The pathophysiology of vascular calcification: are osteoclast-like cells the missing link?

Z.A. Massya-c, R. Mentaverria,a,b A. Mozara,b, M. Braziera,b, S. Kamela,b

a INSERM, ERI-12, 80054 Amiens, France
b University of Picardie-Jules Verne, 80000 Amiens, France

Amiens University Hospital, Divisions of Clinical Pharmacology and Nephrology, avenue Rene Laennec, 80054 Amiens, France

Received: October 13th 2007; accepted: October 30th 2007

Abstract

There is increasing evidence to suggest that the initiation of vascular calcification is an active process involving vascular smooth muscle cell (VSMC) apoptosis and trans-differentiation into calcifying cells. This active process results in the deposition of an osteogenic extracellular matrix and may be exacerbated by a reduction in the levels of one or more native calcification inhibitors (such as fetuin A and pyrophosphate). Here, we present data which strongly suggest that the regression of vascular calcification might also be an active cellular process involving osteoclast-like cells. However, the presence of osteoclast like cells in the vascular wall is rather limited. To explain this rarity of osteoclast-like cells, we recently observed that the same factors, which promote the trans-differentiation of VSMCs into osteoblast-like cells are also capable of inhibiting the in vitro differentiation of monocytes/macrophages into osteoclast-like cells. An imbalance between osteoblast-like and osteoclast-like cell activities would therefore favour the occurrence of a pathological calcification process in vessel walls. Our new data are strongly evocative of a vascular remodelling process similar to that observed in bone tissue. To confirm this hypothesis, strategies for activating osteoclasts in the vascular wall (with a view to preventing or reversing vascular calcifications) are required.

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Résumé

Physiopathologie des calcifications cardiovasculaires: les cellules ostéoclast-like sont-elles le chaînon manquant ?

Il est désormais bien établi que le processus de calcification vasculaire est un processus actif impliquant l’apoptose des ostéoblastes ainsi que leur transdifférentiation en cellules calcifiantes. Ce processus conduit au dépôt d’une matrice extracellulaire de type ostéogénique et pourrait être aggravé par la baisse de la quantité d’inhibiteurs de calcification tels la fétuine A et/ou le pyrophosphate. Dans cette revue, nous discutons du fait que la régression des calcifications vasculaires pourrait également être un processus cellulaire actif faisant intervenir les cellules ostéoclastiques. Néanmoins, la présence de cellules ostéoclast-like a été difficilement démontrée dans les parois vasculaires jusqu’ici. Récemment, nous avons mis en évidence que les mêmes facteurs qui induisent la minéralisation de cellules musculaires lisses inhibent la différenciation des précurseurs monocytes-macrophages en ostéoclast-like. Ainsi, ces données pourraient expliquer la faible présence d’ostéoclast-like dans les parois vasculaires. Un déséquilibre entre l’activité des cellules ostéoblastiques-like et ostéoclastiques-like pourrait favoriser la survenue de calcifications dans les parois vasculaires pathologiques. Ces nouveaux éléments suggèrent fortement la possibilité d’un remodelage au niveau vasculaire, semblable à celui observé dans le tissu osseux. Des stratégies visant...
1. Introduction

Cardiovascular calcification is frequent in the general population, in diabetic patients, and in patients with chronic kidney disease and is associated with an increased cardiovascular risk. [1-11] Cardiovascular calcification in the vascular walls is accompanied by the deposition of a mineralized osteogenic protein matrix. These calcium deposits are essentially localized in the media of the vascular wall but are also found in subintimal atherosclerotic plaques [12]. Both types of deposit are often observed simultaneously in humans and probably share common cellular and molecular mechanisms in their genesis [13].

2. The physiological development of bone calcification

Bone derives from intramembranous and/or endochondral cartilage ossification. In the latter process, longitudinal bars of cartilage matrix become calcified and thus form the growth plate of cortical bones. Hypertrophic chondrocyte death in this zone means that the extracellular chondroid matrix becomes impregnated with calcium salts. Moreover, hypertrophic chondrocytes release matrix vesicles - small membrane-bound bodies equipped with sophisticated cellular tools for creating a microenvironment that favours the nucleation of hydroxyapatite crystals (initiation step). This initial calcification process is followed by (i) the invasion of cartilage cells from the blood vessels (ii) the metaphysis/destruction of cartilage and (iii) the formation of bone along the remaining walls of the precalcified cartilage. The bone matrix produced by osteoblasts is subsequently mineralized by the coordinated interaction of several mineralizing-regulating proteins (i.e., the nucleation and crystal growth steps). Bone mineralization is determined partly by the osteoblasts' ability to remove pyrophosphate (a physiological inhibitor of mineralization) from their surrounding bone matrix via tissue non-specific alkaline phosphatase (Tnap) activity and the presence of a fibrillar (type I) collagen-rich network in the bone matrix [15]. Although the genes coding for Tnap and fibrillar collagen are not osteoblast-specific osteoblasts in bone and odontoblasts in teeth are the only cell types in which they are co-expressed. In the final (regression) step, minerals are resorbed by osteoclasts - the only cell type responsible for bone tissue degradation. Mature osteoclasts are large multinuclear cells with a characteristic tartrate-resistant acid phosphatase (TRAP) activity. The regulation of bone resorption involves two major processes: recruitment of new osteoclasts from haematopoietic precursors (monocyte/macrophage cell lineages) and the activation & survival of mature osteoclasts. Hence, bone degradation is a multi-step process which includes cell adhesion to the bone surface, cell polarization and formation of a sub-osteoclastic bone-resorbing compartment where bone degradation occurs [16]. The imbalance of bone remodelling in favour of osteoclast hyperactivity leads to the acceleration of bone demineralization which is not compensated by an increase in osteoblast activity and thus causes osteoporosis [17].

