Dialysis Accelerates Medial Vascular Calcification in Part by Triggering Smooth Muscle Cell Apoptosis

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Background—Vascular calcification is associated with increased morbidity and mortality in stage V chronic kidney disease, yet its early pathogenesis and initiating mechanisms in vivo remain poorly understood. To address this, we quantified the calcium (Ca) load in arteries from children (10 predialysis, 24 dialysis) and correlated it with clinical, biochemical, and vascular measures.

Methods and Results—Vessel Ca load was significantly elevated in both predialysis and dialysis and was correlated with the patients’ mean serum Ca\texttimes HPO\textsubscript{4}\textsuperscript{2-} product. However, only dialysis patients showed increased carotid intima-media thickness and increased aortic stiffness, and calcification on computed tomography was present in only the 2 patients with the highest Ca loads. Importantly, predialysis vessels appeared histologically intact, whereas dialysis vessels exhibited evidence of extensive vascular smooth muscle cell (VSMC) loss owing to apoptosis. Dialysis vessels also showed increased alkaline phosphatase activity and Runx2 and osterix expression, indicative of VSMC osteogenic transformation. Deposition of the vesicle membrane marker annexin VI and vesicle component mineralization inhibitors fetuin-A and matrix Gla-protein increased in dialysis vessels and preceded von Kossa positive overt calcification. Electron microscopy showed hydroxyapatite nanocrystals within vesicles released from damaged/dead VSMCs, indicative of their role in initiating calcification.

Conclusions—Taken together, this study shows that Ca accumulation begins predialysis, but it is the induction of VSMC apoptosis in dialysis that is the key event in disabling VSMC defense mechanisms and leading to overt calcification, eventually with clinically detectable vascular damage. Thus the identification of factors that lead to VSMC death in dialysis will be of prime importance in preventing vascular calcification. (Circulation. 2008;118:1748-1757.)

Key Words: kidney diseases ■ muscles, vascular smooth ■ calcification, vascular ■ vesicles, vascular smooth muscle cell–derived ■ apoptosis ■ cardiovascular disease ■ pediatrics

The most common cause of death in patients with chronic kidney disease (CKD) is cardiovascular disease.1,2 Structural and functional abnormalities in the large vessels begin as early as the first decade of life,3,4 leading to a 700-fold higher mortality rate than that of the healthy childhood population.1 Factors specific to CKD, in particular, disorders in calcium (Ca) and phosphate (P) metabolism,6,7 are associated with vascular calcification. Evidence of vascular changes in patients with CKD obtained from imaging studies have shown indirect evidence of vascular damage, such as increased carotid artery intima-media thickness (IMT)4,8-10 and arterial stiffness (increased pulse wave velocity [PWV]),4,11 and direct evidence of arterial calcification on computed tomography (CT) scans4,12 and autopsy.13 However, studies in adults are complicated by the presence of multiple proatherosclerotic risk factors, such as diabetes, dyslipidemia, hypertension, and, often, the presence of preexisting vascular calcification, and few studies have been able to characterize the earliest events or the natural history of progression of uremic calcification in vivo.

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In vitro studies using human vascular smooth muscle cells (VSMCs) have shown that vascular calcification is a highly regulated process with many similarities to bone formation.14,15 A mineral imbalance can induce VSMC apoptosis as well as cellular adaptation and vesicle release,16 with these small membrane-bound bodies forming a nidus for the
deposition of basic Ca-P in the form of hydroxyapatite. In the healthy vessel wall, vesicles are loaded with physiological inhibitors of calcification such as fetuin-A and matrix Gla-protein (MGP) that limit their mineralization potential, but evidence suggests that these proteins may be deficient or nonfunctional in patients with CKD. Also, as part of the mineralization process, VSMCs change to an osteo/chondrocytic phenotype that is characterized by the up-regulation of bone-specific transcription factors and matrix proteins, including Runx2/Cbfa1, osterix, and alkaline phosphatase (ALK). This phenotypic adaptation is thought to accelerate or regulate calcification with perturbations in physiological calcification inhibitors, leading to an increased expression of osteogenic markers by VSMCs that further enhances the pro-calcific environment. However, the series of events that lead to the initiation and progression of vascular calcification in the context of an intact vessel wall are not known.

To investigate these processes in vivo, we studied the natural history of vascular calcification in children with CKD. Children provide a good opportunity to study uremic influences on the arterial wall because they have fewer pro-atherosclerotic risk factors, which are major confounders in similar adult studies.

