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Role of Inflammation in Arterial Calcification

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AUTHOR'S SUMMARY

Arterial calcification can be divided into intimal calcification and medial calcification which have strong correlations with adverse cardiovascular events. Continuous research on vascular calcification has been performed, and several cellular and molecular mechanisms and therapeutic targets were identified. However, despite clinical trials to evaluate the efficacy of drug therapies to treat vascular calcification, none have been shown to have efficacy until the present. Therefore, more extensive research is necessary to develop appropriate therapeutic strategies based on a thorough understanding of vascular calcification. In this review, we mainly focus on the pathobiology of arterial calcification, and its clinical implications.

ABSTRACT

Arterial calcification, characterized by calcium phosphate deposition in the arteries, can be divided into intimal calcification and medial calcification. The former is the predominant form of calcification in coronary artery plaques; the latter mostly affects peripheral arteries and aortas. Both forms of arterial calcification have strong correlations with adverse cardiovascular events. Intimal microcalcification is associated with increased risk of plaque disruption while the degree of burden of coronary calcification, measured by coronary calcium score, is a marker of overall plaque burden. Continuous research on vascular calcification has been performed during the past few decades, and several cellular and molecular mechanisms and therapeutic targets were identified. However, despite clinical trials to evaluate the efficacy of drug therapies to treat vascular calcification, none have been shown to have efficacy until the present. Therefore, more extensive research is necessary to develop appropriate therapeutic strategies based on a thorough understanding of vascular calcification. In this review, we mainly focus on intimal calcification, namely the pathobiology of arterial calcification, and its clinical implications.

Keywords: Arterial calcification; Pathobiology; Therapeutic strategy; Clinical implication

INTRODUCTION

Calcium phosphate deposition is the hallmark of cardiovascular calcification and can be observed in the blood vessels, myocardium, and cardiac valves.[1] Several research groups have shown that vascular calcification has a strong correlation with cardiovascular events including all-cause mortality.[2,3] Various risk factors have been reported to contribute to the development of vascular calcification. Aging is the major risk factor for vascular calcification; other known major risk factors, such as diabetes, chronic kidney disease, dyslipidemia, hypertension, male gender and smoking, are risk factors for vascular calcification.[4] Arterial calcification is divided into intimal calcification, the predominant form of calcification in coronary artery plaques and medial calcification, which mostly affects peripheral arteries and aortas.[5] Although clinical studies have shown that a classification between intimal and medial calcification is not dichotomous and simple, this classification is still relevant for clinical treatment and diagnosis due to their different clinical consequences. Accumulating evidence suggests that intimal calcification has a strong correlation with atherosclerotic burden in which microcalcification within the fibrous caps of atherosclerotic plaques may increase local stress. This results in an increased risk for plaque instability and myocardial infarction.[6-9] Meanwhile, in contrast to intimal calcification, research suggests that medial calcification may be involved with different pathophysiologic processes such as mechanical stress, cellular senescence, and oxidative stress rather than lipid accumulation and inflammatory cell infiltration.[10] In this review, we mainly focus on intimal calcification, namely the pathobiology of arterial calcification and its clinical implications.

