Impact of Aldosterone on Osteoinductive Signaling and Vascular Calcification

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Key Words

Mineralocorticoid receptor · PIT1 · Chronic kidney disease · Osteoinductive signaling · Klotho · Aldosterone · Spironolactone

Abstract

Vascular calcification is frequently found already in early stages of chronic kidney disease (CKD) patients and is associated with high cardiovascular risk. The process of vascular calcification is not considered a passive phenomenon but involves, at least in part, phenotypical transformation of vascular smooth muscle cells (VSMCs). Following exposure to excessive extracellular phosphate concentrations, VSMCs undergo a reprogramming into osteo-/chondroblast-like cells. Such 'vascular osteoinduction' is characterized by expression of osteogenic transcription factors and triggered by increased phosphate concentrations. A key role in this process is assigned to cellular phosphate transporters, most notably the type III sodium-dependent phosphate transporter Pit1. Pit1 expression is stimulated by mineralocorticoid receptor activation. Therefore, aldosterone participates in the phenotypical transformation of VSMCs. In preclinical models, aldosterone antagonism reduces vascular osteoinduction. Patients with CKD suffer from hyperphosphatemia predisposing to vascular osteogenic transformation, potentially

further fostered by concomitant hyperaldosteronism. Clearly, additional research is required to define the role of aldosterone in the regulation of osteogenic signaling and the consecutive vascular calcification in CKD, but more generally also other diseases associated with excessive vascular calcification and even in individuals without overt disease.

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Introduction

Vascular calcification may develop in the common population [1], but may be dramatically accelerated in several clinical disorders, particularly chronic kidney disease (CKD) [2–14]. Recent observations shed new light on the role of mineralocorticoids in triggering of vascular calcification [6]. The present brief review addresses the role of mineralocorticoids in vascular calcification.

Osteoinductive Signaling in Vascular Tissue

Vascular calcification [15] is not simply the result of passive precipitation of Ca^{2+} and bivalent phosphate, but results from an active and regulated process involving inhibiting proteins, such as fetuin-A [16] and matrix Gla

protein [17], apoptosis of vascular smooth muscle cells (VSMCs) [18, 19] and triggering of an osteoinductive signaling cascade transforming VSMCs into osteo- and chondrogenic phenotypes [18, 20–29].

A key role in the phenotypic transformation of VSMCs plays the type III sodium-dependent phosphate transporter Pit1 [30], but the mechanisms linking Pit1 expression to vascular calcification are still incompletely understood [30, 31]. The location of the carrier protein may not be confined to the cell membrane and its effect on osteogenic signaling is not necessarily due to phosphate transport across the cell membrane [31]. Pit1 and Pit2 play redundant roles and both may cause phenotypic transformation of VSMCs [32].

The transition of VSMCs into chondroblastic/osteoblastic phenotypes is mediated by key osteogenic transcription factors, most notably by Cbfa1/Runx2 (core-binding factor- α 1/runt-related transcription factor 2) [3, 5, 11]. Cbfa1/Runx2 deficiency inhibits vascular calcification and osteogenesis in the vasculature [11]. The signaling cascade involved in phenotypical transformation of VSMCs involves TNF- α (tumor necrosis factor α) [20], the transcription factors NF κ B (nuclear factor- κ B) and Msx2 (homeobox protein Hox-8) [33], Wnt (wingless-type MMTV integration site) and β -catenin [3, 5, 11, 34]. This phenotypical change of VSMCs ultimately leads to stimulation of alkaline phosphatase (Alpl) which degrades pyrophosphate, a powerful inhibitor of calcification. Pyrophosphate is generated by ENPP1 (ectonucleotide pyrophosphatase/phosphodiesterase) which thus counteracts vascular calcification [31, 35]. Pyrophosphate deficiency may contribute to vascular calcification in CKD patients [35].

Vascular Calcification in CKD – A Role of Aldosterone and Klotho/FGF23

The vascular calcification/mineral bone disorder is highly prevalent in CKD patients [25, 36]. CKD is further associated with reduction of klotho, a cofactor for FGF23, and with increase of FGF23 [37]. Klotho-deficient mice (*kl/kl*) mimic the vascular calcification of CKD patients [23]. Klotho (i) is required for the inhibition of 1,25(OH) $_2$ D $_3$ formation by FGF23, (ii) modifies the renal tubular transport of calcium and phosphate, and (iii) influences phosphate uptake of VSMCs [23]. In CKD patients, 1,25(OH) $_2$ D $_3$ is reduced. Klotho deficiency leads to a marked increase of plasma calcium and phosphate concentrations due to increased 1,25(OH) $_2$ D $_3$ formation,

which can be blunted by a vitamin D-deficient diet [23, 38, 39]. The exact role of vitamin D in vascular calcification is still elusive [26, 40]. Independently of the mineralocorticoid receptor, spironolactone upregulates klotho via VDR activation [41].

The vascular calcification in *kl/kl* mice is paralleled by an upregulation of osteogenic signaling (Pit1, TNF- α , Msx2, Cbfa1/Runx2, osterix and Alpl) [23, 25, 29, 38]. The *kl/kl* mice further suffer from hyperaldosteronism which is partially reversed by a salt-rich diet and a calcium-deficient diet [38]. Presumably, the high extracellular Ca $^{2+}$ concentration leads in part, via the calcium-sensing receptor in the thick ascending limb, to inhibition of salt reabsorption thus causing extracellular volume depletion and subsequent stimulation of aldosterone secretion [38]. Blocking of mineralocorticoid receptors with spironolactone decreases vascular osteoinduction in the *kl/kl* mice as apparent by a decline of Pit1, TNF- α , Msx2, Cbfa1/Runx2, osterix and Alpl expression [29].

