process, while collagen IV inhibits it. These influences appear to be mediated through integrins involved in cell/matrix interaction [45]. In addition, CVCs express the transcription factor cbfa1/osf2 [46], a factor both essential and sufficient for differentiation of the mature osteoblast in bone [47]. However, whether the ability to calcify is a characteristic of all VSMCs is not clear. CVC clones calcify spontaneously and rapidly throughout their existence in vitro; in contrast, other VSMCs clones derived from the same explants never do so [48]. This difference may depend on the ability of individual clones to synthesize an appropriate matrix [45]. A number of studies have investigated the process of calcification in this cell line, suggesting that it is a cAMP-mediated mechanism [46] and influenced by a variety of factors (Table 1) [41, 42, 45, 46, 48–55]. Chronic stimulation of CVCs with cAMP causes a confluent calcification throughout the cell monolayer that is strikingly similar to in vivo medial calcification seen in CRF [46].However, if an osteoblast-like cell is indeed central to the etiology of VC, it nevertheless remains to be confirmed by in vivo observation, and this hypothesis raises a number of questions. First, what might be the origin of these cells? Second, what is their primary role, assuming the calcification process to be pathological? Third, how is calcification stimulated? Fourth, what mechanisms lead to their appearance?

Origin of the osteoblast-like cell

It has been suggested that the properties of CVCs are in vitro phenomena; nevertheless, the cells are derived ex vivo, and clearly not all cells derived from medial vascular explants behave similarly in culture, as noted previously in this article. The fact that at least two populations of cells are encountered, one calcifying and one not, strongly suggests the presence of at least two populations in the original tissue. The origin of these CVCs is not clear, however, and remains a matter of debate. It has been hypothesized that that CVCs may be derived from microvascular pericytes in view of a number of common characteristics, including morphology on light and electron microscopy, expression of cell surface and secreted proteins [56], and the ability to calcify [49]. Pericytes, smooth muscle cells, and osteoblasts are all derived from common marrow mesenchymal stem cells. While mature osteoblasts have reached an irreversible state of end differentiation and are unable to re-enter the cell cycle, pericytes, and to a lesser extent smooth muscle cells, retain their pluripotentiality and have the capability to become osteoblasts [40, 57]. However, conclusive proof of this derivation is lacking, in part because no specific marker for pericytes has yet been described. It is also unclear whether these cells with osteoblastic potential are normally resident in the vessel wall (the fact that CVCs are derived from the media of normal vessels suggests this is so [15]); whether they derive from pericytes migrating from, for example, the adventitial microvasculature; or whether from marrow stromal cells reaching the vessel wall via the circulation [58]. In addition, in a review of their own and other work on pericytes, Doherty and Canfield state that pericytes express cbfa1/osf2 [59]. If this were indeed the case, then pericytes would clearly be cells capable of expressing an osteoblastic phenotype; however, as yet there are no published data to support this claim.

Role of the osteoblast-like cell

If an osteoblast-like cell is indeed central to VC, this is unlikely to be its prime purpose in the vasculature, since VC is clearly pathological, as evidenced by the epidemiological data given previously in this article. More likely is that its differentiation fulfills a different role, but that calcification is initiated as an undesired secondary process. We hypothesize that this prime role may be related to vascular repair. Vessel wall repair

involves migration and proliferation of certain cell types, particularly smooth muscle cells. In order to migrate, a cell must release itself from its ECM attachments. One important role of the ECM attachment is to provide a survival signal, and a migrating cell needs to overcome its lack of fixation to the ECM to avoid apoptosis. OPN has been demonstrated to act as an anti-apoptotic signal for osteoclasts in their normal function [60] and for migrating vascular endothelial cells responding to vessel wall injury, functions mediated through up-regulation of osteoprotegerin expression via an $\alpha_v\beta_3$/nuclear factor-κB–dependent pathway [61]. In the clinical setting, OPN is highly up-regulated during neointima formation during VSMC proliferation and migration following angioplasty [62], suggesting that it may play a similar role in this cell type. A further function of OPN that may be relevant is that it can also act as a chemotactic factor for macrophages and monocytes in models of renal disease, and this role is supported by observations in specific human renal diseases [63]. Macrophages have a clearly defined role in the etiology of atheroma, and OPN may contribute to their recruitment in this context. Therefore, if OPN were an appropriate survival or chemotactic factor in the context of vascular injury, then the differentiation of an osteoblast-like cell would serve as an efficient mechanism for its production.

