Eur J Vasc Endovasc Surg (2018) ■, 1–8

Mechanisms of Arterial Calcification: The Role of Matrix Vesicles

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WHAT THIS PAPER ADDS

Extracellular vesicles are involved in cardiovascular disease; here the earliest phase of vascular calcification is described through an in depth ultra-structural analysis of calcifying matrix vesicles that may represent the unifying motif of the various initiation mechanisms proposed for vascular calcification. These vesicles can be generated locally by dying cells, resident osteoprogenitors or alternatively they may even originate from distant sites that reach the vascular matrix as circulating nucleation complexes. Unravelling their composition and phenotype in normal and pathological conditions will be essential for the development of new therapeutic strategies, in order to prevent and treat vascular calcification.

Vascular calcification is related to vascular diseases, for example, atherosclerosis, and its comorbidities, such as diabetes and chronic kidney disease. In each condition, a distinctive histological pattern can be recognised that may influence technical choices, possible intra-operative complications, and procedure outcomes, no matter if the intervention is performed by open or endovascular means. This review considers the classification and initiating mechanisms of vascular calcification. Dystrophic and metastatic calcifications, Monckeberg's calcification, and genetic forms are firstly outlined, followed by their alleged initiation mechanisms; these include (a) ineffective macrophage efferocytosis; (b) ectopic osteogenesis driven by modified resident or circulating osteoprogenitors. As in physiological bio-mineralisation, active calcification starts with the deposition of cell derived matrix vesicles into the extracellular matrix. To substantiate this belief, an in depth ultra-structural documentation of hydroxyapatite crystal deposition on such vesicles is provided in an ex-vivo human vascular cell model. Revealing the vesicle composition and phenotype in normal and pathological vascular conditions will be essential for the development of new therapeutic strategies, in order to prevent and treat vascular calcification.

Article history: Received 12 August 2017, Accepted 6 December 2017, Available online XXX
Keywords: Vascular calcification, Atherosclerosis, Matrix vesicles, Vascular stem cells, Electron microscopy

INTRODUCTION

Vascular calcification (VC), a feature of advanced atherosclerosis, is the heterotopic accumulation of calcium phosphate salts in the vascular tissue. VC is dramatically increased in patients with diabetes and chronic kidney disease (CKD).[1] Severe atherosclerosis and arterial stiffening with massive vascular calcification are key determinants that contribute to increased cardiovascular mortality,[2] which is now considered an active process governed by highly regulated cellular and molecular pathways similar to those involved in bone formation. However, the fine mechanisms of VC remain poorly understood. Considering that VC has important clinical consequences, identifying its initiating mechanisms is essential for devising strategies to prevent, restrain, or dislodge calcium deposits from the arterial wall.

Common forms of VC

VC is classified as dystrophic or metastatic.[3] Dystrophic, also known as inflammatory calcification, develops in a systemic inflammatory milieu and is influenced by traditional risk factors for cardiovascular disease; it involves the intima of large and medium sized elastic arteries being part of the natural history of atherosclerosis, where calcification is a significant predictor of cardiovascular morbidity and mortality.

Metastatic/metabolic calcification occurs in an apparently healthy arterial media because of changes in calcium metabolism. This form of VC, affecting medium sized and small muscular arteries, is associated with Type 2 diabetes and CKD where the indices of mineral metabolism act on the vascular wall either by pleiotropic mechanisms or by enhancing pathways involved in vascular injury or by determining a perturbation of the bone vascular axis.

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https://doi.org/10.1016/j.ejvs.2017.12.009

Please cite this article in press as: Zazzeroni L, et al., Mechanisms of Arterial Calcification: The Role of Matrix Vesicles, European Journal of Vascular and Endovascular Surgery (2018), https://doi.org/10.1016/j.ejvs.2017.12.009

Table 1 outlines the main pathways characterising inflammatory and metabolic VC.

