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REVIEW

Magnesium to prevent kidney disease–associated vascular calcification: crystal clear?

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ABSTRACT

Vascular calcification is a prognostic marker for cardiovascular mortality in chronic kidney disease (CKD) patients. In these patients, magnesium balance is disturbed, mainly due to limited ultrafiltration of this mineral, changes in dietary intake and the use of diuretics. Observational studies in dialysis patients report that a higher blood magnesium concentration is associated with reduced risk to develop vascular calcification. Magnesium prevents osteogenic vascular smooth muscle cell transdifferentiation in *in vitro* and *in vivo* models. In addition, recent studies show that magnesium prevents calciprotein particle maturation, which may be the mechanism underlying the anti-calcification properties of magnesium. Magnesium is an essential protective factor in the calcification milieu, which helps to restore the mineral-buffering system that is overwhelmed by phosphate in CKD patients. The recognition that magnesium is a modifier of calciprotein particle maturation and mineralization of the extracellular matrix renders it a promising novel clinical tool to treat vascular calcification in CKD. Consequently, the optimal serum magnesium concentration for patients with CKD may be higher than in the general population.

Keywords: calciprotein particle, chronic kidney disease, mineral homeostasis, phosphate, vascular smooth muscle cells

INTRODUCTION

Chronic kidney disease (CKD) is characterized by severe mineral–bone disturbances including hyperphosphatemia, which stimulates the development of vascular calcification [1]. The presence of vascular calcification is associated with increased cardiovascular mortality risk [2]. Currently there are no treatment options with proven efficacy for patients to prevent or counteract vascular calcification [3]. Over the past decade, a higher serum magnesium (Mg^{2+}) concentration has been associated with lower cardiovascular mortality risk in patients on haemodialysis treatment [4]. In addition, a higher Mg^{2+} concentration beneficially modifies the association of serum levels of phosphate (Pi) with all-cause mortality in patients treated with dialysis, mitigating risk if Mg^{2+} is high [5]. Besides these epidemiological association studies, Mg^{2+} has been demonstrated to reduce vascular calcification in a multitude of experimental models. In this review we provide an overview of the current perspectives on the role of Mg^{2+} in CKD-induced vascular calcification.

VASCULAR CALCIFICATION IN CKD AND THE ROLE OF MAGNESIUM: OBSERVATIONAL STUDIES

Vascular calcification is a prognostic marker for cardiovascular mortality in CKD patients [6, 7]. The severity of vascular calcification is correlated with, among others, a reduced glomerular filtration rate (GFR) [8]. In dialysis patients, the prevalence of vascular calcifications is >80% [9, 10]. Vascular calcification can be present in both the intimal and the medial layers of arteries. Intimal calcification develops specifically at sites of atheromatous plaques and is typically associated with advanced stages of atherosclerosis [11]. Medial calcification occurs in response to metabolic imbalance of, for instance, minerals and glucose in the larger arteries and generally is associated with ageing, diabetes and CKD [12–14].

Once established, vascular calcification is currently considered to be irreversible [15]. Indeed, there are no confirmed treatment options to regress calcification in arteries of CKD patients. Rather, treatment is directed at preventing the formation of calcification mainly by reducing blood Pi concentrations [16]. The use of calcium (Ca^{2+})-free Pi binders has been shown to reduce the incidence and progression of vascular calcification, and these are most frequently used in CKD patients to limit vascular calcification development [1]. While Pi binders are successful in lowering blood Pi concentrations, a recent meta-analysis indicated that they do not clearly reduce cardiovascular risk [17]. Studies assessing other medications such as

vitamin D_3 derivatives, normally used to improve Ca^{2+} balance, remained inconclusive, as in some cases vascular calcifications worsened rather than improved [18]. In 2011, the ADVANCE trial reported promising effects of low-dose vitamin D_3 treatment in combination with the calcimimetic cinacalcet on coronary, aortic and cardiac valve calcification progression [19]. However, this treatment never proceeded to clinical use for this indication, potentially because the benefits seem inferior to the current treatment standards [18]. Cinacalcet is frequently used for the treatment of secondary hyperparathyroidism. Given the limited treatment options, it is of great clinical importance to identify novel modifiable factors that influence the evolution of vascular calcification.