Mechanism of calcification

The role of these putative osteoblast-like cells in the mineralization process is also a matter of debate. The ability to secrete an appropriate matrix for mineralization as well as alkaline phosphatase, which is essential for mineralization to begin, and other bone-related proteins suggests that they could promote VC, but this is countered by observations from knockout mice. First, mice with a deletion of MGP develop dramatic VC shortly after birth and die in the neonatal period from aortic rupture [64]. Second, OPN knockout mice develop an enlarged and stronger skeleton characterized by larger hydroxyapatite crystals (abstract; Kizer et al, *Am Soc Bone Miner Res* 15:S343, 2000). A similar skeletal pattern is associated with a milder form of VC in osteoprotegerin knockout mice, a protein moderating osteoclastic activity that is, in turn, modulated by OPN [65]. These data suggest that OPN and MGP act primarily to restrain mineralization and, therefore, that VC may occur as a result of failure of these mechanisms. It is likely that osteocalcin, like MGP a γ-carboxylated GLA protein, has a similar function. The explanation for this apparent paradox is likely to be that in the vasculature, as in bone, cells expressing the osteoblastic phenotype both initiate and subsequently regulate calcification, and that the resulting amount of mineralization reflects a fine balance between these opposing influences. In the absence of osteoclastic activity in the vasculature, a small imbalance in favor of mineralization would lead to significant calcification over time, and whereas an acute vascular injury such as that seen after angioplasty would lead to a short-term response, a state of chronic vascular injury such as that seen in CVD would lead to an environment conducive to calcification over a protracted period.

Indeed, by analogy with the function of osteoblasts in normal bone, the very presence of osteoblast-like cells in the vasculature could lead to a procalcific environment. Bone mass varies in response to applied strain; athletic exercise, for example, is a stimulus for increased bone mass, and in contrast, bed-bound patients and astronauts in the weightless environment of space rapidly lose bone mass. Although the mechanism of this response is not well understood, one early component of it is an increase in intracellular calcium concentration mediated by stretch-activated cation channels (SA-CAT) [66], which leads to changes in cytoskeletal architecture [67], up-regulation of expression of markers of bone anabolism such as OPN [68], and increased mineralization in vitro [69]. SA-CAT has also been described in VSMCs [70]. Thus, one can hypothesize that an osteoblast-like cell in the vessel wall would respond to relatively high levels of repetitive strain in relationship to the cardiac cycle in a similar manner, particularly in proximal large vessels, leading to up-regulation of mineralization. In addition, in CRF, hypertension, fluid overload, and an up-regulated renin-angiotensin system (RAS) all increase vessel wall strain, even in distal parts of the circulation less affected by the cardiac cycle per se. Animal studies suggest that an increase in vessel wall strain may be an early event, preceding structural changes in the vasculature in a model of uremia in rats [71]. However, this explanation remains speculative.

Recruitment of the osteoblast-like cell

What causes the osteoblastic cell to occur in the vasculature? The confluent and extensive nature of medial VC in CRF suggests that whatever initiates this process affects the vascular tree widely and relatively evenly. We hypothesize that chronic vascular injury, by factors such as hypertension, chronic fluid overload, and systemic up-regulation of the RAS, is important to this process. All are common in CRF and affect the vascular tree evenly, provoking changes in the vascular wall such as smooth muscle proliferation, medial hyperplasia, and increased vessel wall stress.

One factor that may be crucial to the vascular response to chronic injury and that may influence the calcification process is parathyroid hormone-related peptide (PTHrP). PTHrP was first described as the agent of hypercalcemia of malignancy and has a high degree of sequence homology to PTH at the amino terminus as well as sharing equal affinity for their common receptor, PTH1R [reviewed in 72–74]. However, this region accounts for only

about one third of PTHrP, and the remainder of the protein is divergent from PTH. Several peptides may be derived from both the midregion and carboxy terminal, and although their precise physiological roles remain unclear, they appear to be involved in calcium metabolism [73, 74]. Expression of PTHrP is widespread in vascular tissue, where it acts in an autocrine or paracrine fashion and, importantly, is up-regulated by vessel wall stress and vasoconstricting agents such as angiotensin II [73]. Thus, the situation in CRF of hypertension, fluid overload, and overactivity of the RAS is likely to up-regulate it throughout the vascular tree. Expression is also up-regulated during acute experimental vascular injury [75] in a time-dependent fashion, in parallel with vessel wall repair and VSMC migration and proliferation. The finding of PTHrP in atherosclerotic plaque confirms its relevance to clinical disease [75]. The effects of PTHrP on VSMCs and CVCs are complex [72]. PTHrP has been shown to inhibit VSMC proliferation when acting through PTH1R [72, 76, 77], but has the opposite effect when its gene is experimentally overexpressed within the cells [54, 72]. This "intracrine" effect is mediated by its ability to escape the secretory mechanisms of the cell and translocate to the nucleus [74], where it is able to act as a transcription factor, leading to VSMC proliferation, and thus contributing to vessel wall hypertrophy. The fact that PTHrP up-regulation is followed rapidly by down-regulation of PTH1R suggests that this latter function may be more pathophysiologically relevant. When applied exogenously to CVCs, PTHrP reduces calcification [55] and is able to antagonize the calcifying influence of vitamin D_3 [51]. However, the effects of overexpression of endogenous PTHrP are not known in this context. A further paradox is the fact that exogenous PTHrP acting through PTH1R would be expected to increase cAMP, one of the secondary messengers of this receptor; however, cAMP has been shown to increase calcification in CVCs (discussed previously in this article), whereas PTHrP has the opposite effect.

