Pyrophosphate deficiency in vascular calcification

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Pathologic cardiovascular calcification is associated with a number of conditions and is a common complication of chronic kidney disease. Because ambient calcium and phosphate levels together with properties of the vascular matrix favor calcification even under normal conditions, endogenous inhibitors such as pyrophosphate play a key role in prevention. Genetic diseases and animal models have elucidated the metabolism of extracellular pyrophosphate and demonstrated the importance of pyrophosphate deficiency in vascular calcification. Therapies based on pyrophosphate metabolism have been effective in animal models, including renal failure, and hold promise as future therapies to prevent vascular calcification.

Pathologic cardiovascular calcification is associated with a number of genetic diseases and common conditions such as diabetes, chronic kidney disease, and aging, and is associated with poor clinical outcomes. This calcification, primarily in the form of hydroxyapatite, occurs in arteries and cardiac valves. In arteries, calcified deposits are distinguished by their location in the arterial wall and their different pathophysiologies. Intimal calcification occurs in advanced atherosclerotic plaques and is associated with inflammation, whereas medial calcification (also called "Monckeberg's medial sclerosis") occurs in the medial layer and the internal elastic lamina in the absence of atherosclerosis or inflammation. It is this latter form that is frequently associated with disordered mineral metabolism.

Chemistry of vascular calcification

Hydroxyapatite (principally carbonated-substituted apatite), which is the main calcium phosphate crystal form found in bone and calcified tissues, is not formed directly from calcium and phosphate ions but rather through a solid-phase reaction through intermediaries such as octacalcium-phosphate and amorphous calcium-phosphate (Figure 1).[1] Amorphous calcium-phosphate, which is also found within calcifications, consists of spherical $Ca_9(PO_4)_6$ clusters (called "Posner's clusters"), which appear to be energetically favored compared to $Ca_3(PO_4)$ and $Ca_6(PO_4)_4$ clusters. Therefore, the structure of hydroxyapatite and octacalcium-phosphate can be also be interpreted as an aggregation of different clusters in a second step (Figure 1). The precursors of hydroxyapatite derive from the formation of dicalcium-phosphate dihydrate ($CaHPO_4 2H_2O$; also called "brushite"), the initial event in the calcification process.

Formation of brushite depends on the concentration and forms of calcium and phosphate ions. At physiologic pH, approximately 80% of phosphate is HPO_4^{2-}, and the remaining 20% is $H_2PO_4^{-}$. In addition to ionized calcium, there is also complexed calcium (primarily to citrate and bicarbonate) and protein-bound calcium (primarily to albumin). Based on the activity coefficients of Ca^{2+} and HPO_4^{2-} and the solubility product of brushite, and assuming that 47% of total serum calcium is ionized and that 81% of total phosphorus is HPO_4^{2-}, the product of the total calcium and phosphorus concentrations (Ca X P) in plasma would have to exceed 90 mg^2/dl^2 in order for precipitation to occur. Thus, despite frequent pronouncements to the contrary, extracellular fluid is not supersaturated with respect to calcium and phosphate.[1] Because bone mineralization and ectopic calcification clearly occur at a lower Ca X P, including in patients

