Magnesium as a Calcification Inhibitor

Lucie Hénaut and Ziad A. Massy

Vascular calcification (VC) is associated with elevated cardiovascular mortality rates in patients with CKD. Recent clinical studies of patients with advanced CKD have observed an association between low serum magnesium (Mg) levels on one hand and elevated VC and cardiovascular mortality on the other. These findings have stimulated interest in understanding Mg's impact on CKD in general and the associated VC in particular. In vitro and preclinical in vivo data indicate that Mg has the potential to protect vascular smooth muscle cells against calcification via several different molecular mechanisms. Accordingly, data from pilot interventional studies in the clinic suggest that oral Mg supplementation reduces VC in patients with CKD. The present review provides an overview of our current understanding of the impact of Mg on the development of VC in patients with CKD.

Key Words: Chronic kidney disease, Magnesium, Vascular calcification

INTRODUCTION

Cardiovascular disease is the leading cause of death in patients with CKD. In particular, vascular calcification (VC) is more prevalent and much more severe in this setting than in the general population.[1] This degenerative process is characterized by the accumulation of calcium (Ca) and phosphate salts within the cardiovascular system (notably in the intimal and medial layers of vessels and in cardiac valves) and is associated with elevated cardiovascular mortality.[2] Intimal and medial calcification notably contribute to cardiovascular disease and excess cardiovascular mortality in patients with CKD. Intimal VC (a consequence of atherosclerosis) may favor coronary ischemic events, whereas medial VC (which develops along the elastic lamina) increases vessel stiffness, arterial pulse wave velocity, systolic blood pressure, and pulse pressure and thus favors the development of cardiac failure, left ventricular hypertrophy, and diastolic dysfunction. Calcification of cardiac valves is also common in ESRD and is associated with a worse prognosis. Over the last few decades, studies of CKD patients have shown that when VC is accompanied by vessel stiffness, arterial hypertension, left ventricular hypertrophy, and cardiomyopathy, the severity of the condition is a strong, independent predictor of death. It is widely admitted that VC is irreversible once established; none of the treatments tested to date can unambiguously prevent or reverse VC. Accordingly, understanding the pathophysiologic mechanisms involved in the development of VC is of critical importance for identifying the most effective means of prevention and treatment.

It has been suggested that along with traditional cardiovascular risk factors, nontraditional risk factors (such as those related to uremic status and disturbed CKD mineral and bone disorder [CKD-MBD]) may explain the elevated prevalence of VC and cardiovascular disease in a CKD setting. CKD-MBD is a systemic disorder characterized by persistently elevated parathyroid hormone (PTH) levels, bone abnormalities, extraskeletal calcification, and a broad spectrum of mineral metabolism disorders (including hypocalcemia, hyperphosphatemia, and hypermagnesemia). Over the last few decades, studies of CKD-MBD have focused on the perturbation of Ca/phosphate homeostasis. However, increasing attention had been paid to the impact of magnesium (Mg) homeostasis on CKD-MBD, in view of (1) the metal's important role in the pathophysiology of the cardiovascular system, and (2) the reported association between Mg disorders and elevated cardiovascular morbidity/mortality. In fact, Mg is involved in the regulation of vascular tone, cardiac rhythm, and platelet-activated thrombosis. The maintenance of normal serum Mg levels (0.75-1 mmol/L) is determined by dietary ingestion and absorption, bone and soft tissue deposition and efflux, and kidney excretion. In CKD, urinary Mg excretion is normal or sometimes even elevated as long as the glomerular filtration rate is above 30 mL/min. Below that value, the kidney excretion of Mg may not be able to balance the dietary intake Mg—which then becomes a major determinant of serum and whole-body Mg levels.[3] Although hypermagnesemia in CKD patients is usually mild and asymptomatic (<1.5 mmol/L), severe, symptomatic hypermagnesemia can be induced by exogenous Mg administration. Indeed, administration of Mg-containing drugs (eg, antacids and laxatives) and high-Mg concentrations in dialysate may provoke severe, symptomatic and sometimes even fatal hypermagnesemia. Conversely, the excessive use of diuretics, reduced gastrointestinal uptake (due to acidosis, and poor nutrition and absorption), and a low Mg concentration in dialysate may lead to abnormally low serum Mg levels.[3,4] The results of many recent clinical studies show that low serum Mg levels are associated with increased VC and elevated cardiovascular mortality in patients with ESRD. These

From the INSERM U1088, CURS, Amiens, France; Division of Nephrology, Ambroise Paré University Hospital, APHP, Boulogne-Billancourt/Paris, France; and INSERM U1018, Team 5, CESP, UVSQ, Villejuif, France.

Financial Disclosure: Z.A.M. reports grants for CKD REIN and other research projects from Amgen, Baxter, Fresenius Medical Care, GlaxoSmithKline, Merck Sharp and Dohme-Chibret, Sanofi-Genzyme, Lilly, Otsuka, and the French government as well as personal fees and grants to charities from Amgen, Bayer, and Sanofi-Genzyme. These sources of funding are not necessarily related to the content of the present manuscript. Also see Acknowledgment(s) on page 288.

Address correspondence to Ziad A. Massy, Division of Nephrology, Ambroise Paré University Hospital, 9 Avenue Charles de Gaulle, F-92104 Boulogne Billancourt cedex, France. E-mail: ziad.massy@aphp.fr

1548-5595/$36.00
https://doi.org/10.1053/j.ackd.2017.12.001

findings have stimulated interest in understanding Mg's role in CKD in general and the associated VC in particular. The present review provides an overview of our current understanding of the impact of Mg on the development of VC in patients with CKD.