Methods

Patients

Medium-sized muscular arteries routinely removed at aortic dissection during a peritoneal dialysis catheter insertion or at renal transplantation in 34 patients with CKD were compared with mesenteric arteries removed at planned intra-abdominal surgery in 6 disease-free, age-matched controls. Children with underlying inflammatory disorders, vasculitis, diabetes, or dyslipidemia, as well as smokers, were excluded to keep the patient and control groups free of confounding pro-atherosclerotic risk factors.

Clinical and Vascular Measures

Patients’ clinical and biochemical data were recorded over a 3-year period (Data Supplement Table I) and expressed as mean time-averaged values. Carotid IMT (cIMT), PWV, and coronary calcification score on multi-slice CT scan were measured in all patients older than 5 years of age and compared with 40 healthy age-matched children.

In Vitro Analyses, Histology, and Immunohistochemistry

The Ca content and ALK levels in the vessel wall were measured quantitatively by cresolphthalein complexone chemistry and colorimetrically as the hydrolysis of p-nitrophenyl phosphate, respectively (Data Supplement Table II). Detailed histology and immunohistochemistry were performed to study vessel integrity and cell number (hematoxylin/eosin), calcification (von Kossa), apoptosis (terminal deoxynucleotidyl transferase mediated dUTP nick end labeling [TUNEL] and cleaved caspase-3 antibody), proliferation (Ki67), and inflammation (CD3 and CD68 for macrophage infiltration). Vessel integrity was examined by Verhoff van Geison and Mason’s trichrome stains for elastic lamellae and collagen, respectively, and vessel ultrastructure was studied by electron microscopy. Also, immunohistochemistry for vesicle components (annexin-VI and fetuin-A), osteogenic factors (Runx2 and ostex), and carboxylated and undercarboxylated MGP were performed. Detailed methods and antibodies used are described in the Data Supplement.

Statistics

Data are described as mean±SD or median and range. The Kruskal-Wallis test was used to test significance between the 3 groups, and the Mann–Whitney U test was performed to compare values between groups, adjusting probability values for multiplicity. Linear regression analyses were performed to test associations between vessel Ca load and clinical measures.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Predialysis and Dialysis Vessels Have an Increased Ca Load

The vessel Ca load was significantly higher in predialysis compared with control vessels (20.4±6.5 vs 9.4±2.3 μg/μL), but was highest in dialysis vessels (39.2±6.0 μg/μL) where levels were 2-fold greater than predialysis and 3- to 4-fold greater than in normal vessels (P<0.0001 [ANOVA]; Figure 1A, Data Supplement Table II). The Ca load was significantly and consistently higher in dialysis vessels despite a similar age and level of renal deterioration in the predialysis and dialysis patients and was independent of preservation of residual renal function in dialysis patients.

Calcification was not detectable by von Kossa staining in control or predialysis vessels. In contrast, diffuse speckled calcification in the media and along the internal elastic lamina of dialysis vessels was present in 6 (25%) of 24 dialysis vessels (Figure 1B), suggesting that Ca accumulation/loading within the tunica media begins before overt calcification can be detected by von Kossa.
Vessel Ca Load Is Correlated With Dialysis Vintage, Ca×P Product, and cIMT
Cumulative data on demographic and biochemical parameters (Data Supplement Table I) were correlated with the Ca load in the vessel wall. The Ca load did not increase with the time spent in CKD stages IV and V before dialysis was commenced (\(P=0.29, r^2=0.03\); Figure 2A), but there was an increase in Ca load with increasing time on dialysis (\(P=0.017, r^2=0.22\); Figure 2B) that was independent of age, gender, and dialysis modality. The mean time-averaged serum Ca×P product showed a strong linear relation with Ca load (\(P=0.008, r^2=0.20\); Figure 2C). No correlation was seen with serum Ca, P, or intact parathyroid hormone levels, high-sensitivity C-reactive protein, cumulative Ca intake from P-binders, or alphacalcidol dosage (Data Supplement Table II).