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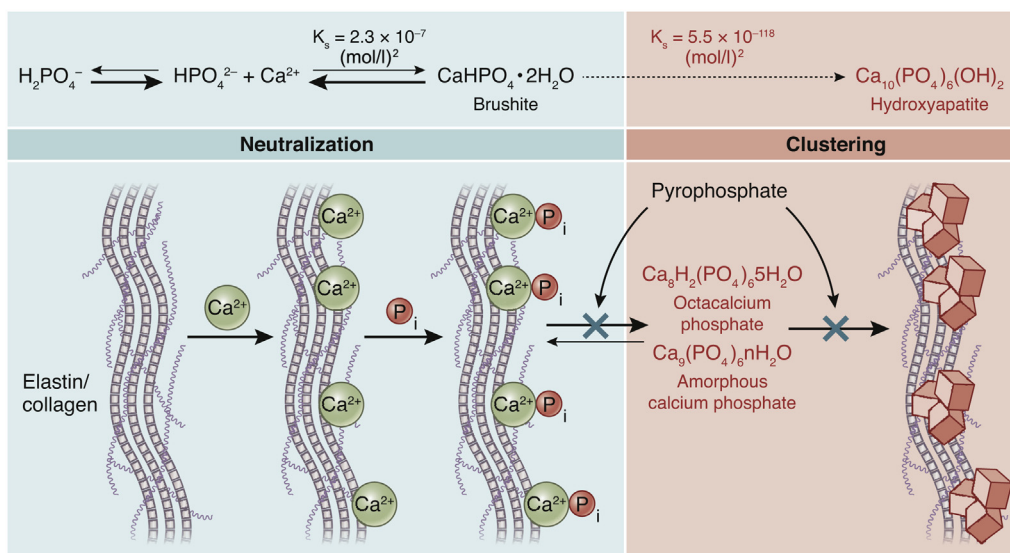
Figure 1 | Schematic representation of the chemistry of vascular calcification. In biological systems, inorganic phosphate (Pi) exists primarily as the hydrogen phosphate ion (HPO_4^{2-}). Although hydroxyapatite is essentially insoluble under physiologic conditions, its formation requires the initial formation of dicalcium-phosphate dehydrate or brushite (upper panel). Because this reaction is not thermodynamically favored in extracellular fluids *in vivo* under normal conditions, hydroxyapatite is not formed. However, matrix proteins (lower panel) serve as solid-phase catalysts by binding calcium non-ionically, with subsequent charge neutralization by phosphate, in an orientation that promotes crystalline hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$), through the 2 precursors, octocalcium-phosphate ($Ca_8H_2[PO_4]_6 5H_2O$) and amorphous calcium-phosphate ($Ca_9[PO_4]_6 nH_2O$). Pyrophosphate directly inhibits this orientation by binding to nascent crystals. K_s, solubility product constant.

with chronic kidney disease, it is clear that a process must exist for calcification to occur below the solubility product of brushite. This is mediated by matrix proteins that essentially catalyze this reaction.[2] According to the charge neutralization theory of calcification, the high glycine content of these matrix proteins favors the formation of beta-turns which bind calcium ions nonionically, enabling phosphate to bind the calcium in a configuration that favors apatite formation under physiologic conditions (Figure 1).[2] In bone and arteries, this process occurs predominantly on Type I collagen and elastic fibers, respectively. Any proposed mechanism of vascular calcification, including osteochondrogenic differentiation or apoptosis of vascular smooth muscle, must take this chemistry into account. Recent studies demonstrate that calcium-phosphate deposits can induce the transition to a bone-forming phenotype as well as apoptosis in vascular smooth muscle cells, suggesting that these processes may instead be a result of the initial calcification.[3]

It is clear, then, that mineralization, including vascular calcification, can occur at physiological calcium and phosphate concentrations and that inhibitory mechanisms must be in place to restrict this to bone and cartilage. A number of genetic diseases and animal models have identified endogenous inhibitors of calcification that are required to prevent vascular calcification under normal conditions. These include proteins such as the matrix Gla protein, fetuin-A, and osteopontin and the low-molecular-weight compound pyrophosphate. Pathologic calcification occurs when inhibitors are deficient or when over-whelmed by factors such as hyperphosphatemia. When the solubility product for $CaHPO_4$ is actually exceeded, precipitation in the circulation is prevented

by binding of fetuin-A to nascent crystals to form colloidal calcium-phosphate (also termed "calciprotein" particles), which is then cleared by the reticuloendothelial system.

Pyrophosphate is a direct inhibitor of calcification

Pyrophosphate is a potent inhibitor of calcium crystallization and deposition that has long been used industrially for this purpose and is the active ingredient in plaque-preventing toothpaste. It acts by avidly binding to nascent hydroxyapatite crystals, being sequestered in the process,[4] with complete inhibition at micromolar concentrations that are more than 1,000-fold less than physiologic calcium or phosphate concentrations.[4,5] Extracellular pyrophosphate is present at levels sufficient to completely prevent hydroxyapatite formation and, although there is local production by vascular smooth muscle,[6] it is the circulating pyrophosphate that is primarily responsible for inhibiting calcification.[7] Its ability to prevent vascular calcification was originally demonstrated in an animal model of vitamin D toxicity[8] and subsequently in aortic rings and aortic valves in culture.[9,10] This action is mimicked by various polyphosphates including bisphosphonates,[11–13] which were originally developed as nonhydrolyzable pyrophosphate analogs to prevent ectopic calcification. A normal pyrophosphate concentration is sufficient to prevent calcification when the phosphate concentration is in the normal range but may not be sufficient in the face of hyperphosphatemia.[5] Pyrophosphate may also contribute to the inhibition of calcium-phosphate precipitation in the circulation as serum levels correlate with the formation of calciprotein particles *in vitro*.[14]