Magnesium and cardiovascular mortality risk

Hypomagnesaemia (serum Mg^{2+} concentration <0.7 mmol/L) is a well-known risk factor for cardiovascular disease, events and mortality in the patients with higher baseline cardiovascular risk and in patients with dialysis-dependent CKD [4, 20–23]. Besides its blood concentrations, low dietary Mg^{2+} intake is also associated with all-cause mortality, risk of stroke, heart failure and diabetes mellitus type 2 in the general population [24]. Lower blood Mg^{2+} concentration is associated with an increased risk of death from heart failure and coronary heart disease, even at concentrations (0.7–0.8 mmol/L) that fall within the reference range for serum Mg^{2+}, which is typically between 0.7 and 1.0 mmol/L [25, 26].

In both CKD and dialysis patients, similar associations exist, as a number of epidemiological studies have revealed an inverse association between serum Mg^{2+} concentration and (cardiovascular) mortality risk [4, 27, 28]. Observational studies in large groups of dialysis patients reported that a higher serum Mg^{2+} concentration is associated with a reduced risk of developing vascular calcification [29]. Dialysis patients with a Mg^{2+} concentration <1.14 mmol/L showed significantly more risk for mortality [30]. In other studies, blood Mg^{2+} concentrations <1.23 mmol/L [31], 1.15 mmol/L [27] and 1.21 mmol/L [32] were associated with increased cardiovascular and all-cause mortality in dialysis patients. Of note, these concentrations are all above the reference value for serum Mg^{2+}. This suggests that supranormal Mg^{2+} concentrations beneficially impact survival rate in CKD. Importantly, a large Japanese cohort study reports a U-shaped curve for this association, as a blood Mg^{2+} concentration >1.27 mmol/L was associated with increased cardiovascular mortality [20]. Together, these observational studies suggest that a blood Mg^{2+} concentration between 1.14 and 1.27 mmol/L may be optimal and maintaining blood Mg^{2+} concentrations in this range in CKD and dialysis patients might be of importance. Of note, it may be of importance to determine the optimal Mg^{2+} concentration on an individual level.

While the relevance of Mg^{2+} is increasingly recognized in the context of CKD, Mg^{2+} status is infrequently assessed in clinical practice. Given recent clinical trials that demonstrate a beneficial effect of Mg^{2+} supplementation on intermediate endpoints like calcification propensity or coronary artery calcification [33, 34], the time seems right to study the effect of Mg^{2+} supplementation on clinically relevant outcomes, which so far has not been done.

Magnesium homoeostasis in CKD

The kidney is the central organ for maintaining Mg^{2+} balance, in addition to less pronounced roles for the intestinal tract and bone. Within the kidney, 90% of the filtered load of Mg^{2+} is reabsorbed paracellular in the proximal tubule and thick ascending limb of Henle's [35]. Fine-tuning takes place in the distal convoluted tubule (DCT) [36, 37]. Disturbed Mg^{2+} reabsorption in the DCT inevitably results in Mg^{2+} wasting, since Mg^{2+} cannot be reabsorbed downstream. Consequently DCT-targeting diuretics, e.g. thiazides, are associated with hypomagnesaemia [38].

In patients with CKD, Mg^{2+} balance is disturbed, initially mainly due to limited ultrafiltration of this mineral. However, patients with early-stage CKD have normal blood Mg^{2+} concentrations due to an effective increase in fractional excretion of Mg^{2+} [39]. Additionally, the use of loop diuretics in CKD patients indirectly reduces paracellular reabsorption of Mg^{2+} in the thick ascending limb of Henle's loop [40]. Type 2 diabetes, often present in CKD patients, has also been associated with hypomagnesaemia and renal Mg^{2+} wasting [41, 42]. In more severe CKD, blood Mg^{2+} concentrations tend to increase, especially if the GFR drops to <10 mL/min/1.73 m^2 [43]. In dialysis patients, the blood concentration of Mg^{2+} is dependent on the dialysate Mg^{2+} concentrations [39]. The mean serum Mg^{2+} concentration was in the high–normal range of normal blood Mg^{2+} concentrations (0.98 mmol/L) in a European cohort of haemodialysis patients [4]. However, on an individual level, hypomagnesaemia and hypomagnesaemia may be present depending on the patient's status and disease severity, possibly depending on dietary intake [44].

