REVIEW

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New mediators of vascular damage in dialysed patients

I. A. CHECHERIŢĂ[1)], C. DAVID[1)], L. STOICA[1)], P. POPESCU[1)], A. CIOCÂLTEU[1)], I. LASCĂR[2)]

[1)]*Nephrology Clinic, "St. John" Emergency Hospital, Bucharest*

[2)]*Department of Plastic Surgery and Reconstructive Microsurgery, Floreasca Emergency Hospital, Bucharest*

"Carol Davila" University of Medicine and Pharmacy, Bucharest

Abstract

Cardiovascular events are the main causes of mortality in dialysed patients. Traditional risk factors such as hypertension, aging, smoking, diabetes, and abnormal lipid metabolism does not fully explain the high frequency of cardiovascular disease in renal patients, indicating that some other distinct pathogenesis may be involved. Vascular calcification have been associated with high cardiovascular morbidity and mortality in chronic kidney disease (CKD) patients. It is an active process that resembles osteogenesis, regulated by bone proteins and osteoblast-like cells. Elements involved in the pathogenesis are: the risk factors that initiates the process, the promoters released and overexpressed and the dysregulation of the inhibitor factors of extraskeletal calcifications. Although researches in the past decade have greatly improved our knowledge of the multiple factors and mechanisms involved in vascular calcification, many questions remain unanswered.

Keywords: vascular calcifications, end stage renal disease, risk factors, promoters, inhibitors.

Introduction

Cardiovascular events are the main causes of mortality in dialysed patients. The contribution of traditional risk factors such as hypertension, aging, smoking, diabetes, and abnormal lipid metabolism does not fully explain the high frequency of cardiovascular disease in renal patients, indicating that some other distinct pathogenesis may be involved. CKD is characterized by accelerated atherosclerosis and particularly by pronounced vascular calcification. Both progressive circumscript calcification of atherosclerotic plaques and diffuse calcification of the media of central arteries are frequent findings. The hemodynamic consequences of this process are the loss of arterial elasticity, increase in pulse wave velocity, development of left ventricular hypertrophy, decrease in coronary artery perfusion, and myocardial ischemia and failure. Calcified atherosclerotic plaques of CKD patients are susceptible to rupture [1]. Although researches in the past decade have greatly improved our knowledge of the multiple factors and mechanisms involved in vascular calcification, many questions remain unanswered.

Vascular calcification is not a passive, but a very active process with osteoblastic transdifferentiation of vascular smooth muscle cells (VSMC); the formation of hydroxyapatite resembles the process normally occurring in bone. Many key regulators of bone formation and bone structural proteins are expressed in both types of vascular lesions. There is, also, a growing evidence that physiologic inhibitors of vascular calcification such as fetuin-A, osteopontin, and bone morphogenic protein 7 play an important role in the process [2].

Promoters of extraskeletal calcifications

Metabolic insults in end stage renal disease (ESRD) – uremia, dyslipidemia, oxidative stress and hyperphosphatemia – initiates formation of "osteoblast-like" cells in the vessel wall and the recruitment of undifferentiated progenitors of the osteochondrocyte lineage. The last decades studies highlighted a large list of promoters of the calcification process: extracellular P^{2-}, uremic toxins, oxidative stress and inflammatory cytokines, Cbfa1/Runx2, membrane-bound matrix vesicles, apoptotic bodies, ROS hydrogen peroxide, leptin and glucocorticoids.

The altered Ca^{2+} and P^{2-} metabolism is the most important contributor to the progression of vascular calcification in ESRD. It was previously thought that high serum phosphate levels caused vascular calcification by simply exceeding ($Ca^{2+} \times P^{2-}$) solubility, resulting in precipitation. Recent studies showed that high extracellular phosphate levels induce VSMCs to transform into osteoblast-like cells [3]. Influx of P^{2-} leads to the induction of osteoblastic-differentiation factors such as *Cbfa1/Runx2,* osteopontin (OPN), osteocalcin (OC) and alkaline phosphatase. The blockade of Pit-1, a type III sodium-dependent phosphate co-transporter, impairs this mineralization, suggesting that elevated extracellular P^{2-} concentrations induce the mineralization of VSMCs through the activation of Pit-1. It was recently demonstrated that the activation of Pit-1 is produced also by impaired extracellular Ca^{2+} concentrations.

Membrane-bound matrix vesicles and apoptotic

bodies released by apoptotic VSMC in ESDR are the nidus for mineral nucleation.

Uremic toxins increase the mineralization of VSMCs and upregulates the expression of Cbfa1/Runx2 and its target protein OPN, regardless of the serum P^{2-} concentration. They also increases the secretion of a crucial mediator of osteoblastic differentiation, bone morphogenic protein-2 (BMP-2), resulting in the mineralization of VSMCs [3].

The *ROS hydrogen peroxide* – enhanced in ESDR patients' serum – was recently shown to promote osteogenic transdifferentiation of VSMCs, including upregulation and activation of Runx2/Cbfa1 in concert with matrix mineral deposition.