CLINICAL IMPORTANCE OF ARTERIAL CALCIFICATION

The degree of coronary calcification can be measured non-invasively by computed tomographic measurements of coronary artery calcium. Studies have shown that increasing coronary calcium score is associated with adverse cardiovascular prognosis. In an analysis of 6,722 men and women enrolled in the multi-ethnic study of atherosclerosis, coronary calcium scores of 1–100, 101–300 and > 300 were associated with hazard ratios of 9.67, 7.73 and 3.61, respectively, compared to subjects with a calcium score of 0.[11] Ironically, heavily calcified plaques are more likely to be stable and are less likely to undergo plaque rupture and rapid progression. The answer to this paradox lies in the types of calcification and their role in mediating the risk of plaque instability. Histologically, intimal calcification is divided into 1) microcalcification (< 15 µm), which cannot be seen by radiography; 2) punctate/fragmented calcification (< 3 mm), which corresponds to speckled (< 2 mm) or fragmented calcification (< 5 mm) on radiographs; and 3) sheet/nodular calcification, which corresponds to diffuse calcification on radiographs.[5] Microcalcification and punctate/fragmented calcification increase plaque stress by several fold, increasing the risk for plaque rupture.[12] Documented well in a study by Mori et al.[5] This group demonstrated that microcalcification and punctate/fragmented calcification were more likely to be associated with thin cap fibroatheroma, plaque erosion or plaque rupture; sheet calcification was more likely to be associated with healed plaque rupture or fibrocalcified plaques. Although subjects with high calcium score have many areas in the coronary arteries with diffuse sheet calcification, a high calcium score represents a high degree of plaque burden. Therefore, a subject with a high calcium score will more than likely have many areas in the coronary artery that harbor microcalcification or punctate/fragmented calcification. Hence, this provides an explanation for the increased risk of cardiovascular outcomes in subjects with higher calcium score. The clinical importance

of coronary calcium can be summarized in 2 key points. First, coronary calcium score is used clinically to determine the degree of coronary plaque burden and for risk stratification in subjects who are being considered for primary prevention. In the recent 2018 American Heart Association/American College of Cardiology guideline on the management of blood cholesterol, coronary calcium score is recommended for use when the decision for treatment with statins is uncertain in subjects with intermediate risk for cardiovascular disease.[13] Second, in early stages of coronary calcification, such as microcalcification and punctate/fragmented calcification, an increased risk of plaque erosion and plaque rupture exists. Therefore, understanding the pathobiology of coronary intimal calcification is important in identifying potential therapeutic targets to modulate coronary calcification, which subsequently may be beneficial in reducing acute coronary events.

MECHANISM OF VASCULAR CALCIFICATION IN TERMS OF VASCULAR BIOLOGY

Recent studies support that vascular calcification is an active and complex process, not a passive and degenerative process.[14] Although a number of new determinants and mechanisms have been suggested, current results and hypotheses to explain the pathogenesis of vascular calcification are far from complete. The common determinants and mechanisms of vascular calcification are summarized below.

COMMON DETERMINANTS OF VASCULAR CALCIFICATION

During the past few decades, extensive research has suggested cellular and molecular mechanisms for the development of vascular calcification.[15][16] A number of cell types including resident cells and circulating cells have been reported to contribute to vascular calcification. Damaged macrophages, stromal cells, and smooth muscle cells secrete extracellular vesicles that are critical for initiation of vascular calcification in atheroma.[17] Although the mechanisms are not fully understood, Yao et al.[18] demonstrated that endothelial cells also contribute to vascular calcification through endothelial-mesenchymal transitions. In addition, researchers revealed that circulating stem cell progenitors can contribute by their calcifying/decalcifying potentials.[19][20] Under pathological conditions, vascular smooth muscle cells (VSMCs) can play important roles in intimal and medial calcification in which exposure to a high-calcium and phosphate milieu can induce osteogenic/chondrogenic trans-differentiation of VSMCs through activation of various signaling pathways.[21][22] Inflammation, apoptosis, oxidative stress, and mitochondrial dysfunction are critical inducers for VSMC trans-differentiation in intimal calcification.[10][23] Molecular mechanism and phenotypic changes are usually associated with bone-forming osteoblasts in the pathological process of VSMC trans-differentiation in vascular calcification.[24] Bone biomarkers such as cathepsin K, fibroblast growth factor 23-Klotho, bone morphogenic protein (BMP) 2, alkaline phosphatase (ALP), and Runx2 have been shown to be inducers contributing to osteoblastic differentiation in vascular calcification.[25-29] However, osteoprotegerin, fetuin-A, BMP7, and other biomarkers are inhibitors of vascular calcification.[30-32]