Hypomagnesaemia is also common in dialysis patients. A relatively low dialysate Mg^{2+} concentration of 0.5 mmol/L is most frequently used [45]. In 34 Dutch haemodialysis patients, the mean plasma Mg^{2+} concentration was 0.88 mmol/L prior to dialysis, which declined post-dialysis to a mean value of 0.78 mmol/L when the dialysate Mg^{2+} was 0.5 mmol/L [46]. The Mg^{2+} concentration declined in most individuals. Obviously a dialysate concentration of 0.25 mmol/L Mg^{2+} increases the risk for hypomagnesaemia even more [39]. In CKD, blood Mg^{2+} concentrations are further affected by the use of medication (e.g. proton pump inhibitors, thiazides and loop diuretics), the prescription of diets low in potassium (food products rich in potassium are generally also rich in Mg^{2+}) and the presence of diabetes [47]. Monitoring and adjusting the blood Mg^{2+} concentration in CKD patients might be relevant, as hypomagnesaemia is associated with progression to end-stage renal disease in diabetic nephropathy patients [48, 49].

Bone serves as the body's Mg^{2+} store. In total, 60% of the total body Mg^{2+} is embedded at the surface of the hydroxyapatite crystals [50]. In bone, Mg^{2+} directly contributes to healthy bone growth. In animal models with Mg^{2+} deficiency, hydroxyapatite crystals grow tighter and more brittle, resulting in less flexible bone that is more prone to fracture [51]. Multiple studies suggest effects of Mg^{2+} on bone cell function and fate.

However, a consensus is lacking, as results have indicated that Mg^{2+} both promotes and impairs osteoblastogenesis and osteoclastogenesis in different *in vitro* studies [52–55]. A recent study showed that mild hypomagnesaemia is associated with a lower risk to bone fracture in late-stage CKD patients [56]. However, too highly elevated blood Mg^{2+} concentrations may compromise bone mineralization, as Mg^{2+} is known to interfere with hydroxyapatite formation [57, 58]. Triggered by ageing and uraemia, bone abnormalities such as osteoporosis, osteomalacia and other mineralization defects are common in CKD [59]. As such, properly maintaining Mg^{2+} status may be important for the already compromised health of bones.

PATHOGENESIS OF VASCULAR CALCIFICATION AND THE ROLE OF CALCIPROTEIN PARTICLES

Medial calcification, as observed in CKD patients, was originally thought to be the result of passive precipitation and deposition of accumulating blood Ca^{2+} and inorganic Pi in the vessel wall. Almost two decades ago, genes and proteins normally restricted to bone tissue were found to be expressed in calcified arteries of CKD patients [60]. This finding led to the premise that vascular calcification is an active cell-orchestrated process by transdifferentiation of vascular smooth muscle cells (VSMCs) into osteoblast-like cells. VMSCs are triggered by the uremic milieu, well established for Pi and indoxyl sulphate, to change their transcriptional repertoire and lose their contractile phenotype [61]. These transformed VSMCs are characterized by the expression of genes such as Runt-related transcription factor 2 (*RUNX2*), bone morphogenetic proteins (*BMP*), osteopontin (OPN encoded by *SPP1*) and osterix (OSX encoded by *SP7*), as well as higher alkaline phosphatase (ALP) activity [62, 63], thereby resembling osteoblasts. These cells orchestrate the local spread of vascular calcification by increasing extracellular matrix synthesis and releasing Ca^{2+}-rich exosomes [64]. Experimental evidence strongly suggests that these processes are directly initiated at least partially by excess extracellular Pi and its increased cellular uptake [65]. Interestingly, the results of other studies suggest that VSMC transdifferentiation is a consequence rather than a cause of the vascular calcification process. For example, *in vitro* studies suggest that VSMC transdifferentiation may be induced primarily by formation of calciprotein particles or nanocrystals in the extracellular space [66] and exosome release facilitating Ca–Pi crystal nucleation [64], DNA damage and cellular senescence [67].

Calciprotein particle formation mediate phosphate-induced calcification

For some years it has been established that elevated blood Pi concentrations give rise to colloidal particles in the circulation [68]. In healthy individuals, despite supersaturated concentrations of soluble Ca^{2+} and Pi in the circulation, the step from soluble Pi towards amorphous Ca–Pi rarely occurs, due to the presence of inhibitors (e.g. fetuin-A, albumin, osteopontin). In CKD, the Pi concentration increases even more and the expression of inhibitory proteins or colloidal components of these particles, such as fetuin A, decreases [69]. Therefore in the circulation of CKD patients, this protective mineral-buffering system is compromised [70]. When this occurs, Pi, Ca^{2+} and serum proteins aggregate to form amorphous, soluble Ca–Pi particles or primary calciprotein particles (CPP1). Depending on the local milieu, CPP1 mature to crystalline secondary calciprotein particles (CPP2). The maturation time of CPP1–CPP2, which is measured *ex vivo* in serum samples in the recently developed T_{50} test, has been proposed as a measure for the calcification propensity in individuals [71].