Monckeberg's calcification

Monckeberg's calcification (MC), after decades of misconception wherein it had been considered as an end stage fibroatheroma, was subsequently recognised as a distinct non-atheromatous vascular lesion.[4] MC develops in proximity with the internal elastic lamina or less commonly in the media of muscular arteries in the absence of changes in calcium metabolism.[5] Its clinical significance is still unknown; recent reports[4,5] assimilate MC with metabolic VC. However, another study[6] performed on 143 consecutive histologically normal femoral arteries from healthy young multi-organ donors seems to rule this possibility out; 25% of this population (mean age 38 years, range 14—59 years) had MC in the absence of any CVD risk factor. Hence, MC arises early in life, possibly as an expression of dysregulated osteogenic commitment of vascular progenitors.

Figure 1 highlights the histological characteristics of different VC.

Genetic forms of VC

In VC, a genetic component is also present, as multiple genes are candidates to contribute to calcification, leading to heterogeneous diseases.[7] The most common monogenic disorder associated with VC is pseudoxanthoma elasticum (PXE), a rare autosomal recessive connective tissue disease caused by mutations in the ABCC6 gene. Other disorders associated with VC are PXE like syndrome with multiple coagulation factor deficiency, generalised arterial calcification of infancy, Keutel Syndrome, idiopathic basal ganglia calcification, and arterial calcification because of CD73 deficiency. These genetic disorders are reviewed by De Vilder et al.[7]

Initiating mechanisms of VC

Different mechanisms (Fig. 2A and B) have been proposed for initiating VC, including ineffective efferocytosis, the main mechanism operating in fibroatheroma, and active calcification with bone formation resulting from osteogenic differentiation of resident or circulating calcifying vascular cells (CVCs).

Ectopic osteogenesis involves the deposition of matrix vesicles (MVs), a cell product that act as sites for hydroxyapatite crystal precipitation; these vesicles may even originate from distant sites that reach the vascular matrix as substrate circulating nucleation complexes. The vesicle content and micro-environment composition address the fate of MV towards the deposition of calcium or not.

Ineffective efferocytosis

Almost all cell types populating fibroatheroma (endothelial cells, vascular smooth muscle cells (VSMCs), lymphocytes, and macrophages) undergo apoptotic cell death. The loss of macrophages has as a consequence the accumulation of apoptotic bodies followed by local increase in pro-inflammatory mediators; the end result is the formation of foci of secondary necrosis and the establishment of a vicious loop in the inflammatory response. A rapid phagocytic clearance of the apoptotic bodies, efferocytosis, prevents subsequent post-apoptotic necrosis. The inefficient removal of apoptotic bodies leads to the formation of necrotic debris. The formation of fibroatheroma necrotic core is fueled by this process; however, part of the apoptotic/necrotic dust serves as template for the inflammatory type

Table 1. Characteristics of inflammatory and metabolic VC.

	Inflammatory	Metabolic Ageing[38]	Diabetes[14]	CKD[11]
Main target	Elastic arteries	Elastic, muscular arteries	Elastic, muscular arteries, arterioles and capillaries	Muscular arteries
Calcification site	Intima	Media	Intima and media	Media
Morphology	Fibrous cap, necrotic/lipidic core, cholesterol crystals, inflammatory cells, neo-angiogenesis	Pathological intimal thickening	Fibroatheromas can be associated with predominantly calcified peripheral vascular lesions w/o inflammatory cells; hyaline material in small arteries; thickening of capillary basal lamina	Calcification of the media, sometimes along the internal elastic lamina, coexists with fibroatheromas
Metabolic pathways	Oxidative stress; pro-inflammatory microenvironment; ox-LDL; ineffective efferocytosis	Dysregulated autophagy; mitochondrial dysfunction; altered redox homeostasis and nutrient sensing	Overproduction of reactive oxygen species by mitochondria. Several pathways are triggered: polyol and hexosamine flux, advanced glycation end products (AGEs), protein kinase C (PKC), and nuclear factor-κB mediated vascular inflammation	Altered bone—vascular axis; biochemical factors involved are: FGF-23, BMP2, OPG, MGP, ectonucleotide pyrophophatase/phosphodiesterase 1 (Enpp1), bone/liver/kidney ("tissue non-specific") ALP, and lipid oxidation products

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