In addition, PTHrP is clearly involved in bone physiology [73]. It is known to be involved in normal skeletal development, controlling chondrocyte proliferation and maturation, and orchestrating generation of an appropriate ECM for subsequent calcification. PTHrP has also been shown to increase proliferation of an osteoblast cell line in vitro, but appears to restrain differentiation [78]. The same study showed that PTHrP expression was associated with down-regulation of BMP-2 and alkaline phosphatase, changes likely to inhibit mineralization [78].

Taken together, these data suggest that PTHrP has the potential to respond to the chronic vascular injury characteristic of CRF by promoting VSMC proliferation. In addition, it can prepare the way for calcification by promoting osteoblastic proliferation and directing synthesis of an appropriate matrix; however, which of its autocrine, paracrine or intracrine functions predominate in vivo is not known, and as discussed later in this article, other components of the uremic milieu are likely to influence its behavior.

INFLUENCE OF CRF AND ITS TREATMENT ON VC

A number of issues relatively specific to CRF may influence the processes described previously in this article, which may in turn account for the striking medial VC seen. First, hyperparathyroidism is very common in CRF, and PTH has been demonstrated to increase intracellular calcium; as noted previously, this is a stimulus for calcification in the osteoblast. PTH is likely to interact with PTHrP function since PTH acts to down-regulate the common PTH1R receptor. This would impair the antimineralizing influence of PTHrP and may cause it to act preferentially through other pathways that encourage calcification. In addition, the function of many subpeptides of PTHrP and the receptors through which they act is not known. These peptides may be relevant since the carboxy-terminal fragments accumulate in CRF. Other receptors, for which PTH and PTHrP have differential affinity, have been described and hypothesized [74, 79] but need further characterization. In addition, PTH has also been linked with intimal proliferation, which may be significant in the etiology of calciphylaxis.

Second, a high calcium/phosphate ($CaxPO_4$) product has consistently been associated with increased VC [3, 12]. Patients with CRF often fail to achieve their target phosphate level, and a high $CaxPO_4$ product is maintained in part by the widespread use of calcium carbonate as a phosphate binder, which in the context of concurrent use of vitamin D analogues often leads to a mild hypercalcemia. The prescribed dose of calcium carbonate has been correlated to degree of VC as well as vascular stiffness and LVH in a recent clinical study [13].

Third, vitamin D analogues, also widely prescribed for ROD, may have adverse effects, but the issue needs clarification. Vitamin D has long been known to cause VC in humans and other species [80], characteristically in the media of the vessel wall. VSMCs express vitamin D receptors, and vitamin D inhibits proliferation of these cells by acute influx of calcium into the cell; however, in a cell expressing an osteoblast-like phenotype, this may be a stimulus to initiate mineralization [66]. It has been shown that vitamin D_3 increases expression of alkaline phosphatase in VSMCs in culture [51] and to increase calcification by CVCs in vitro [25, 51]. On the other hand, vitamin D_3 levels have been inversely correlated with coronary calcification [81, 82], and vitamin D_3 has been shown to up-regulate MGP expression in cultured osteoblasts [83, 84] and rat VSMCS [85]. In addition, vitamin D_3 is able to down-regulate collagen I [86].

These factors would tend to restrain calcification. These paradoxical influences are likely to depend on cell type, differentiation state, and species, as well as the balance of particular vitamin D moieties present, and require clarification.

Fourth, in vitro data have linked the presence of advanced glycation end products (AGEs) to VC [53]. While this has obvious implications for diabetic patients with poor glycemic control, it has recently been shown that even nondiabetic CRF patients have increased levels of AGE formation, attributable to carbonyl stress [87], and this may, therefore, have wider implications for VC in CRF.