MOLECULAR MECHANISMS OF VASCULAR CALCIFICATION IN CKD

VC is a complex process that involves not only the precipitation of minerals (due to supersaturated phosphate and Ca concentrations in the extracellular milieu, that is, an inorganic component) but also tightly regulated, cell-mediated process including apoptosis, osteochondrogenic differentiation, matrix vesicle release, and elastin degradation (ie, a cellular component).[5] Despite the significant recent growth in our knowledge of VC, the order of appearance and time course of these 2 steps in vivo is still subject to debate.

Under normal physiological conditions, blood vessels and cardiac valves are protected from supersaturated concentrations of serum Ca and phosphate by a number of active inhibitors. Pyrophosphate, adenosine, matrix Gla protein (MGP), fetuin-A, bone morphogenetic protein-7 (BMP7) and other factors have been shown to prevent the transformation of soluble, amorphous calcium phosphate (Ca/P) complexes into harmful, stable hydroxyapatite crystals. In the CKD population, a decrease in levels of active inhibitors and a simultaneous increase in levels of active inducers of calcification (including hyperphosphatemia, hypercalcemia, inflammatory cytokines, oxidative stress, uremic toxins, and advanced glycation end products) are responsible for the extremely high prevalence of intimal and medial VC and valvular calcification. Hyperphosphatemia (a typical manifestation of CKD-MBD) is the calcification inducer most strongly associated with VC in CKD. Within the vascular system, elevated levels of inorganic phosphate (Pi) and extracellular Ca and the deposition of amorphous Ca/P lead to the conversion of vascular smooth muscle cells (VSMCs), resident fibroblasts, and quiescent valve interstitial cells into osteochondrogenic cells. During this conversion, the vascular cells downregulate the expression of VC-inhibiting genes (such as MGP and BMP7) and start to express bone-promoting genes (such as BMP2, RUNX2, and alkaline phosphatase) that act to transform amorphous Ca/P deposits into a well-organized, calcified, crystalline structure.[6] This phenomenon is associated with (1) the secretion of a procalcifying matrix rich in type I collagen and (2) the production of matrix metalloproteinases 2 and 9 (known to promote elastin degradation and the subsequent release of highly procalcifying elastin peptides). According to a recent report, the elevated number of Ca/P nanocrystals formed in response to CKD-related loss of circulating inhibitors and hyperphosphatemia may directly promote the osteo/chondrogenic conversion of vascular cells.[7] These Ca/P nanocrystals can also be taken up via endocytosis into VSMCs, where subsequent lysosomal degradation increases Ca and Pi levels within the intracellular milieu. In an attempt to compensate for this increase, VSMCs release matrix vesicles loaded with Ca/P products. Once this compensatory mechanism is overwhelmed, the intracellular Ca/Pi overload triggers apoptosis, resulting in the formation of Ca/P-containing apoptotic bodies in the VSMCs. This phenomenon can be accentuated by additional Pi uptake through the sodium-dependent phosphate transporter Pit-1. The apoptotic bodies and matrix vesicles ultimately contribute to a positive feedback loop by releasing nanocrystals into the surrounding milieu and thus amplifying the calcification process.

CLINICAL SUMMARY

- Vascular calcification (VC) is a major contributor to cardiovascular disease and excess cardiovascular mortality in patients with CKD.
- Epidemiologic studies indicate a possible link between low serum magnesium (Mg) levels and the development of VC in both the general population and patients with CKD.
- Data from in vitro studies and animal models suggest that Mg supplementation protects vascular cells against calcification.
- The results of pilot clinical studies suggest that oral Mg supplementation reduces cardiovascular calcification.

MAGNESIUM AS A CALCIFICATION INHIBITOR IN CKD

Lessons Learned from In Vitro Studies

In early reports, Posner and associates demonstrated that Mg could directly interfere with the process whereby Ca and P crystallize into hydroxyapatite; the researchers found that the addition of Mg to metastable Ca/P solutions inhibited the formation of Ca/P apatite and Ca-acidic phospholipid–phosphate complexes.[8,9] This inhibition stabilized amorphous Ca/P and favored the formation of the Mg-containing crystal whitlockite [$(Ca,Mg)_3(PO_4)_2$]. Hydroxyapatite had long been thought to be the sole mineral phase present in human uremic VC.[10] However, a recent synchrotron study revealed the colocalization of whitlockite with hydroxyapatite in the arteries of patients with moderately impaired kidney function (CKD Stages 2-4).[11] This observation suggests that Mg (like the calcification inhibitors fetuin-A, MGP, and osteopontin) is closely associated with microcalcification.

In a series of in vitro experiments, the use of $MgCl_2$ reportedly prevented Pi-induced calcification and the osteogenic differentiation of human, bovine, and rodent VSMCs by increasing or restoring the activity of the Mg transporter TRPM7. These effects were associated with greater expression of anticalcification proteins (including osteopontin, BMP7, and MGP) and a decrease in VSMC apoptosis. $MgCl_2$ was also shown to reduce Ca entry into VSMCs; this is of particular interest because elevated Ca is known to upregulate Pit-1, which in turn increases Pi uptake.[12] According to recent studies, the Mg-associated inhibition of VSMC calcification is mainly mediated by inhibition of the Pi-induced Wnt/β-catenin signaling