The cIMT showed a strong independent correlation with Ca load (\(P=0.01, r^2=0.28\); Figure 2D). Despite the increased Ca load in all predialysis and dialysis patients, the IMT was increased in only 18 dialysis patients (75%) and remained within age-related normal limits (0.40 mm) in all the predialysis patients. On cardiac CT scan, calcification was seen only in the 2 patients who had the highest Ca loads and macrocalcification visible by von Kossa (Agatston score 1221 and 631; patients 35 and 39 in Data Supplement Table I). The PWV was not correlated with the Ca load in the overall cohort (not shown) and was increased in only the 2 patients with coronary calcification on CT.

Dialysis But Not the Vessel Ca Load Is Associated With VSMC Apoptosis
Despite the increased Ca load in all the predialysis vessels, there was no difference in the number of VSMCs between predialysis and control vessels (median, 122 [range, 112 to 129] versus 126 [range, 118 to 144] cells per unit area, \(P=0.08\); Figure 3A). However, there was a significant reduction in VSMC numbers in dialysis vessels (median, 80 [range, 58 to 106] VSMCs per unit area; Figure 3A). To determine whether the reduced cell number was a result of a significantly greater Ca load in the dialysis vessels or was induced by exposure to dialysis, predialysis and dialysis vessels with a similar Ca load of 25 to 35 \(\mu\)g/\(\mu\)L (patients 13 to 22 in Data Supplement Table II; \(n=4\) predialysis and \(n=6\) dialysis vessels) were compared. Significantly fewer VSMCs were present in dialysis compared with predialysis vessels (median, 88 [range, 67 to 110] versus 120 [range, 114 to 126] cells per unit area; \(P=0.004\)) and this was confirmed on histology (Figure 3C). In addition, cystic areas were observed in von Kossa positive regions, suggesting that lost VSMCs were not replaced. Ki67 staining showed that few cells were proliferating (<0.5% positivity; results not shown), with no difference between control and dialysis vessels, implying that VSMC proliferation was not induced in response to cell loss.

To determine whether apoptosis was contributing to VSMC loss, TUNEL staining was performed. Significant apoptosis was evident only in dialysis vessels (0.4±0.4%, 0.27±0.5%, and 3.16±1.0% in normal, predialysis, and dialysis vessels, respectively; \(P=0.008\)). Vessels with fewer VSMCs had the greatest percentage of TUNEL positive areas (Figure 3B and 3C). Importantly, areas of apoptosis were seen in the same region of the vessel as von Kossa-positive calcified areas in adjacent sections (Figure 3C). Histology was performed to explore further the nature of dialysis-induced vascular damage. A neointima was seen only in the
most severely affected dialysis patients (Data Supplement Table II). Calcification was exclusively medial on von Kossa staining. There was no evidence of an inflammatory response or macrophage infiltration in any vessel, the internal and external elastic laminae were intact, and there was no evidence of increased collagen deposition, suggesting that the vascular changes in dialysis patients were not atherosclerotic (data not shown).

VSMCs Undergo Osteogenic Differentiation in Dialysis Vessels

Osteoblastic conversion of VSMCs, measured by ALK activity, is an early event in vascular calcification. Despite an increased Ca load, there was no increase in ALK activity in predialysis (median, 6.4 [range, 3.7 to 12.0] IU/μL) compared with control vessels (median, 4.9 [range, 2.8 to 10.5] IU/μL), but dialysis vessels had -2-fold higher ALK levels (median, 15.0 [range, 8.6 to 20.1] IU/μL; Figure 4A). Again, it was not clear whether the greater Ca load in dialysis vessels or factors specific to dialysis per se were responsible for triggering an osteoblastic phenotypic change. There was no correlation between the Ca load and ALK in the overall cohort (P=0.08), but dialysis vessels with comparable Ca loads to predialysis vessels (25 to 35 μg/μL, patients 13 to 22 in Data Supplement Table II; n=4 predialysis and n=6 dialysis vessels) had greater ALK levels (median, 13.3 [range, 10.4 to 16.2] IU/μL versus 6.4 [range, 4.1 to 7.2] IU/μL, P=0.009). However, ALK levels did not increase with time on dialysis (P=0.39).

Immunohistochemistry for the osteogenic transcription factors showed that all vessels had some positivity for Runx2 and osterix, but the levels and distribution varied. Control and predialysis vessels showed diffuse staining for Runx2 in a predominantly nuclear distribution (2% and 4.4%/unit area, respectively) whereas dialysis vessels showed an upregulation of Runx2 staining (13.4%/unit area, P＜0.0001), with a marked cytoplasmic distribution (Figure 4B and 4C). Staining for osterix showed a similar distribution (Figure 4C). Overall, the pattern of Runx2 and osterix staining mirrored ALK levels, with increased cytoplasmic staining in vessels with the highest ALK activity.