OXIDATIVE STRESS AND REDUCED NITRIC OXIDE (NO) AVAILABILITY

Oxidative stress is a key phenomenon in causing aging of endothelium and endothelial dysfunction. Aged endothelium produces increased free radicals that may form a vicious cycle to further accelerate vascular aging. Direct evidence of endothelial oxidative stress with aging in humans was provided by the demonstration that increased levels of nitrotyrosine were observed in human aged vascular endothelial cells along with oxidative stress markers. This suggests that aging is associated with increased formation of reactive oxygen species.[33][34] In young healthy individuals, reduced nicotinamide adenine dinucleotide phosphate, an essential electron donor in all organisms, contributes to superoxide (O_2^-) generation in vascular endothelial cells. However, the superoxide anions are soon detoxified to H_2O_2 by manganese superoxide dismutase (MnSOD) present in the mitochondria. However, in conditions of elevated oxidative stress, such as with aging, O_2^- binds to NO and leads to formation of a potent free radical known as peroxinitrite ($ONOO^-$) that further damages macromolecules in the endothelial cells. Furthermore, peroxinitrite can inactivate both MnSOD and endothelial nitric oxide synthase (eNOS) in the endothelial cells.[35] The switch of eNOS from an NO-generating enzyme to a superoxide-generating enzyme (NO synthase uncoupling) leads to a further increase of superoxide anions and aggravates oxidative stress in aged endothelial cells. Increased peroxynitrite penetrates the phospholipid membrane causing DNA damage, lipoprotein oxidation, disruption of mitochondrial activities, and further depletion of plasma antioxidants, mostly NO. Consequently, reduced NO bioavailability promotes migration, proliferation and phenotypic change of VSMCs in the calcification of vascular cell process. Another molecule associated with vascular calcification is homoarginine.[36] Homoarginine is an endogenous amino acid which is reduced in patients with increased cardiovascular risk.[37] Homoarginine has been described as an inhibitor of ALP.[38] Homoarginine blood concentrations can be enhanced by dietary lysine, which decreases arterial calcification.[39] Therefore, homoarginine could, at least in theory, inhibit vascular calcification. However, homoarginine supplementation has been shown to paradoxically stimulate osteogenic/chondrogenic transformation of VSMCs and vascular calcification.[36] One possible reason for this may be low efficacy of NO formation by homoarginine. Homoarginine was a less potent substrate for NO formation by all 3 NOS isoforms,[40] resulting in reduced NO-producing activity in the homoarginine utilization process. Thus, homoarginine exerted a partial antagonistic effect like the NOS inhibitor L-NAME that was reversed by NO donors. The NOS inhibition likely contributed to acceleration of VSMC calcification.[41]

EXTRACELLULAR MATRIX DISRUPTION AND STIFFNESS ACCELERATE VASCULAR CALCIFICATION

Another mechanism that has recently gained interest is the transition of endothelial cells to osteoblasts via endothelial to mesenchymal transition.[42] Endothelial to mesenchymal transition can be promoted by a myriad of factors, and the transforming growth factor-β/BMP family of factors are the strongest promotors.[43]

Vascular calcification and stiffness reinforce one another, forming a vicious cycle in which trans-differentiation of endothelial cells and VSMCs play a central role. Also, mechanical stress disrupts the extracellular matrix; elastic fibers are fragmented and replaced by collagen

fibers either by activation of matrix metalloproteinases (MMPs) or by material fatigue.[44] Certain risk factors such as high phosphate and calcium levels, inflammation, or oxidative stress cause VSMCs to trans-differentiate into calcifying vascular cells with upregulation of Runx2 and BMP2. These VSMCs produce calcifying exosomes, initiating a cascade effect by stimulating neighboring VSMCs to release intracellular endoplasmic reticulum calcium stores. This release induces mineralization and stiffness of the extracellular matrix.[42] Extracellular matrix stiffness influences VSMC behavior and stimulates activation of the integrin-FAK-Src pathway, potentially inducing Runx2 and β-catenin expression.[45] Indirectly, endothelial cells produce less NO when the extracellular matrix stiffens. Also, the activation of Runx2 induces fibrosis/stiffness and mineralization of the extracellular matrix while activation of MMPs induces VSMC phenotypic switching.[46] The acceleration of arterial stiffness is associated with aging, hypertension, atherosclerosis and increased angiotensin II signaling.[47] Angiotensin II has been suggested to accelerate vascular senescence by the ERK/p38 MAPK cysteine-rich angiogenic protein 61 pathway.[48]