The osteoblastic differentiation of VSMCs was also demonstrated to be induced by *TNF-α and inflammatory cytokines*, in a dosage-dependent manner [4]. A recent study showed that the administration of infliximab, a specific inhibitor of TNF-α signaling, significantly reduced high-fat diet-induced aortic calcium accrual [5].

Inhibitors of ectopic calcifications

Osteoprotegerin (OPG) belongs to the tumor necrosis factor (TNF) receptor superfamily, which has a range of pleiotropic effects on bone metabolism, endocrine function and the immune system. Based on the observations that mice lacking OPG gene develop extensive calcifications of the vascular system and other soft tissues, it was initially considered as protective against excess calcification. However, several clinical observations revealed a positive association between OPG and vascular calcification, the advancement of coronary artery disease expressed semi-quantitatively and even mortality. Some authors suggested that serum OPG might be the marker of low-turnover bone disease, which in turn is a well-recognized risk factor for developing vascular calcification. Further studies are needed to clarify the role of OPG in vascular calcification and atherosclerosis, but in our opinion, this factor may link these processes with bone metabolism [5, 6].

A recent study coming from Coen G *et al.* demonstrates that OPG has multiple roles in vascular damage. One pathways of involvement in the development of atherosclerosis is *via* its second ligand: TNF-related apoptosis inducing ligand (TRAIL). TRAIL is a potent activator of apoptosis. OPG could influence vascular disease by inhibiting TRAIL-induced apoptosis of vascular cells. Another mechanism whereby OPG could be associated with vascular damage independent of VC could be *via* its association with endothelial dysfunction [6]. OPG has been related to endothelial cell survival, apoptosis and modulation of endothelial inflammatory response. This association between OPG and endothelial dysfunction has been demonstrated clinically in type II diabetes [7, 8].

Matrix γ-carbaglutamic acid protein (MGP) acts in different ways to inhibit vascular calcifications. In the first place, it binds BMP-2 to mediate the osteoblastic differentiation of VSMCs and inhibits the activity of BMP-2 in the differentiation of mesenchymal cells. It has been demonstrated that MGP also binds Ca^{2+} crystals and inhibits crystal growth. Considering these facts, MGP has a role in maintaining the normal phenotype of VSMCs and in preventing their osteoblastic differentiation [5].

Another inhibitor of extraskeletal calcifications, *osteopontin* (OPN), has multifunctional roles in vascular physiology. This phosphoprotein found in mineralized tissues is acting as an inhibitor of apatite crystal growth by binding to the mineralized crystal surface, and its actions are independent of extracellular P^{2-} concentration and ALP activity. Secondary, OPN has emerged as a proinflammatory cytokine that enhances vascular remodeling and angiogenesis, in part through the activation of MMPs [9].

The role of *Fetuin-A* is very controversial. It was classically described as a circulating inhibitor of vascular calcification. VSMCs can take up serum fetuin-A and pool it in intracellular membrane-bound matrix vesicles. These vesicles are released from VSMCs and become the nidus for mineral nucleation. These released vesicles have abundant fetuin-A and abrogate the ability of regular membrane-bound matrix vesicles to form hydroxyapatite crystal [2]. Recent studies, though, failed to determine a strong relationship between its concentration and inhibition of extraskeletal calcifications [10].

Pyrophosphate (Ppi) is generated from the hydrolysis of nucleotide triphosphates by the nucleotide pyrophosphatase phosphodiesterase family. It is an important inhibitor of vascular calcification and acts by inhibiting hydroxyapatite crystal formation. It had been demonstrated that blocking PPi generation is necessary to induce aortic ring calcification even with high concentrations of Ca^{2+} and P^{2-} [11].

The role of *vitamin D* in the process of vascular calcification is yet incompletely specified. Vitamin D compounds have an important role in this relationship, because they are widely used for the treatment of secondary hyperparathyroidism and have calcemic and phosphatemic actions. Apparently, these compounds can induce vascular calcification through their calcemic and phosphatemic actions. Oversuppression of PTH by vitamin D compounds leads to low-turnover bone disease, typically adynamic bone disease, which is associated with vascular calcification. Even though vitamin D analogs were designed to suppress PTH with less calcemic and phosphatemic actions, they sometimes induce hypercalcemia and hyperphosphatemia. Both hypercalcemia and hyperphosphatemia associate with vascular calcification in patients with CKD. As well as *in vitro* and *in vivo* studies; however, there is no clear evidence that vitamin D compounds directly induce vascular calcification in patients with CKD [12].

The pathway of vascular calcification in ESRD

We tried to compose a complete and understandable scheme for extraskeletal calcification in ESRD, to summarizing all the components and the mechanisms known by now to be implicated in the process (Figure 1).