Vessel Calcification Is Associated With Undercarboxylated MGP and Fetuin-A Deposition

MGP and fetuin-A are inhibitors of calcification released by VSMCs in vesicles and apoptotic bodies. Fetuin-A–positive dialysis vessels with comparable Ca loads to predialysis vessels (25 to 35 μg/μL, patients 13 to 22 in Data Supplement Table II; n=4 predialysis and n=6 dialysis vessels) had greater ALK levels (median, 13.3 [range, 10.4 to 16.2] IU/μL versus 6.4 [range, 4.1 to 7.2] IU/μL, P=0.009). However, ALK levels did not increase with time on dialysis (P=0.39).

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Figure 3. Cell number and apoptosis in vessels. (A) VSMC nuclei per unit area of vessel were counted on a hematoxylin-eosin stained sample to determine the number of VSMCs in different vessel types. There was no reduction in the number of VSMCs in predialysis compared with control vessels, but cell numbers were significantly reduced in dialysis vessels. (B) There was no increase in the number of apoptotic cells in predialysis as compared with normal vessels, but dialysis vessels showed significantly more apoptosis. (C) Staining for α smooth muscle cell actin (top) was reduced, as was cell number in dialysis vessels compared with controls. Arrows indicate areas of cell loss in the dialysis vessel. TUNEL staining (middle) was present in dialysis vessels but absent in controls. Von Kossa staining (bottom) showed that areas of medial calcification in dialysis vessels localized to regions that were also TUNEL–positive in adjacent sections. No TUNEL positivity or calcification were observed in control vessels. VSMC indicates vascular smooth muscle cells; M, media; Ad, adventitia; α-SM actin, α smooth muscle cell actin; and TUNEL, terminal deoxynucleotidyl transferase mediated dUTP nick end labeling.
Calcification In Vivo Is a Vesicle-Mediated Process

The presence of MGP and fetuin-A at sites of calcification is highly suggestive of a vesicle-mediated calcification process, and this was confirmed by staining for annexin VI, a Ca-binding protein concentrated in both chondrocyte and VSMC (our unpublished data) matrix vesicles. Annexin VI staining was localized in the same region of the vessel as von Kossa-positive areas on adjacent sections (not shown).

Transmission electron microscopy ultrastructural analysis revealed that in the normal vessel wall, VSMCs were morphologically contractile, there was no evidence of extracellular vesicles, and the nuclei showed normal appearance and distribution of heterochromatin (Figure 6C, a). However, VSMCs in dialysis vessels showed apoptosis and damage characterized by increased electron density of nuclear heterochromatin, cell shrinkage, and/or vesicle release (Figure 6C, b and c). This damage was evident in >60% of VSMCs in 1 patient with a Ca load of 33 µg/µL (patient 21, Data Supplement Table II) and no evidence of calcification on von Kossa, suggesting that it is an early event preceding overt calcification. Interestingly, VSMCs with heteropycnotic nuclei did not have all the hallmarks of typical apoptosis or necrosis, nor did they exhibit characteristics of “synthetic” VSMCs, consistent with the absence of proliferation and suggesting that apoptosis of contractile VSMCs may have unique features. In patients without overt calcification, extracellular vesicles did not contain any evidence of hydroxyapatite nanocrystals, but vessels with calcification had vesicles containing microcrystalline structures consistent with hydroxyapatite. Vesicles deposited in the extracellular matrix were in the size range of 0.1 to 1.0 µm, which is consistent with their derivation from both apoptotic bodies and plasma membrane budding of matrix vesicles (Figure 6C, c through e).

Discussion

In this study, we provide quantitative evidence that Ca accumulation in the vessel wall begins predialysis, and factors specific to the dialysis milieu trigger accelerated calcification. We hypothesize that Ca accumulation in the vessel begins in response to increased Ca and P, but protective mechanisms such as adequate mineralization inhibitor levels and extrusion of intracellular Ca via vesicle release preserve normal VSMC function. In the dialysis milieu, damage-inducing agents that include continued exposure to high, and possibly worsening, Ca and P lead to apoptosis. This in turn increases local Ca levels and reduces local levels of VSMC-derived mineralization inhibitors, which potentiates osteo/chondrocytic differentiation of smooth muscle cells.
and the release of pro-calcific vesicles that form a nidus for calcification (Figure 7).