INFLAMMATION

Atherosclerosis is a chronic inflammatory process characterized by interactions among macrophages, endothelial cells and VSMCs. Inflammation is the key process that links the risk factors of cardiovascular disease with atherogenesis.[49] Inflammation is not only important for progression of atherosclerosis but is an important trigger for vascular calcification. Studies have demonstrated that inflammation of the arteries precedes the development of arterial calcification. In a study by Aikawa et al.,[50] a strong association between macrophage burden and osteogenic activity was demonstrated in vivo in the arteries of apolipoprotein E$^{-/-}$ mice. Also, in a study by Abdelbaky et al.,[51] 137 patients who underwent positron-emission tomography/computed tomography 1–5 years apart were analyzed. The results demonstrated that an increased inflammatory signal at baseline was associated with subsequent vascular calcification at the corresponding segments of the aortic wall. The results of the above-mentioned studies suggest that inflammation is the key driver and precursor of vascular calcification.

As mentioned previously, osteoblastic trans-differentiation of VSMCs is a key process in vascular calcification. Specific knockout of Runx2, the key transcription factor for osteoblastic differentiation, in VSMCs has been shown to be significantly associated with inhibition of vascular calcification.[52][53] Osteoblastic transformation of VSMCs is characterized by loss of SMC markers, such as SM22α and SM α-actin, and gain of osteogenic markers such as Runx2, osteopontin and ALP.[10] Matrix vesicles released by osteoblast-like cells are important in hydroxyapatite crystal formation and subsequent calcification.[54] In normal states, VSMCs are maintained in contractile phenotype usually in the media layer. However, VSMCs have diverse plasticity and may trans-differentiate into foam cell-like cells, osteoblast-like cells and chondrocyte-like cells in response to various stimuli such as vascular injury and inflammation.[10] Inflammation is the key process that mediates osteoblastic trans-differentiation of VSMCs. M1 macrophages, by secreting pro-inflammatory cytokines, promote vascular calcification by inducing osteoblastic trans-differentiation of VSMCs. Cytokines derived from M1 macrophages, such as oncostatin M, interleukin-1β, interleukin-6, and tumor necrosis factor (TNF)-α, induce osteoblastic trans-differentiation.[55-57] In a study by Menini et al.,[58] 62 human carotid plaques obtained after carotid endarterectomy were assessed for plaque instability, type of calcification, markers of inflammation and presence

of markers of osteogenesis. The study demonstrated higher expression of receptor for advanced glycation end-product (RAGE), interferon-γ and TNF-α in unstable plaques with microcalcification compared to stable plaques with macrocalcification. This suggests that inflammation is the key process that precedes the development of vascular calcification and microcalcification that increases the risk of plaque progression by association with increased inflammation and directly increasing plaque stress.

POTENTIAL TREATMENT OPTIONS FOR VASCULAR CALCIFICATION

Because hypertension, dyslipidemia, diabetes, and chronic kidney disease are important risk factors of vascular calcification, the therapeutic agents for these diseases were evaluated as potential anti-calcifying agents. The calcium channel blocker nifedipine showed a slower progression of coronary calcification in hypertensive patients compared with amiloride-hydrochlorothiazide in a clinical trial[59]; however, a subsequent study failed to replicate the findings.[60] In contrast, renin-angiotensin-inhibiting agents have demonstrated promise. Tissue angiotensin II is increased in aged vascular tissue,[61] and exogenous increase in angiotensin II accelerates vascular senescence.[62] In a preclinical study in rabbits fed an atherogenic diet with vitamin D2 over a period of 12 weeks, angiotensin II receptor blocker significantly inhibited arterial calcification.[63] However, angiotensin II inhibition is only effective in prevention of calcification, not reversing or resolving already-formed calcification.

