Mechanisms of arterial calcifications and consequences for cardiovascular function

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Cardiovascular complications are the leading cause of mortality in chronic (CKD) and end-stage renal disease (ESRD). The risk of developing cardiovascular complications is associated with changes in the structure and function of the arterial system, which are in many aspects similar to those occurring with aging. The presence of traditional risk factors does not fully explain the extension and severity of arterial disease. Therefore, other factors associated with CKD and ESRD must also be involved. Arterial calcification (AC) is a common complication of CKD and ESRD, and the extent of AC in general population as well as in patients with CKD is predictive of subsequent cardiovascular mortality beyond established conventional risk factors. AC is an active process similar to bone formation that implicates a variety of proteins involved in bone and mineral metabolism and is considered part of a systemic dysfunction defined as CKD-associated mineral and bone disorder (CKD-MBD).

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Premature vascular aging and arterial stiffening are observed with progression of chronic kidney disease (CKD) and in end-stage renal disease (ESRD).[1] Damage of large arteries is a major contributory factor to cardiovascular complications, and a leading cause of mortality in CKD including ESRD.[2,3] Arterial stiffening in such patients is of multifactorial origin, with extensive arterial calcifications (ACs) representing a major covariate.[4–6] ACs are predictive of cardiovascular mortality beyond established conventional risk factors.[7,8] ACs are largely contributing to myocardial ischemia, cardiac hypertrophy, and microvascular disease in brain and kidney.[9,10]

MECHANISMS OF AC

Long time considered a passive process associated with cellular aging and death, and high extracellular fluid calcium–phosphate concentrations, the results of many recent studies have indicated that cardiovascular calcifications are an active process, in many aspects similar to embryonic bone formation, which is regulated by a variety of genes and proteins implicated in mineral and bone metabolism. This process involves differentiation of contractile vascular smooth muscle cells (VSMCs), and pericytes into distinct, 'osteoblast-like' cells with a secretory phenotype. VSMC synthesize bone-associated proteins, including alkaline phosphatase, osteocalcin, osteopontin, and a coat of collagen-rich extracellular matrix, via the formation of matrix vesicles, nodules, and apoptotic bodies, which serve as initiation sites for apatite nanocrystal deposition (but also with bone formation and real ossification).[11–16] Clinical and experimental data show that this process can be triggered by many factors including dyslipidemia,[17] oxidative stress,[18] advanced glycation end-products and hyperglycemia,[19] hyperphosphatemia,[20] increased serum aldosterone levels,[21] and age-associated cellular senescence.[22–24] These risk factors have in common that they all converge to a final pathway, that is, inflammation and nuclear factor (NF)-κB activation.[25–28] Molecular imaging *in vivo* has demonstrated inflammation-associated osteogenesis in early-stages of atherosclerosis,[29] confirming the role of inflammation in triggering the metabolic cascade leading to the transformation of VSMC into an osteogenic phenotype. Macrophage activation releases proinflammatory cytokines (such as interleukin-6 and tumor necrosis factor-α), and proteolytic enzymes (matrix metalloproteinase-2, matrix metalloproteinase-9,

and cathepsin S) whose release is associated with osteochondrocytic VSMC transdifferentiation.[29,30] Interleukin-6 and tumor necrosis factor-α are the first steps for the activation of BMP2:BMP4 and Msx2, which promotes calcification by activating paracrine Wnt signals and nuclear activation and localization of β-catenin, an indispensable coregulator of expression of Runx2, osterix, and Sox9, which are all transcription factors associated with the osteochondrogenic phenotype conversion of VSMC and pericytes.[30–32] The second aspect of inflammation-related calcification is the proteolytic activation of elastolysis and degradation of extracellular matrix. The fragmentation of elastic lamellae and release of biologically active elastin-derived peptides also promote VSMC dedifferentiation and calcium deposition.[29,33]

Aging is the most typical condition associated with the development of vascular calcifications. VSMC senescence is associated with the switch to a secretory phenotype (senescence-associated secretory phenotype) that initiates osteoblastic transition with calcifications and artery-wall remodeling.[22–24] Senescence-associated secretory phenotype is linked to low-grade arterial inflammation with increased NF-κB activation.[34] NF-κB activity, inflammation, and excessive production of reactive oxygen species are associated with several features of the progeroid syndrome such as accumulation of prelamin A,[35] low telomerase activity and telomere shortening,[36] and DNA damage,[24,35] all conditions being associated with the development of an osteogenic program and AC.