CPP2 have been identified in the circulation of CKD patients and their presence is associated with aortic stiffness [72–74]. CPP2, in contrast to CPP1, have been shown to directly induce VSMC calcification in cultured VSMCs [75]. Only nanocrystals and CPP2, but not Pi in solution, induced upregulation of osteogenic proteins in VSMCs [66, 76]. Accordingly, vascular calcification can conceptually be prevented by inhibiting hydroxyapatite formation despite persistently high extracellular Pi concentrations in VSMCs [77]. Of note, it remains unknown whether the CPP2 concentrations used *in vitro* are comparable to concentrations circulating in CKD patients. Obviously the exposure time *in vitro* is shorter than in *in vivo* conditions. Altogether, these findings suggest that not Pi as such, but rather the formation of CPP2, is the true culprit in vascular calcification initiation and progression [70, 78]. Studies in animal models and patients are required to further confirm the causal role of CPP in vascular calcification.

VASCULAR CALCIFICATION IN CKD AND THE ROLE OF MAGNESIUM: EXPERIMENTAL STUDIES

Since a higher serum Mg^{2+} concentration is associated with improved cardiovascular morbidity and mortality in CKD and dialysis, the potentially preventive effect of Mg^{2+} has been investigated in a multitude of experimental models. In cellular and rodent models, Mg^{2+} supplementation prevented high Pi-induced vascular calcification. Mg^{2+} supplementation to Pi and Ca^{2+}– enriched culture medium effectively suppressed calcification in human, bovine and rat VSMCs *in vitro* [79–81]. Moreover, Mg^{2+} prevented calcification in rat aortic rings treated with high Pi and in a rat model of CKD [82, 83]. Additionally, vascular calcification in Klotho knock-out mice was abrogated by a high-Mg^{2+} diet [84]. A note of caution, Mg^{2+} may increase the risk for osteomalacia in CKD by disturbing bone mineralization when used in too high concentrations or during development [45, 58, 84]. Although longer-term studies are warranted, a 28-day increase (1.0 versus 0.5 mmol/L) in Mg^{2+} concentration in dialysate did not lead to a change of bone turnover markers [85].

The anti-calcifying properties of Mg^{2+} are operational at the medial layer of arteries [86, 87]. A substantial body of studies has investigated potential mechanisms underlying these protective properties. Recent insights regarding the impact of Mg^{2+} on CPP maturation expanded the spectrum of mechanisms that underlie the anti-calcifying properties of Mg^{2+} (Figure 1).

Influence of magnesium on VSMC transdifferentiation

As discussed in a previous section, VSMC transdifferentiation towards an osteoblast-like cell has often been suggested to