Fifth, warfarin is commonly prescribed in ESRD and also theoretically may promote calcification since MGP and osteocalcin are both γ-glycosylated, warfarin-sensitive inhibitors of mineralization [85]. Rodent studies have demonstrated that the enzyme system for regenerating oxidized vitamin K in the vascular wall is much less efficient than the hepatic system, and thus is far more sensitive to the effects of warfarin [88]. By analogy with other enzyme systems, this system may be further impaired in uremia, which may lead to a procalcifying environment in the vasculature even at relatively low levels of anticoagulation. It is interesting to note the anecdotal association between warfarin and calciphylaxis [89]. Nevertheless, it is difficult to draw emphatic conclusions from rodent data since there are clear and important interspecies differences relating to MGP. Whereas MGP knockout mice have florid VC leading to premature death [64], as noted previously in this article, patients with the human equivalent of this situation, Keutel's syndrome, have no evidence of VC despite ectopic calcification at other cartilaginous sites [90].

FUTURE DIRECTIONS

These considerations suggest that VC in CRF at least in part may be an iatrogenic phenomenon, relating to the use of calcium-containing phosphate binders, vitamin D analogues, and anticoagulation. The introduction into clinical practice of alternative phosphate binders that do not contain calcium [91], newer vitamin D analogues that are less calcemic [92], and calcimimetic compounds for the control of hyperparathyroidism [93] may ameliorate this problem. The optimal control of fluid balance and hypertension also may contribute to an improvement.

In addition, recent work in our laboratory has suggested that the differentiation factor BMP-7 may be useful in the treatment of both tubulointerstitial fibrosis [14] and ROD (abstract; Gonzalez, *J Am Soc Nephrol* 11:554A, 2000). BMP-7 is widely expressed in development and is required for tubular epithelial cell differentiation in the kidney and the normal program of osteoblast differentiation in bone [94]. In the adult, the main site of BMP-7 synthesis is the renal collecting duct, and patients with CRF are deficient in this protein. ROD is characterized by a failure of full osteoblast differentiation and maturation, which may reflect BMP-7 deficiency; BMP-7 therefore may be a potential treatment for this condition. In addition, BMP-7 has been shown to ameliorate tubulointerstitial fibrosis and preserve glomerular filtration rate in rat models of both obstructive uropathy and diabetic nephropathy, to a greater extent than angiotensin-converting enzyme inhibitors [14]. However, one of its target genes is cbfa1/osf2, and if this factor is important in the etiology of VC, as noted previously in this article, then BMP-7 may have an adverse effect on cardiovascular morbidity and mortality that may outweigh its benefits. Clearly, this issue needs clarification before BMP-7 can be considered for clinical trials.

CONCLUSION

In conclusion, VC is a widespread phenomenon that has important consequences for long-term cardiovascular outcome. The mechanisms of its etiology are poorly understood, but are likely to reflect a balance between a number of processes relating to vascular repair in response to chronic injury. In addition, VC is likely to have a significant iatrogenic component in many patients. There are currently virtually no data concerning VC in CRF where strong circumstantial evidence suggests it has an extremely important impact. ROD and its treatment, the use of warfarin, and possibly other poorly defined elements of the uremic milieu may contribute to its etiology in this setting. Better understanding of the processes underpinning VC may have important consequences for the management of patients with CRF and ESRF, both currently and in the future, as any therapy to retard or improve VC would be likely to have an important impact on mortality in this patient group, as well as in a wider context.

Perhaps the most significant problem with the state of knowledge at present is the dearth of human data: The majority of the work referred to here is from rodent or bovine models, mostly in vitro, and the example of MGP suggests that there may be important interspecies differences in the processes described. We advocate a focused research effort in this area.

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Reprint requests to Matthew R. Davies, M.B., B.S., B.Sc, M.R.C.P., Department of Pediatrics, 660 S. Euclid Avenue, Campus Box 8208, St. Louis, Missouri 63110, USA.
E-mail: daviesmat@hotmail.com

APPENDIX

Abbreviations used in this article are: AGEs, advanced glycation end products; BMP-7, bone morphogenetic protein-7; $CaxPO_4$, calcium/phosphate; CRF, chronic renal failure; CVC, calcifying vascular cells; CVD, cardiovascular disease; DM, diabetes mellitus; ECM, extracellular matrix; ESRD, end-stage renal disease; LVH, left ventricular hypertrophy; MGP, matrix GLA-protein; OPN, osteopontin; PTHrP, parathyroid hormone-related peptide; RAS, renin-angiotensin system; ROD, renal osteodystrophy; SA-CAT, stretch-activated cation channels; VC, vascular calcification; VSMC, vascular smooth muscle cells.