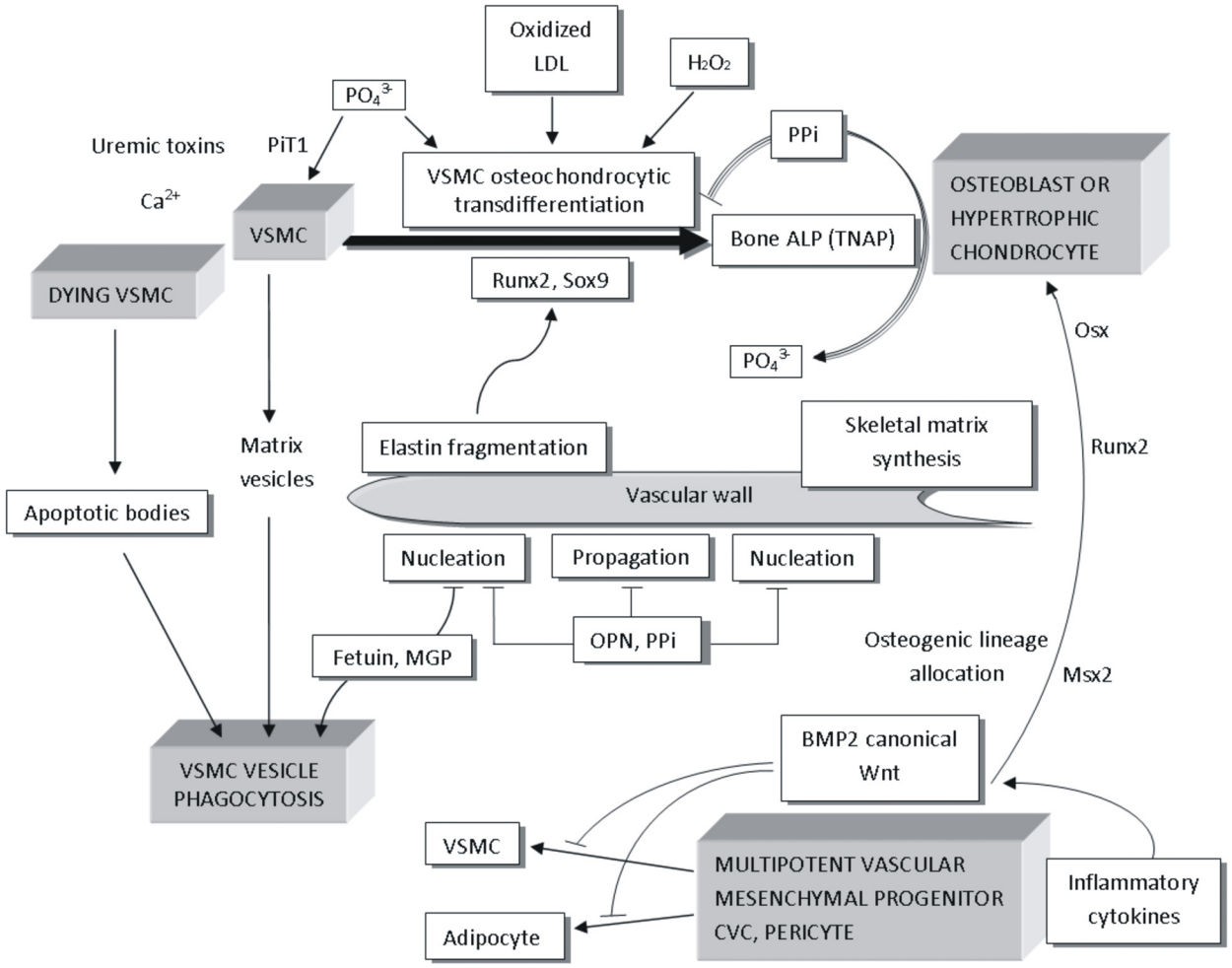

Figure 1 – ***Vascular calcification in ESRD: VSMC – vascular smooth muscle cell; MGP – matrix γ-carbaglutamic acid protein; OPN – osteopontin; PPi – pyrophosphate; BMP2 – bone morphogenetic protein-2; OPG – osteoprotegerin; OC – osteocalcin.***

The risk factors, such as hypertension, hyperlipidemia, diabetes, uremic-specific and, the most important, hyperphosphatemia, initiates the process, by stimulating the osteogenic differentiation of VSMCs and thermodynamic mechanisms. Then, the promoters of extraskeletal calcification are released and overexpressed and the dysregulation of endogenous calcification inhibitors favorites the process.

Innovative clinical studies addressing the combined use of inhibitors that work through distinct molecular mechanisms on vascular calcification will be necessary to reduce significantly vascular calcification and cardiovascular mortality in CKD.

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Corresponding author
Ionel Alexandru Checheriță, Junior Assistant, MD, PhD student, Nephrology Clinic, "St. John" Emergency Hospital, 13 Vitan–Bârzeşti Highroad, Sector 4, 042122 Bucharest, Romania; Phone +4021–334 50 75, Fax +4021–334 59 70, e-mail: fizij@yahoo.com

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