Importantly, the clinical detection of VSMC damage and calcification was only possible in patients with the most severe calcification. Calcification was inexorable and extremely rapid on dialysis, with a dialysis vintage of even 2 months sufficient to induce histologically overt calcification and VSMC damage, emphasizing the need to avoid dialysis and perform preemptive renal transplantation wherever possible. The identification of factors specific to dialysis that trigger the accelerated wave of VSMC death will be key in minimizing the detrimental effects of arterial calcification in renal failure.

**Ca Load in CKD Vessels Is Medial and Is Correlated With Dysregulated Mineral Metabolism**

Calcification in dialysis involved multiple vascular beds, including the carotid, coronary, omental, and inferior epigastric vessels. Histology showed that calcification was entirely medial in distribution, and there was no evidence for inflammation, suggesting that calcification, at least in its early stages and in young patients with CKD, is an arteriosclerotic rather than an atherosclerotic process. Ca loading was evident in both predialysis and dialysis, but evidence for vascular remodeling (ie, increased cIMT and neointima formation) was observed only in dialysis vessels with the highest Ca loads. Potentially, this implies that there may be a causal relationship between Ca loading and increased susceptibility to vessel wall damage and remodeling; however, currently available clinical tools are not sensitive enough to detect what may be functionally significant vascular damage in CKD.

The Ca load showed a strong correlation with the patients’ serum Ca×P product in all CKD vessels. Numerous adult studies have previously shown an association between the serum Ca×P product and an increased cIMT or vascular calcification in dialysis patients, but there are conflicting reports in predialysis patients. However, these adult studies were complicated by preexisting vascular disease as well as other risk factors for calcification, whereas the children in our study were free of the major confounders for cardiovascular disease, diabetes, dyslipidemia, and uncontrolled hypertension. Also, unlike adult studies, the increased Ca load was independent of the patients’ age and related only to their time on dialysis. There was no increase in Ca load.
with increasing time spent in CKD stages IV or V before initiating dialysis, but significantly greater calcification was seen with increasing time on dialysis and was correlated with the induction of apoptosis. Dialysis was associated with more severe dysregulation of mineral metabolism, and our earlier work has shown that VSMC calcification in vitro increases markedly when in addition to phosphate, Ca is added to the culture medium,17 as it triggers apoptosis and vesicle release.29 It is plausible that transient fluctuations in serum Ca, as often seen after hemodialysis, that are not reflected in serum Ca levels may contribute to calcification. Treatment regimens such as calcium-based phosphate binders4,8,12 and vitamin D4,5,8,13 have also been shown to increase hypercalcemic episodes and Ca load; however, we did not find any association between these and the vessel Ca load in this study. Animal studies suggest roles for oxidative stress, inflammatory mediators,30 and advanced glycation end products in promoting VSMC injury,31 but these have yet to be explored in a human in vivo model. However, although inflammation plays a key role in intimal calcification and in medial calcification in patients with diabetes,32,33 its role in uremic medial calcification, at least in our cohort of pediatric patients free of diabetes, appeared minimal given the lack of inflammatory cell infiltrate in the vessel wall and the absence of any correlation between calcium load and high-sensitivity C-reactive protein. Thus other factor(s) specific to the dialysis milieu that may be responsible for VSMC death remain to be identified.