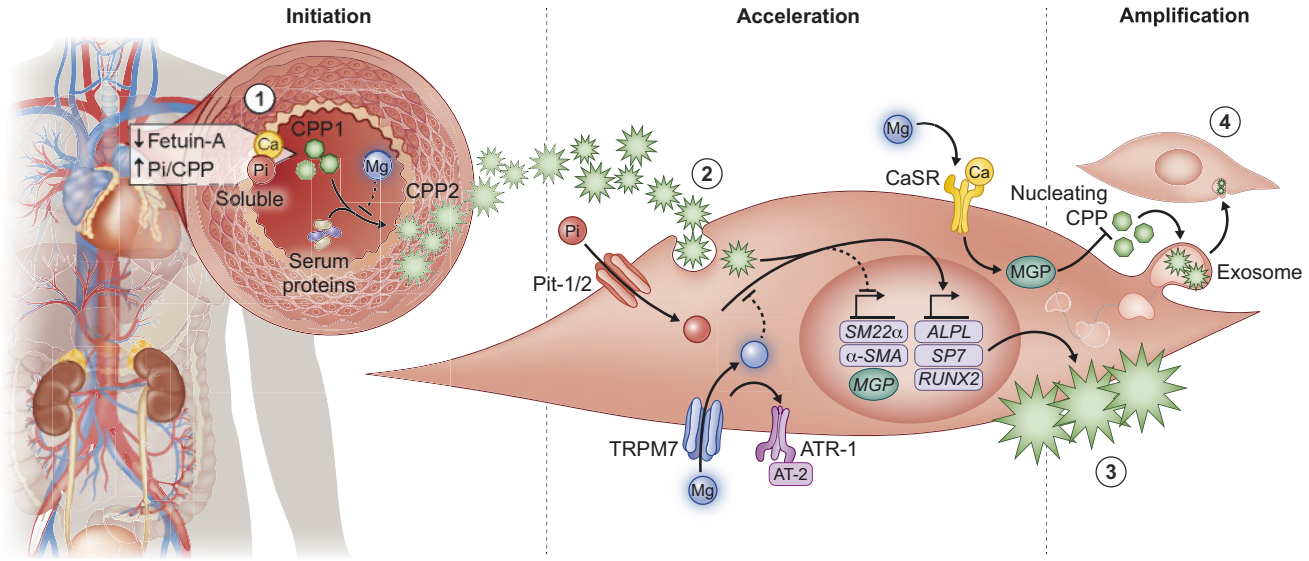

FIGURE 1: Overview of the mechanisms by which magnesium prevents vascular calcification.
1 – High Pi concentrations and the absence of circulating inhibitors such as fetuin-A stimulate the formation of CPP1 in the circulation of CKD patients. Subsequently CPP1 transitions into CPP2 due to a lack of circulating crystallization inhibitors. **2** – *In vitro* studies suggest that CPP2 and Pi induce calcification in VSMCs and stimulate expression of pro-calcification genes such as *RUNX2*, ALP (*ALPL*) and osterix (*SP7*). Simultaneously, contractility genes such as transgelin (SM22α) diminish. **3** – Combined, this cascade results in VSMC transdifferentiation and calcification and loss of VSMC function and contractility. **4** – The resulting calcified VSMCs amplify the calcification process by shedding Ca-loaded exosomes that are engulfed by neighbouring VSMCs. Mg^{2+} is proposed to interfere with the calcification process on multiple levels. First, Mg^{2+} inhibits the transition from CPP1 towards CPP2, preventing VSMC calcification. Second, increased Mg^{2+} entry facilitated by TRPM7 and possibly angiotensin receptor 2 (AT-2) may directly interfere with Pi-mediated VSMC osteoblast-like transdifferentiation. Third, Mg^{2+} may restore CaSR activity and MGP γ-carboxylation or protein expression.

be the driving event of vascular calcification, manifestation and progression [88]. Consequently the leading hypothesis has been that Mg^{2+} prevents vascular calcification primarily by downregulating pathways involved in the transcription of osteogenic genes. A large number of studies have reported that Mg^{2+} directly downregulates pro-calcification genes and proteins, among others *RUNX2*, *BMP2* and *OSX* (reviewed in detail in [79, 81, 89–92]). Other studies report that Mg^{2+} prevents VSMC calcification through restoring activity of the Ca^{2+}-sensing receptor (CaSR), important for matrix Gla protein (MGP) synthesis [93]. An increase in MGP after Mg^{2+} supplementation has been reported previously [92]. As such, Mg^{2+} may increase the abundance of calcification inhibitors. Although these results are often interpreted as direct effects of Mg^{2+} on gene transcription, it is important to note that the experimental setups are often insufficient to distinguish intracellular from extracellular modes of action. In general, these experiments rely on increasing Pi concentrations in the experimental culture media, which will result in CPP formation in the culture medium. Consequently the effects of Mg^{2+} may be intracellular, or may depend on extracellular inhibition of CPP maturation, preceding osteogenic transdifferentation [76]. In these setups, measurements of osteoblast-like gene or protein expression is therefore insufficient to draw firm conclusions on the mechanisms involved.