REFERENCES

1. US Renal Data System (USRDS): *1999 Annual Data Report.* Bethesda, National Institute of Diabetes and Kidney Diseases, 1999
2. Raine AEG, Margreiter R, Bruner KP: Report of management of renal failure in Europe XXII, 1991. *Nephrol Dial Transplant* 7: 7–35, 1992
3. Block GA, Port FK: Re-evaluation of risks associated with hyperphosphatemia and hyperparathyroidism in dialysis patients: Recommendations for a change in management. *Am J Kidney Dis* 35: 1226–1237, 2000
4. Lindner A, Charra B, Sherrard DJ, *et al*: Accelerated atherosclerosis in prolonged maintenance hemodialysis. *N Engl J Med* 290: 697–701, 1974
5. Nicholls AJ, Edward N, Catto GRD, *et al*: Accelerated atherosclerosis in long-term dialysis and renal-transplant patients: Fact or fiction? *Lancet* 1:276–278, 1980
6. Ibels LS, Alfrey AC, Huffer WE, *et al*: Arterial calcification and pathology in uremic patients undergoing dialysis. *Am J Med* 66:790–796, 1979
7. Burke JF Jr, Francos GC, Moore LL, *et al*: Accelerated atherosclerosis in chronic-dialysis patients-another look. *Nephron* 21:181–185, 1978
8. Thomas FT, Lee HM: Factors in the differential rate of arteriosclerosis (AS) between long surviving renal transplant recipients and dialysis patients. *Ann Surg* 184:342–351, 1976
9. Foley RN, Parfre PS, Sarnak MJ: Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis* 32(Suppl): S112–S119, 1998
10. Ma KW, Greene EL, Raij L: Cardiovascular risk factors in chronic renal failure and hemodialysis populations. *Am J Kidney Dis* 19: 505–513, 1992
11. Margolis JR, Chen JTT, Kong Y, *et al*: The diagnostic and prognostic significance of coronary artery calcification. *Radiology* 137: 609–616, 1980
12. Goodman WG, Goldin J, Kuizon BD, *et al*: Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med* 342:1478–1483, 2000
13. Guerin AP, London GM, Marchais SJ, *et al*: Arterial stiffening and vascular calcifications in end-stage renal disease. *Nephrol Dial Transplant* 15:1014–1021, 2000
14. Hruska K, Guo G, Wozniak M, *et al*: Osteogenic protein-1 prevents renal fibrogenesis associated with ureteral obstruction. *Am J Physiol* 279:F130–F143, 2000
15. Boström K, Watson KE, Horn S, *et al*: Bone morphogenetic protein expression in human atherosclerotic lesions. *J Clin Invest* 91:1800–1809, 1993
16. Virchow R: Sclerosis and ossification of arteries, in *Cellular Pathology: As Based Upon Physiological and Pathological Histology*, New York, Dorer, 1971, pp 404–408
17. Wexler L, Brundage B, Crouse J, *et al*: Coronary artery calcification: Pathophysiology, epidemiology, imaging methods, and clinical implications. *Circulation* 94:1175–1192, 1996
18. Edmonds ME: Medial arterial calcification and diabetes mellitus. *Z Kardiol* 89(Suppl 2):II/101–II/104, 2000
19. Ejerblad S, Ericsson JLE, Eriksson I: Arterial lesions of the radial artery in uraemic patients. *Acta Chir Scand* 145:415–428, 1979
20. Nishizawa Y, Shoji T, Kawagishi T, *et al*: Atherosclerosis in uremia: Possible roles of hyperparathyroidism and intermediate density lipoprotein accumulation. *Kidney Int* 52:90–92, 1997
21. Fitzgerald PJ, Ports TA, Yock PG: Contribution of localized calcium deposits to dissection after angioplasty. *Circulation* 86:64–70, 1992
22. Letsov GV, Baldwin JCWS: Surgery for coronary artery disease, in *The Oxford Textbook of Surgery*, edited by Morris PJ, Mall RA, 1994, pp 1763–1780
23. London GM, Guerin AP, Marchais SJ, *et al*: Cardiac and arterial interactions in end-stage renal disease. *Kidney Int* 50:600–608, 1996
24. Niederhoffer N, Lartaud-Idjouadiene I, Giummelly P, *et al*: Calcification of medial elastic fibers and aortic elasticity. *Hypertension* 29:999–1006, 1997