In contrast, several anti-diabetic medications have potential calcification-resolving effects. Patients with diabetes have a greater degree of vascular calcification. The mechanisms may be a direct effect of hyperglycemia on vascular cells.[64] Advanced glycation end-products (AGEs) and the RAGE are also associated with vascular calcification in diabetic patients, and these have been shown to exert similar effects in chronic kidney disease.[65] A long-term intervention study in diabetic patients suggests that metformin treatment started from the prediabetic period for an average duration of 14 years significantly lowered coronary calcium scores, especially in men.[66] The results showed that glycemic control in early stages of diabetes mainly prevents accumulation of AGEs, delaying the development of vascular calcification.

Calcifying vascular cells are morphologically identical to VSMCs, but differ from primary smooth muscle cell cultures when cultured in an approximately 10-fold enrichment for calcium nodule formation. These cells also differ in the expression of molecular markers such as osteopontin, type I collagen, and the epitope for monoclonal antibody 3G5.[67] However, there are more primitive calcifying progenitor cells that have potential to turn into specialized cells capable of either promoting or reversing calcium accumulation (osteoblasts or osteoclasts, respectively).[19] Both types of cells may transform into osteoblast-like cells and thereby promote atherosclerotic calcium build-up. However, cells expressing both Sca-1 and platelet-derived growth factor receptor α are more committed to differentiate into an osteoblastic lineage, but those expressing only Sca-1 can be bi-directional. These Sca-1-expressing cells can differentiate into either osteoblast- and osteoclast-like cells. Drugs that stimulate the nuclear protein peroxisome proliferator activated receptor-gamma (PPARγ) are known to promote the formation of osteoclasts and inhibit the formation of osteoblasts, primarily transforming bi-directional cells into osteoclast-like cells. This suggests that PPARγ may prevent and potentially reverse calcium accumulation in blood vessels.[19]

In chronic kidney disease patients, there were strong associations between low serum magnesium levels and elevated vascular calcification and cardiovascular mortality. In vitro and preclinical in vivo data indicate that magnesium has the potential to protect against vascular calcification through the blockade of the inorganic phosphate-induced Wnt/β-catenin signaling pathway.[68] Accordingly, data from pilot clinical interventional studies suggest that oral magnesium supplementation reduces vascular calcification in patients with chronic kidney disease.[69]

Interestingly, not all cardiovascular medication reduces vascular calcification. Most surprisingly, HMG-CoA reductase inhibitors ('statins') promote the progression of coronary calcification,[70] an effect increased with duration of statin use.[71] Such an effect of statins was unexpected given that their cardiovascular protective effects were well proven[72,73] and that hyperlipidemia is associated with calcification.[74] Similarly, although associated with reduction in all-cause mortality in patients with coronary artery calcification, high intensity exercise paradoxically accelerates coronary artery calcification.[75-77] In these cases, the anti-inflammatory effect of statins or exercise may induce microcalcification of the intima into diffuse sheet calcification associated with stable, fibrocalcified plaques.[5]

Regarding pharmacologic treatment, vitamin K supplementation has been under consideration for decades. The rationale is that a post-translational modification of matrix GLA protein (MGP) by γ-glutamyl carboxylase, which enables MGP to inhibit bone BMP2, requires a vitamin K-dependent reaction.[78] Studies have shown that vitamin K deficiency and low vitamin K intake are more common in patients with cardiovascular and renal disease and are associated with increased risk of cardiovascular and all-cause mortality.[79] Also, warfarin, a vitamin K inhibitor, has been shown to accelerate vascular calcification.[80]

One potential interventional therapy is intravascular lithotripsy.[81] Extracorporeal lithotripsy is currently used to disintegrate gallstones and renal stones. The technology is now adapted to intravascular catheters and is being tested for its ability to convert coronary calcium deposits into smaller pieces without removal.[82] However, current indication is limited due to facilitation of stent deployment and expansion in heavily calcified coronary arteries.[83]

CONCLUSION

In this review, we showed that arterial calcification is an active and complex pathobiological process. Understanding new paradigms in cardiovascular calcification is important for future directions of therapeutic strategy in arterial calcification. We updated the latest findings ranging from the cellular and molecular pathophysiology of arterial calcification to clinical implications and potential treatment options.

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