Several studies provide more advanced approaches that support an intracellular role of Mg^{2+} in the prevention of VSMC transdifferentiation. Knock-down or inhibition of the divalent cation channel transient receptor potential melastatin 7 (TRPM7) impaired the preventive effect of Mg^{2+} on VSMC calcification [94]. Since TRPM7 is the major Mg^{2+} channel in VSMCs, these findings demonstrate that inhibition of osteogenic transcription depends on intracellular Mg^{2+} levels. Several studies indicate that TRPM7 is required for the anti-calcifying effects of Mg^{2+} in VSMCs. In rat VSMCs, 2-aminoethoxydiphenyl borate (2-APB), a non-selective TRPM7 blocker, abrogated the protective effects of Mg^{2+} in human high Pi–treated VSMCs supplemented with Mg^{2+} [91]. Similar results were reported by another study using 2-APB in human VSMCs [80]. In addition, treatment of human VSMCs with a small interfering RNA directed at TRPM7 resulted in calcification despite Mg^{2+} supplementation [92]. Combined, these studies demonstrate the need for intracellular Mg^{2+} to limit VSMC calcification. Interestingly, other studies have shown that TRPM7 and increased Mg^{2+} influx may mediate the anti-calcifying properties of angiotensin type 2 [95].

However, the obligate involvement of TRPM7 remains uncertain, as results from our group demonstrated that Mg^{2+} actually did prevent bovine VSMC calcification even when TRPM7 was blocked using the channel inhibitor 2-APB [77]. While similar concentrations were used, the use of bovine VSMCs rather than human VSMCs might explain these contradictory findings. Importantly, in most studies it was not accurately assessed whether Mg^{2+} entry had been modified by TRPM7 blocking, as no validated intracellular Mg^{2+} probes or patch-clamp techniques were used. Other studies may suggest

an alternative role for TRPM7 in VSMC calcification. Its activation increased after treating VSMCs with interleukin-18, a cytokine that stimulated VSMC calcification effectively *in vitro* [96]. Taken together, the impact of intracellular Mg^{2+} on VSMC gene transcription should be studied in more detail by using validated intracellular Mg^{2+} probes and more specific methods of TRPM7 blocking.

Influence of magnesium on calciprotein particle formation

In light of recent data demonstrating that CPP2 are important drivers of VSMC transdifferentiation and vascular calcification, the model describing a major role for Mg^{2+} on intracellular pro-calcifying pathways might be incomplete [66, 75]. The formation of CPP2 may precede transdifferentiation of VSMCs into an osteoblast-like phenotype. Consequently the inhibition of CPP2 maturation by Mg^{2+} will then result in reduced osteogenic gene expression and maintain VSMCs in their contractile phenotype.

The effect of Mg^{2+} on hydroxyapatite formation was widely studied in the 1970s. Although not in the context of CKD, the interference on Ca–Pi crystallization by Mg^{2+} has been established in several experimental studies [97–100]. First, Mg^{2+} can substitute Ca^{2+} in hydroxyapatite formation, favouring the formation of Mg^{2+}-containing whitlockite instead [101–103]. Indeed, whitlockite is sometimes observed in calcified arteries of CKD patients and uraemic rats and has been found to be less pathogenic compared with hydroxyapatite [104–107]. Second, Mg^{2+} may prevent the formation of hydroxyapatite altogether [77]. In aqueous solution, Mg^{2+} shields amorphous Ca–Pi particles from transition into crystalline mature hydroxyapatite, thus stabilizing the amorphous phase, which is likely to be applicable during CPP2 formation in the serum [97].

The importance of these mechanisms in the context of CKD-induced vascular calcification was recently tested by our group and by others. Interestingly, Mg^{2+} did not inhibit initial CPP1 formation from supersaturated concentrations of Ca^{2+} and Pi but prevented maturation into crystalline CPP2 [76, 98, 99, 108]. Once CPP2 was formed, Mg^{2+} was incapable of preventing CPP2-induced VSMC mineralization and osteogenic protein expression [76]. Therefore these findings suggest that Mg^{2+}-dependent inhibition of CPP2 maturation is instrumental in the prevention of VSMC calcification (Figure 2). The potential strength of the stabilizing effect of Mg^{2+} after CPP1 formation is illustrated by the observation that nucleation of amorphous Ca–Pi in aqueous solution is delayed by 2 h after the addition of 57 µmol/L Mg^{2+} [100]. Given that the normal physiological range of blood Mg^{2+} is 0.7–1.1 mmol/L, even minor differences in Mg^{2+} availability may greatly affect the formation of CPP2 from Ca–Pi particles *in vivo*. In patient sera, supplemental Mg^{2+} from concentrations as low as 0.2 mmol/L (the average Mg^{2+} concentration of the serum pools was 0.83 mmol/L) dose-dependently reduced serum calcification propensity [71, 76]. Of note, the required concentration of Mg^{2+} may depend on the exact composition of the calcifying milieu. Indeed, others reported previously that Mg^{2+} did not inhibit hydroxyapatite formation during VSMC calcification using high Pi concentrations in the medium [109]. Interestingly, a recent study reported that increasing dialysate Mg^{2+} for 28 days resulted in a 21% reduction of CPP1 and a 68% reduction of CPP2 [85]. These results suggest that Mg^{2+} not only inhibits the formation of