25. Niederhoffer N, Bobryshev YV, Lartaud-Idjouadiene I, *et al*: Aortic calcification produced by vitamin D_3 plus nicotine. *J Vasc Res* 34:386–398, 1997
26. Edwards RB, Jaffe W, Arrowsmith J, *et al*: Calciphylaxis: A rare limb and life threatening cause of ischaemic skin necrosis and ulceration. *Br J Plast Surg* 53:253–254, 2000
27. Oh DH, Eulau D, Tokugawa DA, *et al*: Five cases of calciphylaxis and a review of the literature. *J Am Acad Dermatol* 40:979–987, 1999
28. Hafner J, Keusch G, Wahl C, *et al*: Uremic small-artery disease with medical calcification and intimal hyperplasia (so-called calciphylaxis): A complication of chronic renal failure and benefit from parathyroidectomy. *J Am Acad Dermatol* 33:954–962, 1995
29. Bleyer AJ, Choi M, Igwemezie B, *et al*: A case control study of proximal calciphylaxis. *Am J Kidney Dis* 32:376–383, 1998
30. Shioi A, Mori K, Jono S, *et al*: Mechanism of atherosclerotic calcification. *Z Kardiol* 89(Suppl 2):II/75–II/79, 2000
31. Boström KI: Cell differentiation in vascular calcification. *Z Kardiol* 89(Suppl 2):II/69–II/74, 2000
32. Kockx MM, Muhring J, Bortier H, *et al*: Biotin- or digoxigenin-conjugated nucleotides bind to matrix vesicles in atherosclerotic plaques. *Am J Pathol* 148:1771–1777, 1996
33. Kim KK: Calcification of matrix vesicles in human aortic valve and aortic media. *Fed Proc* 35:156–162, 1976
34. Rekhter MD, Zhang K, Narayanan AS, *et al*: Type I collagen gene expression in human atherosclerosis. *Am J Pathol* 143:1634–1648, 1993
35. Hirota S, Imakita M, Kohri K, *et al*: Expression of osteopontin messenger RNA by macrophages in atherosclerotic plaques. *Am J Pathol* 143:1003–1008, 1993
36. O'Brien ER, Garvin MR, Stewart DK, *et al*: Osteopontin is synthesized by macrophage, smooth muscle, and endothelial cells in primary and restenotic human coronary atherosclerotic plaques. *Arterioscler Thromb* 14:1648–1656, 1994
37. Shanahan CM, Cary NRB, Metcalfe JC, *et al*: High expression of genes for calcification-regulating proteins in human atherosclerotic placques. *J Clin Invest* 93:2393–2402, 1994
38. Ikeda T, Shirasawa T, Esaki Y, *et al*: Osteopontin mRNA is expressed by smooth muscle-derived foam cells in human atherosclerotic lesions of the aorta. *J Clin Invest* 92:2814–2820, 1993
39. Fitzpatrick LA, Severson A, Edwards WD, *et al*: Diffuse calcification in human coronary arteries. *J Clin Invest* 94:1597–1604, 1994
40. Shanahan CM, Proudfoot D, Tyson KL, *et al*: Expression of mineralisation-regulating proteins in association with human vascular calcification. *Z Kardiol* 89(Suppl 2):II/63–II/68, 2000
41. Shioi A, Nishizawa Y, Jono S, *et al*: β-Glycerophosphate accelerates calcification in cultured bovine vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 15:2003–2009, 1995
42. Wada T, McKee MD, Steitz S, *et al*: Calcification of vascular smooth muscle cell cultures. *Circ Res* 84:166–178, 1998
43. Jakoby MGI, Semenkovich CF: The role of osteoprogenitors in vascular calcification. *Curr Opin Nephrol Hypertens* 9:11–15, 2000
44. Stein GS, Lian JB, Stein JL, *et al*: Transcriptional control of osteoblast growth and differentiation. *Physiol Rev* 76:593–617, 1996
45. Watson KE, Parhami F, Shin V, *et al*: Fibronectin and collage I matrixes promote calcification of vascular cells in vitro, whereas collagen IV matrix is inhibitory. *Arterioscler Thromb Vasc Biol* 18:1964–1971, 1998
46. Tintut Y, Parhami F, Boström K, *et al*: cAMP stimulates osteoblast-like differentiation of calcifying vascular cells. *J Biol Chem* 273:7547–7553, 1998