In vitro, calcium and phosphate promote both synergistically and independently VSMC calcification.[37] Recent findings indicate that hyperphosphatemia, through activation of mitochondrial respiration, stimulates the production of reactive oxygen species with final activation of NF-κB, enhancing Runx2 (Cbfa1) activation and matrix vesicle release and promoting the differentiation of mesenchymal cells into osteoblastic lineage.[38]

In presence of normal serum, VSMC do not calcify. Serum inhibits spontaneous calcium and phosphate precipitation in solution,[39] indicating that systemic calcification inhibitors are present in the serum and also in VSMC, which constitutively express potent local or systemic inhibitors of calcification,[40] such as matrix GLA protein,[41] which may limit AC by binding to bone morphogenic proteins.[42] Osteopontin and osteoprotegerin are potent inhibitors of AC *in vivo*, and inactivation of their genes enhances the calcification process.[43] Fetuin-A (AHSG or α_2-HS glycoprotein) is a potent circulating AC inhibitor that is abundant in plasma.[44] Pyrophosphate is another potent inhibitor. *In vitro*, phosphate-stimulated apatite production can be completely prevented by adding pyrophosphates.[45]

In CKD and ESRD patients, the relationships between AC and changes in phosphate and calcium homeostasis are associated with disruption of endocrine and humoral pathways, including parathyroid hormone (PTH), calcitriol, and the fibroblast growth factor-23—Klotho–vitamin D axis. The increase in bone resorption observed in CKD patients with secondary hyperparathyroidism is frequently associated with AC. Excessive phosphate and calcium efflux from bone probably has an important role. The direct intervention of PTH is less clear. Chronically elevated PTH upregulates RANKL, downregulates OPG gene expression, and raises the RANKL/OPG ratio.[46] However, intermittent increases in serum PTH exert an anabolic action on bone, and intermittent PTH administration has been shown to prevent AC.[47] Low Klotho expression and resistance to the phosphaturic effect of fibroblast growth factor-23 are also associated with AC.[20,48–50]

CLINICAL IMPACT OF ACs

Both intima and media calcifications are associated with increased morbidity and mortality,[7] but they alter arterial functions by different pathological mechanisms.[1] Intima plaque calcification occurs in the context of common atherosclerosis.[2] Intima calcification induces arterial dysfunction resulting from the narrowing of the arterial lumen with ischemia affecting the tissues and organs downstream.[9] As calcification advances with the progression of atherosclerosis, it is uncertain whether the calcification itself represents a risk factor or whether it is only a surrogate marker of plaque burden and disease extension. Acute coronary events and myocardial infarction are more related to the biomechanical stability of atherosclerotic plaques and rupture of the fibrous cap of the plaque. Although a higher coronary AC score is associated with a poorer cardiovascular prognosis, the influence of calcification on plaque stability is controversial. The results of several studies indicate that AC does not increase plaque vulnerability, which seems more attributable to a large lipid pool, thin fibrous cap, and intensity of local inflammation.[51–53]

Media calcification (Mönckeberg's sclerosis or media calcinosis) is characterized by diffuse mineral deposits within the arterial tunica media. Media calcification is concentric, not extending into arterial lumen in its typical pure form and is associated with abnormal cushioning function of blood vessels (arteriosclerosis–arterial hardening) by promoting arterial stiffness.[5] The first, principal consequence of arterial stiffening is increased systolic pressure, resulting in elevated cardiac afterload and left ventricular hypertrophy. The second consequence is decreased diastolic pressure and impaired coronary perfusion.[9] As the two forms of AC are frequently associated in patients with CKD abnormalities of conduit and cushioning function are often associated as well.

MANAGEMENT AND PREVENTION

Several experimental studies have shown that the development of AC calcification can be prevented or delayed by several interventions such as treatment with pyrophosphates,[54] inhibition of receptor activator of NF-κB,[55] treatment with bisphosphonates,[56] estrogens,[57] cinacalcet,[58] angiotensin-converting enzyme inhibitors,[59] and mineralocorticoid receptor antagonists,[60] and inhibition of bone morphogenic proteins signaling.[61]