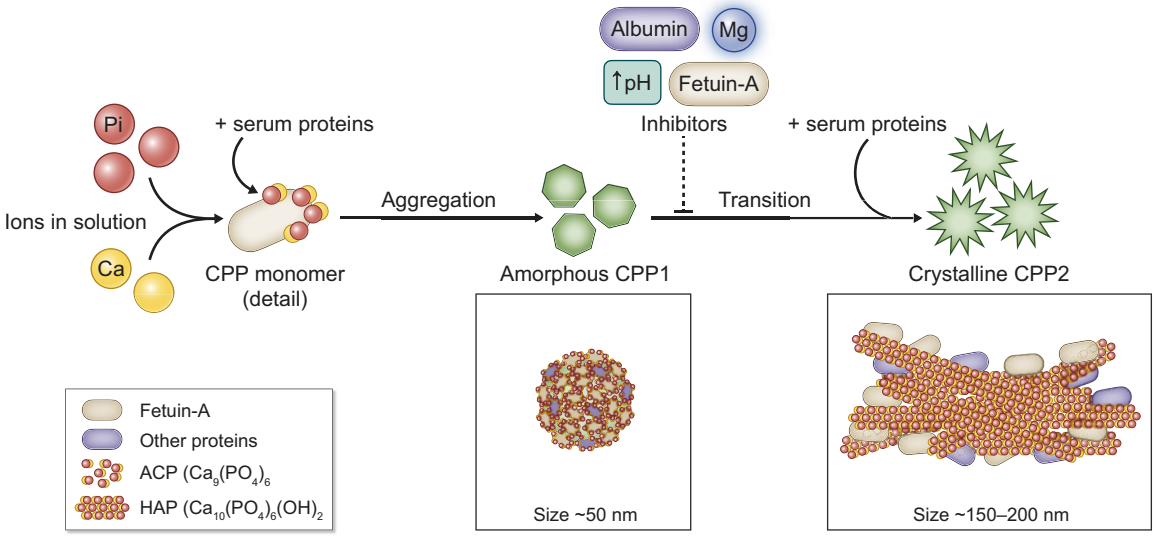

FIGURE 2: Close-up of calciprotein particle formation and phase transition.
Soluble Pi and Ca^{2+} in combination with fetuin-A and other serum proteins can aggregate into calciprotein monomers and subsequently these monomers cluster in CPP1 [110]. CPP1 are composed of complexes of fetuin-A and other serum proteins and aggregated amorphous Ca–Pi particles. CPP1 are approximately 50 nm in size and kept stable by serum components. While being initially harmless, the disbalance between calcification promotors (Ca^{2+}, Pi) and inhibitors (fetuin-A, albumin, Mg^{2+} and alkaline pH) in CKD promote further maturation and transition of CPP1 into crystalline CPP2. CPP2 are larger (150–200 nm) and consist of a crystalline hydroxyapatite core in a protein-covered shell. Mg^{2+} prevents the maturation of CPP1 into CPP2 but not initial CPP1 formation.

CPP2 formation, but also CPP1, and translates to patients, lowering the overall CPP burden in CKD.

In this context, it is important to realize that intracellular transcriptional effects of Mg^{2+} and extracellular Mg^{2+} effects on CPP maturation are not mutually exclusive. Both mechanisms may contribute to reduced vascular calcification in CKD.

A CLINICAL PERSPECTIVE OF MODIFYING MAGNESIUM BALANCE

As CPP formation mainly depends on Ca^{2+}, Pi and fetuin-A concentrations, CPP may explain Pi-stimulated vascular calcification in CKD and thus mediate Pi-induced VSMC calcification. Consequently these pathological effects of Pi may be reduced by preventing CPP2 formation by Mg^{2+}. Indeed, many experimental vascular calcification inhibitors were shown to delay CPP2 maturation [71]. The introduction of the calcification propensity test (T_{50} test), which measures the transition time from CPP1 to CPP2 *ex vivo* in serum samples, has created an opportunity to quantify the mineral-buffering capacity of CKD patients to withstand vascular calcification (Figure 3) [71]. A higher calcification propensity (T_{50}) is associated with mortality and the presence and severity of vascular calcification in patients with CKD [111, 112]. Factors reducing calcification propensity are of interest as potential treatment options for progressive vascular calcification.