3. The pathophysiological development of vascular calcification

There is increasing evidence to suggest that matrix deposition in the vascular walls results from an active cellular process leading to the accumulation of osteogenic extracellular material and which is initiated (at least in part) by the materials activation of an apoptotic process and the release of apoptotic bodies and matrix vesicles [18]. This active process may be exacerbated by a reduction in the levels of one or more native calcification inhibitors (such as fetuin A pyrophosphate, matrix GLA protein, osteopontin and osteoprotegerin) [19-22] and/or by a partial defect in phagocytosis [23].

This process also appears to be intimately related to VSMC trans-differentiation into vascular calcifying cells. To date, several factors have been shown to promote in vitro, binding inorganic phosphate, inflammation, and oxidation products. These factors act through a common mechanism involving core binding factor a1 (Cbfa1) [24, 25], a transcription factor which is specific for the osteoblastic phenotype. Similar changes in the VSMC phenotype have been observed in animal models [24, 26] and human biopsy specimens [25]. However, the recent demonstration of the association of chondrocyte-like cells with media calcification in both rat and human arteries indicates that in addition to VSMC trans-differentiation, a process resembling endo-
chondral bone formation is a second mechanism by which vascular calcification may occur [27]. It is still unclear to what extent the formation of calcifying vascular cells is due to trans-differentiation of VSMCs resident in the local media or cell recruitment from the adventitia after the transformation of pericytes by Wnt signalling [28].

Osteoclast differentiation is tightly coupled to the presence of cell-cell contacts between the osteoclast precursors and osteoblasts in the bone tissue (the receptor activator of NF-κB (RANK)/RANK ligand (RANKL)/osteoprotegerin system). In the calcified vascular wall the presence of (i) monocyte/macrophage cell types that are able to differentiate directly into osteoclasts [29] and (ii) VSMCs that have an osteoblast-like phenotype and secrete factors involved in osteoclast differentiation (such as RANKL macrophage colony-stimulating factor (M-CSF) or pro-inflammatory cytokines) strongly suggests that osteoclastogenesis might occur [13; 30; 31]. The coexistence of these two differentiated cell types led us to hypothesize a vascular remodelling process similar to that observed in bone tissue. Hence an imbalance between the two processes in favour of the osteoblast-like phenotype could promote calcification [32; 33].

Preliminary data support the presence of cells with an osteoclast-like phenotype in the calcified arterial wall [31 34]. Moreover Bas et al. demonstrated that calcitriol-induced vascular tissue calcification in rats was partially reverted shortly after withdrawal of calcitriol treatment and that this process was associated with the presence of activated macrophages in the media [35]. Furthermore in a recent study bone-marrow-derived mature allogenic osteoclasts have been seen to interact with calcified aortic elastin and reduce its mineral content in the absence of detectable elastin degradation [36]. Taken as a whole these data indicate that an active cellular process may be involved in the regression of vascular calcification and that osteoclast-like cells may play a central role in this process.

4. The potential role of osteoclast-like cells in vascular calcification

to those observed in patients with CKD inorganic phosphate (one of the most important uraemic toxins inducing cardiovascular calcifications) significantly and dose-dependently decreased in vitro osteoclast monocyte/macrophage progenitor differentiation into osteoclast-like cells [37]. Inorganic phosphate was shown to affect RANKL-induced signalling mainly via the down-regulation of the RANKL-induced JNK Akt and NF-κB activation pathways. We also recently obtained similar results with other uraemic toxins such as oxidized LDL (data not published). Other researchers have shown that calcifying vascular cells restrict osteoclast differentiation via modulation of the release of matrix proteins (such as osteopontin) and soluble factors (such as osteoprotegerin and interleukin 18) [38]. It therefore appears reasonable to assume that a reduction in the activity of osteoclast-like cells in the arterial wall could be involved in the emergence and persistence of vascular calci-

Fig. 1: Inorganic phosphate not only promotes the trans-differentiation of vascular smooth muscle cells into calcifying osteoblast-like cells but also inhibits the differentiation of monocytes/macrophages into mature osteoclast-like cells and thus blocks calcified matrix resorption. RANK: receptor activator of NF-κB, RANKL: receptor activator of NF-κB ligand, M-CSF: macrophage colony-stimulating factor.
vascular remodelling in the presence of mineral deposit

vascular remodelling in the absence of mineral deposit

formation (osteoblast-like cells) degradation (osteoclast-like cells)

increased formation (osteoblast-like cells) decreased degradation (osteoclast-like cells)

uremic toxins (e.g. inorganic phosphate, and oxidized LDL)

No potential conflict of interest relevant to this article was reported.

Acknowledgements

We are very grateful to the Picardy Regional Council for its unrestricted and precious assistance in supporting the INSERM, ERI-12 research unit.

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