**Dialysis Induces VSMC Apoptosis and Osteo/Chondrocytic Differentiation**

Previous in vitro studies have shown that apoptosis precedes the development of VSMC calcification. Apoptotic bodies form a nidus for the deposition of hydroxyapatite,16,17 and apoptosis has been shown to increase the local concentrations of Ca to >30 mmol/L, with this elevation potentially inducing further VSMC death, vesicle release, and calcification.29 A reduction in the number of VSMCs would also reduce local production of mineralization inhibitors such as MGP, resulting not only in accelerated calcification but enhanced osteo/chondrocytic differentiation.18,19,34 Further evidence for this comes from the incremental increases in the deposition of vesicle proteins annexin VI, MGP, and fetuin-A through predialysis to dialysis, as well as the presence of vesicles and
dying VSMCs within dialysis vessels that had not yet developed overt calcification as shown by TEM. Studies have shown that VSMCs release Ca-loaded vesicles in response to Ca overload potentially to prevent apoptosis and that these vesicles are loaded with calcification inhibitors, including fetuin-A and MGP, that act to limit their calcification potential. However, with time in the dialysis milieu, vesicle release and VSMC damage increases, resulting in a reduced capacity of the VSMCs to handle Ca overload and to produce or incorporate inhibitors. The circulating protein fetuin-A is greatly reduced in dialysis and is protective against VSMC apoptosis, whereas the form of MGP in the calcified dialysis vessels was the unmodified Glu form that has a much reduced capacity to inhibit calcification; its lack of modification may be due to progressive VSMC loss or dysfunction, resulting in reduced γ-carboxylase enzymatic activity in the cells. However, although evidence supports a causal relationship between apoptosis and the calcification cascade, we cannot rule out the possibility that some VSMCs may have undergone hypertrophy, and thus cell loss may be overestimated. Hypertrophy has been shown to occur as a response to injury and is associated with an irreversible modulation of VSMC phenotype, which may also contribute to vessel remodeling in dialysis.

Is Osteoblastic Conversion Protective or Detrimental?

Dialysis vessels with the highest Ca loads also had the highest levels of ALK, suggesting that ALK levels were enhanced by dialysis-induced VSMC injury despite the reduction in VSMC number. The importance of vascular damage in inducing calcification has been suggested in a previous in vitro study in which rat aortic rings were induced to calcify only if subjected to mechanical damage, implying that in injured vessels, an upregulation of ALK plays a key role in inducing calcification. ALK can promote calcification by hydrolysis of pyrophosphate, and this may be an additional mechanism for accelerated calcification in dialysis. In humans, elevated serum ALK levels have been associated with calcific uremic arteriopathy, and a genetic deficiency in pyrophosphate levels causes infantile idiopathic arterial calcification.

We also found significantly greater Runx2 and osterix positivity in dialysis vessels implying in vivo osteoblastic conversion of VSMCs. The expression of Runx2 and osterix in the control vessels was unexpected but may reflect a developmental pattern of expression for these proteins in the still immature vasculature of children. The cytoplasmic distribution of Runx2 in dialysis vessels was also striking and may reflect VSMC damage or the expression of alternate

Figure 7. Summary figure showing Ca accumulation in the vessel wall begins predialysis, and factors specific to the dialysis milieu trigger accelerated calcification. MGP indicates matrix Gla-protein; Gla, carboxylated; Glu, undercarboxylated; Ca, calcium; P, phosphate; IMT, intima-media thickness; and CT, computed tomography.
isoforms of this protein in response to calcium/calcification, and this observation requires further analysis.\textsuperscript{41,42} Expression of osteoblastic markers by VSMCs preceded calcification, and it remains unclear whether the expression of osteo/chondrocytic proteins by VSMCs is an adaptive response aimed at regulating mineralization or whether these changes, which, interestingly in this study, occurred in contractile VSMCs, act to enhance the mineralization process.

The sequence of events that leads to VSMC injury, osteo/chondrocytic transformation, and ALK upregulation, as well as the factors that initiate VSMC apoptosis, will be crucial to our further understanding of the calcification process.

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Disclosures
None.

References


**CLINICAL PERSPECTIVE**

The present study, for the first time, provides quantitative evidence that calcium (Ca) accumulation in the vessel wall begins predialysis and that factors specific to the dialysis milieu trigger accelerated calcification. We found that the vessel Ca load is strongly correlated with the mean serum Ca × phosphate (P) product, suggesting that stringent measures to control the serum P levels and also to limit the Ca load to patients (from P binders and dialysate) should be practiced, beginning from the predialysis stages. The currently available clinical measures of vascular damage and calcification (carotid intima-media thickness, pulse wave velocity, and multi-slice computed tomography scan) are not sensitive enough to detect early stages of calcification, and a normal/negative test should be interpreted with caution. Importantly, although Ca accumulation begins predialysis, it progresses extremely rapidly on dialysis: a dialysis vintage of even 2 months was sufficient to induce overt calcification. Histologically, vessels from dialysis patients showed apoptotic vascular smooth muscle cell damage, osteo/chondrocytic conversion, and vesicle release, suggesting that in the dialysis milieu, damage-inducing agents in addition to continued exposure to high and possibly worsening Ca and P lead to progressive vascular smooth muscle cell damage and calcification. Thus this study further emphasizes the need to avoid dialysis and perform preemptive renal transplantation wherever possible.
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