The identification of Mg^{2+} as a targetable factor to modify CPP2 maturation provides an interesting clinical tool for vascular calcification in CKD. Increasing dialysate Mg^{2+} concentration from 0.5 to 1.0 mmol/L led to an increase in serum Mg^{2+} concentration of 0.34 mmol/L and improved T_{50} significantly [113]. In another study, *ex vivo* addition of Mg^{2+} to serum samples of pre-transplant CKD patients, dose-dependently improved T_{50} [76]. In 2019, a clinical trial was published in which MgO was supplemented in 96 pre-dialysis CKD patients, leading to decreased progression of coronary artery calcification, although no effect was seen on thoracic aorta calcification [34]. Now the stage is set for clinical trials in patients with CKD and on dialysis to assess the effectiveness and safety of Mg^{2+} supplementation in CKD patients on clinically relevant endpoints. However, proper monitoring of efficacy is challenging because establishing differences in clinically relevant endpoints requires long-term follow-up of a substantial number of study participants. It is tempting to assume that, for instance, changes in the T_{50} antedate changes in these clinical endpoints. While T_{50} is an informative indicator of calcification propensity in CKD, it is of importance to determine whether Mg^{2+} supplementation ultimately results in lower calcification scores and its associated clinical events in CKD patients.

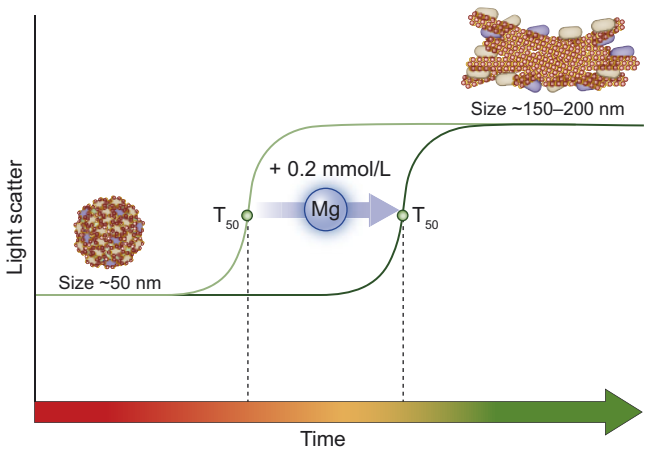

FIGURE 3: T_{50} as a measure of calcification propensity and effects of magnesium.
The formation of colloidal CPP1 is caused by an imbalance between blood Pi concentrations and calcification inhibitors. The transition time from CPP1 to CPP2, referred to as T_{50}, can be monitored by time-resolved nephelometry and is a measure for the mineral-buffering system of the serum and the calcification propensity. Mg^{2+} increments of 0.2 mmol/L [76] delay the transition from CPP1 to CPP2, increases T_{50} and therefore reduces calcification propensity in the serum of CKD patients [71].

CONCLUDING REMARKS

Mg^{2+} supplementation is a promising treatment option for vascular calcification. Inhibition of CPP2 maturation may be the key molecular mechanism that explains the prevention of vascular calcification by Mg^{2+}, but beneficial effects on gene expression profiles of VSMCs may operate in parallel. As such, Mg^{2+} is an essential factor in the calcification milieu, which helps to restore the mineral-buffering system overwhelmed by Pi in CKD patients. Consequently the optimal serum Mg^{2+} concentration for patients with CKD may be higher than in the general population. Future studies are warranted to determine a safe and effective reference range for serum Mg^{2+} concentrations in CKD patients.

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CONFLICT OF INTEREST STATEMENT

M.G.V. has received lecture fees and scientific support from Amgen, Vifor Fresenius Medical Care Renal Pharma, Kyowa Kirin, Medice, AbbVie and Fresenius Medical Care. He is an advisor for Amgen, Vifor Fresenius Medical Care Renal Pharma, Kyowa Kirin and Medice and serves on the advisory board of Otsuka. M.G.V. is also a member of the European Renal Association–European Dialysis and Transplantation Association working group on CKD-MBD and a member of the Kidney Disease: Improving Global Outcomes committee on CKD-MBD. All the other authors have declared no competing interests.

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