Whether klotho plays a role in cardiovascular tissue is still incompletely understood. Klotho is not only expressed in the kidney, but in a variety of further cell types including VSMCs [42]. Klotho is required for the vascular effects of FGF23 [42] and interferes with osteoinductive signaling [23]. On the other hand, in a CKD model, neutralization of FGF23 has led to increased phosphate levels and mortality in a CKD model [43]. Dysregulation of the klotho/FGF23 duo presumably plays an important role in CKD [25, 36]. In CKD models and CKD patients, aortic klotho expression is reduced [42, 44]. Stimulatory effects of FGF23 on vascular calcification have been described [45], but other studies could not show a functional klotho/FGF23 system in vascular tissue [46, 47]. Klotho directly influences cardiomyocytes [48] and FGF23 exerts direct effects on the heart independent of klotho [49].

Mineralocorticoid Signaling in Vascular Tissue

Aldosterone exerts detrimental effects in the cardiovascular system [50]. The mineralocorticoid receptor is expressed in VSMCs and mineralocorticoids stimulate vascular calcification [51, 52]. Interestingly, angiotensin II can directly activate the vascular mineralocorticoid receptor [53]. Aldosterone upregulates a wide variety of genes involved in vascular injury and calcification [54]. Accordingly, excessive plasma aldosterone concentrations are associated with vascular stiffening and atherosclerosis [55–57]. The development of atherosclerosis is slowed by inhibition of mineralocorticoid receptors [58].

Aldosterone triggers phenotypic reprogramming of smooth muscle cell via TNF- α /Msx2/Cbfa1 signals. These effects of aldosterone are independent of bone morphogenetic protein 2 (BMP2) [51]. Aldosterone causes phenotypic transformation of VSMCs via stimulation of PIT1 [29]. The promoter sequence of the PIT1 gene harbors putative mineralocorticoid response elements MRE/GRE and aldosterone increases the expression of PIT1 in a spironolactone-sensitive manner [29]. Phosphate treatment apparently activates the mineralocorticoid receptor, as spironolactone downregulates PIT1 and CBFA1/RUNX2 gene expression even in the absence of exogenous aldosterone [29]. FGF23 ameliorates the effects of aldosterone on Pit1 expression via klotho [29]. Vascular stiffness is mediated by the mineralocorticoid receptor in smooth muscle cells [59].

The mineralocorticoid receptor is further expressed in endothelial cells where it downregulates the expression of the endothelial NO synthase [60, 61]. Aldosterone leads to stiffening of endothelial cells which compromises endothelial cell deformation and subsequent activation of NO synthase [62, 63]. This effect is again reversed by spironolactone [64]. Nitric oxide counteracts osteogenic transformation in part by modifying *Pai1* expression [24]. Furthermore, aldosterone promotes the generation of oxidative stress and activation of NF κ B [54], an effect partially due to upregulation of serum- and glucocorticoid-inducible kinase SGK1 [65]. SGK1 phosphorylates and thus activates the NF κ B inhibitory protein I κ B kinase IKK, which phosphorylates I κ B leading to degradation of I κ B and subsequent disinhibition and nuclear translocation of NF κ B [65].

Aldosterone and Vascular Calcification in CKD

In CKD the impaired renal elimination of phosphate [66] leads to increased plasma phosphate concentrations, which trigger detrimental changes in the vasculature [18, 22]. Expression of klotho is decreased correlating with enhanced plasma phosphate levels and an unfavorable course of the disease [37]. Markers of osteogenic transformation are found in arteries of CKD patients [18, 67]. The vascular calcification leads to vascular events which presumably promote the high mortality of CKD patients [68]. Elevated plasma phosphate levels are associated with a higher risk of death and conversely some studies documented an increased lifespan of CKD patients on phosphate binders [66, 69]. Several mechanisms contribute to the phosphate-induced vascular

changes [3, 21, 24, 25, 28, 33] and hyperaldosteronism may be a further contributor to vascular calcification [29, 52]. Hyperaldosteronism is frequently found in CKD patients [70, 71] so that aldosterone might contribute to enhanced vascular osteoinduction in the vessels of such patients [6].

Recent studies documented a beneficial effect of aldosterone antagonism [50, 72–98]. In peritoneal dialysis patients, spironolactone treatment showed protective effects on cardiac function [99]. Furthermore, spironolactone treatment showed beneficial effects on endothelial dysfunction in hemodialysis patients without heart failure [100]. Most importantly, there is recent documentation that spironolactone treatment reduced the risk of both cardiovascular morbidity and death in hemodialysis patients [101]. Unfortunately, aldosterone antagonism is associated with the risk of hyperkalemia [102] in patients with and without remaining renal function. In CKD patients, an initial decline of estimated glomerular filtration rate was noted [103]. The occurrence of hyperkalemia is a risk, but occurs only in a low percentage of patients [101, 102]. Clearly, further studies are required to assess the risks and benefits of aldosterone antagonism in CKD.

Conclusions

Aldosterone upregulates the type III sodium-dependent phosphate transporter *Pit1* and thus contributes to the osteoinductive cascade involved in vascular calcification [29, 52]. The effect is reversed by the mineralocorticoid receptor antagonist spironolactone. Spironolactone or other aldosterone antagonists are thus expected to delay the onset of vascular calcification by reducing transdifferentiation of VSMCs into osteo-/chondroblast-like cells. Future analysis will reveal whether mineralocorticoid antagonism can accomplish reduction of vascular calcification without untoward side effects [70].

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