47. Ducy P, Zhang R, Geoffroy V, *et al*: Osf2/Cbfa1: A transcriptional activator of osteoblast differentiation. *Cell* 89:747–754, 1997
48. Watson KE, Boström K, Ravindranath R, *et al*: TGF-β1 and 25-hydroxycholesterol stimulate osteoblast-like vascular cells to calcify. *J Clin Invest* 93:2106–2113, 1994
49. Schor AM, Allen TD, Canfield AE, *et al*: Pericytes derived from the retinal microvasculature undergo calcification in vitro. *J Cell Sci* 97:449–461, 1990
50. Mori K, Shioi A, Jono S, *et al*: Dexamethasone enhances in vitro vascular calcification by promoting osteoblastic differentiation of vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 19: 2112–2118, 1999
51. Jono S, Nishizawa Y, Shioi A, *et al*: 1,25-Dihydroxyvitamin D_3 increases in vitro vascular calcification by modulating secretion of endogenous parathyroid hormone-related peptide. *Circulation* 98:1302–1306, 1998
52. Balica M, Boström K, Shin V, *et al*: Calcifying subpopulation of bovine aortic smooth muscle cells is responsive to 17-β-estradiol. *Circulation* 95:1954–1960, 1997
53. Yamagishi S, Fujimori H, Yonekura H, *et al*: Advanced glycation end products accelerate calcification in microvascular pericytes. *Biochem Biophys Res Commun* 258:353–357, 1999
54. Massfelder T, Dann PM, Wu TL, *et al*: Opposing mitogenic and anti-mitogenic actions of parathyroid hormone-related protein in vascular smooth muscle cells: A critical role for nuclear targeting. *Proc Natl Acad Sci USA* 94:13630–13635, 1997
55. Jono S, Nishizawa Y, Shioi A, *et al*: Parathyroid hormone-related peptide as a local regulator of vascular calcification. *Arterioscler Thromb Vasc Biol* 17:1135–1142, 1997
56. Doherty MJ, Ashton BA, Walsh S, *et al*: Vascular pericytes express osteogenic potential in vitro and in vivo. *J Bone Miner Res* 13:828–838, 1998
57. Campbell GR, Campbell JH: Vascular smooth muscle and arterial calcification. *Z Kardiol* 89(Suppl 2):II/54–II/62, 2000
58. Bostrom K, Watson KE, Stanford WP, *et al*: Atherosclerotic calcification: Relation to developmental osteogenesis. *Am J Cardiol* 75:88B–91B, 1995
59. Doherty MJ, Canfield AE: Gene expression during vascular pericyte differentiation. *Crit Rev Eukaryot Gene Expr* 9:1–17, 1999
60. Chellaiah M, Hruska KA: Osteopontin stimulates gelsolin associated phosphoinositide levels and PtdIns 3-hydroxyl kinase. *Mol Biol Cell* 7:743–753, 1996
61. Malyankar UM, Scatena M, Suchland KL, *et al*: Osteoprotegerin is an $\alpha_v\beta_3$-induced, NF-κB-dependent survival factor for endothelial cells. *J Biol Chem* 275:20959–20962, 2000
62. Giachelli CM, Bae N, Almeida M, *et al*: Osteopontin is elevated during neointima formation in rat arteries and is a novel component of human atherosclerotic plaques. *J Clin Invest* 92:1686–1696, 1993
63. Okada H, Moriwaki K, Konishi K, *et al*: Tubular osteopontin expression in human glomerulonephritis and renal vasculitis. *Am J Kidney Dis* 36:498–506, 2000
64. Luo G, Ducy P, McKee MD, *et al*: Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature* 386:78–81, 1997
65. Bucay N, Sarosi I, Dunstan CR, *et al*: Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev* 12:1260–1268, 1998
66. Kizer N, Guo XL, Hruska KA: Reconstitution of osteoblast stretch-activated channels by expression of alpha-rENaC. *Proc Natl Acad Sci USA* 94:1013–1018, 1997
67. Chen NX, Ryder KD, Pavalko FM, *et al*: Ca^{2+} regulates fluid shear-induced cytoskeletal reorganization and gene expression in osteoblasts. *Am J Physiol Cell Physiol* 278:C989–C997, 2000
68. Owan I, Burr DB, Turner CH, *et al*: Mechanotransduction in bone: Osteoblasts are more responsive to fluid forces than mechanical strain. *Am J Physiol* 273:C810–C815, 1997
69. Wozniak M, Fausto A, Carron CP, *et al*: Mechanically strained cells of the osteoblast lineage organize their extracellular matrix through unique sites of $\alpha_v\beta_3$-integrin expression. *J Bone Miner Res* 15:1731–1745, 2000
70. Kirber MT, Walsh JV Jr, Singer JJ: Stretch-activated ion channels in smooth muscle: A mechanism for the initiation of stretch-induced contraction. *Pflügers Arch* 412:339–345, 1988
71. Savage T, McMahon AC, Mullen AM, *et al*: Increased myogenic tone precedes structural changes in mild experimental uraemia in the absence of hypertension in rats. *Clin Sci* 95:681–686, 1998
72. Vasavada RC, Garcia-Ocana A, Massfelder T, *et al*: Parathyroid hormone-related protein in the pancreatic islet and the cardiovascular system. *Recent Prog Horm Res* 53:305–340, 1998
73. Strewler GJ: The physiology of parathyroid hormone-related protein. *N Engl J Med* 342:177–185, 2000
74. Wysolmerski JJ, Stewart AF: The physiology of parathyroid hormone-related protein: An emerging role as a developmental factor. *Annu Rev Physiol* 60:431–460, 1998
75. Ozeki S-I, Ohtsuru A, Seto S, *et al*: Evidence that implicates the parathyroid hormone-related peptide in vascular stenosis. *Arterioscler Thromb Vasc Biol* 16:565–575, 1996
76. Maeda S, Wu S, Juppner H, *et al*: Cell-specific signal transduction of parathyroid hormone (PTH)-related protein through stably expressed recombinant PTH/PTHrP receptors in vascular smooth muscle cells. *Endocrinology* 137:3154–3162, 1996
77. Ishikawa M, Akishita M, Kozaki K, *et al*: Amino-terminal fragment (1-34) of parathyroid hormone-related protein inhibits migration and proliferation of cultured vascular smooth muscle cells. *Atherosclerosis* 136:59–66, 1998
78. Du P, Ye Y, Seitz L, *et al*: Endogenous parathyroid hormone-related peptide enhances proliferation and inhibits differentiation in the osteoblast-like cell line ROS 17/2.8. *Bone* 26:429–436, 2000
79. Juppner H: Receptors for parathyroid hormone and parathyroid hormone-related peptide: Exploration of their biological importance. *Bone* 25:87–90, 1999
80. Stehbens WE: Atherosclerosis and degenerative diseases of blood vessels, in *Vascular Pathology*, edited by Stehbens WE, Lie JT, London, Chapman, 1995
81. Watson KE, Abrolat ML, Malone L, *et al*: Active serum vitamin D levels are inversely correlated with coronary calcification. *Circulation* 96:1755–1760, 1997
82. Doherty T, Tang W, Dascalos S, *et al*: Ethnic origin and serum levels of 1α25-dihydroxyvitamin D sub3 are independent predictors of coronary calcium mass measured by electron-beam computed tomography. *Circulation* 96:1477–1481, 1997
83. Fraser JD, Otawara Y, Price PA: 1,25-Dihydroxyvitamin D_3 stimulates the synthesis of matrix gamma-carboxyglutamic acid protein by osteosarcoma cells. Mutually exclusive expression of vitamin K-dependent bone proteins by clonal osteoblastic cell lines. *J Biol Chem* 263:911–916, 1988
84. Fraser JD, Price PA: Induction of matrix Gla protein synthesis during prolonged 1,25-dihydroxyvitamin D_3 treatment of osteosarcoma cells. *Calcif Tissue Int* 46:270–279, 1990
85. Shanahan CM, Proudfoot D, Farzaneh-Far A, *et al*: The role of Gla proteins in vascular calcification. *Crit Rev Eukaryot Gene Expr* 8:357–375, 1998
86. Pavlin D, Bedalov A, Kronenberg MS, *et al*: Analysis of regulatory regions in the COL1A1 gene responsible for 1,25-dihydroxyvitamin D_3-mediated transcriptional repression in osteoblastic cells. *J Cell Biochem* 56:490–501, 1994
87. Miyata T, Kurokawa K, Van Ypersele De Strihou C: Advanced glycation and lipoxidation end products: Role of reactive carbonyl compounds generated during carbohydrate and lipid metabolism. *J Am Soc Nephrol* 11:1744–1752, 2000
88. Wallin R, Cain D, Sane DC: Matrix Gla protein synthesis and gamma-carboxylation in the aortic vessel wall and proliferating vascular smooth muscle cells. *Thromb Haemost* 82:1764–1767, 1999
89. Coates T, Kirkland GS, Dymock RB, *et al*: Cutaneous necrosis from calcific uremic arteriolopathy. *Am J Kidney Dis* 32:384–391, 1998
90. Munroe PB, Olgunturk RO, Fryns J-PVML, *et al*: Mutations in the gene encoding the human matrix Gla protein cause Keutel syndrome. *Nat Genet* 21:142–144, 1999
91. Hergesell O, Ritz E: Phosphate binders on iron basis: A new perspective? *Kidney Int* 56(Suppl 73):S42–S45, 1999
92. Slatopolsky E, Dusso A, Brown A: New analogs of vitamin D_3. *Kidney Int* 56(Suppl 73):S46–S51, 1999
93. Coburn JW, Elangovan L, Goodman WG, *et al*: Calcium-sensing receptor and calcimimetic agents. *Kidney Int* 56(Suppl 73):S52–S58, 1999
94. Hogan BLM: Bone morphogenetic proteins: Multifunctional regulators of vertebrate development. *Genes Dev* 10:1580–1594, 1996