Osteopontin protects against high phosphate-induced nephrocalcinosis and vascular calcification

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Pathologic calcification is a significant cause of increased morbidity and mortality in patients with chronic kidney disease. The precise mechanisms of ectopic calcification are not fully elucidated, but it is known to be caused by an imbalance of procalcific and anticalcific factors. In the chronic kidney disease population, an elevated phosphate burden is both highly prevalent and a known risk factor for ectopic calcification. Here we tested whether osteopontin, an inhibitor of calcification, protects against high phosphate load-induced nephrocalcinosis and vascular calcification. Osteopontin knockout mice were placed on a high phosphate diet for 11 weeks. Osteopontin deficiency together with phosphate overload caused uremia, nephrocalcinosis characterized by substantial renal tubular and interstitial calcium deposition, and marked vascular calcification when compared with control mice. Although the osteopontin-deficient mice did not exhibit hypercalcemia or hyperphosphatemia, they did show abnormalities in the mineral metabolism hormone fibroblast growth factor-23. Thus, endogenous osteopontin plays a critical role in the prevention of phosphate-induced nephrocalcinosis and vascular calcification in response to high phosphate load. A better understanding of osteopontin's role in phosphate-induced calcification will hopefully lead to better biomarkers and therapies for this disease, especially in patients with chronic kidney disease and other at-risk populations.

Kidney International (2016) ■, ■–■; http://dx.doi.org/10.1016/j.kint.2015.12.046

KEYWORDS: calcium; fibroblast growth factor-23; parathyroid hormone; phosphate; uremia; vascular calcification

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Received 11 May 2015; revised 8 December 2015; accepted 30 December 2015

Pathologic calcification of soft tissues has deleterious effects depending on the organ system involved. Nephrocalcinosis is the deposition of calcium within the renal parenchyma and tubules. This accumulation of calcium in the kidney is due to increased urinary excretion of calcium, phosphorus, and/or oxalate in combination with a loss of protective urinary inhibitors of mineralization. There are many underlying diseases that cause nephrocalcinosis, and depending on the etiology, nephrocalcinosis can cause progressive renal dysfunction and end-stage renal disease.[1] Similar to nephrocalcinosis, vascular calcification (VC) is caused by an imbalance in procalcific and anticalcific factors. In the vasculature, calcification is associated with increased cardiovascular morbidity and mortality.[2,3] Whereas VC is seen as a component of aging, the increased cardiac risk is especially prevalent in certain disease states such as diabetes mellitus and chronic kidney disease (CKD).[4–6] VC can involve either the intimal or the medial layer of the arterial wall. Intimal calcification is typically secondary to atherosclerosis, whereas medial calcification is much more characteristic of patients with diabetes mellitus and CKD.[7–10] Once thought to be a benign finding, arterial medial calcification is associated with increased vessel wall stiffness, decreased vascular compliance, increased pulse wave velocity, and systolic hypertension.[11,12] Together these symptoms lead to decreased coronary perfusion, left ventricular hypertrophy, and eventually to congestive heart failure or myocardial infarction.[13] The mainstay of VC therapy involves attempting to maintain mineral homeostasis with the use of oral phosphate binders, activated vitamin D or vitamin D analogs, and calcimimetics, but at present there is no effective treatment for this disease.

An elevated phosphate load is a known risk factor for the development of both renal and vascular calcification. Administration of high doses of phosphate to rodents causes nephrocalcinosis and significant renal damage.[14,15] A higher phosphate diet leads to urinary calcium phosphate supersaturation and an increase in calcium phosphate kidney stones in settings of genetic hypercalciuria.[16] In humans, elevated phosphate loading from colonoscopy preps or tumor lysis syndrome causes phosphate nephropathy and acute kidney injury, which is now known to be a major risk factor for the development of CKD.[17–19] In uremic animal models, phosphate loading is known to cause VC and in patients with preexisting CKD, hyperphosphatemia is a significant risk factor

for VC and for cardiovascular mortality.[20–22] Even in patients without CKD, elevated serum phosphate levels are associated with increased cardiovascular risk.[23] This is particularly concerning as the phosphorus content of the U.S. food supply continues to increase and correlates with the rising use of phosphate additives, which are mostly inorganic phosphate salts with rapid intestinal absorption and high bioavailability.[24]

The underlying mechanisms that lead to pathologic calcification are complex and thought to involve active, regulated processes that are common to bone formation. Loss of anticalcific factors is considered a critical element contributing to both bone mineralization and pathologic calcification.[25] One important anticalcific protein is osteopontin (OPN). OPN, a multifunctional phosphoprotein and member of the SIBLING (small integrin-binding ligand N-linked glycoproteins) family, was initially discovered as a major noncollagenous component of cortical bone.[26] OPN is produced by multiple cell types in bone and plays an integral role in bone remodeling largely due to its function in osteoclast adhesion and bone resorption.[27] Osteopontin is rich in aspartic acid, glutamic acid, and a polyaspartic acid motif, and it can be highly phosphorylated at serine and threonines, all of which allows it to bind to both ionic calcium and to hydroxyapatite, making it a potent inhibitor of calcification.[28–30] As OPN is a multifunctional protein, it has been studied in a wide range of disease models including cancer, osteoporosis, liver disease, and infectious diseases, but despite the large number of studies utilizing OPN-deficient mice, spontaneous vascular calcification has never been described in these animals.

Another important molecule involved in mineralization is fibroblast growth factor-23 (FGF-23), which is primarily produced by osteocytes and functions to maintain phosphorus homeostasis in the body.[31] Increased phosphorus and 1,25-dihydroxy vitamin D (1,25 $(OH)_2D$) stimulate FGF-23 release from osteocytes under the control of PHEX and DMP1 proteins.[32] Intact FGF-23 binds with its coreceptor α-klotho, which is secreted by renal tubular cells, and this complex activates FGF receptors in the renal proximal tubule and reduces expression of the type II sodium/phosphate cotransporters (NaPi2).[33] In this fashion, FGF-23 prevents hyperphosphatemia by decreasing urinary phosphate reabsorption. This effect is recognized prominently in CKD, where FGF-23 levels increase to counter decreased glomerular filtration of phosphorus as glomerular filtration rate declines.[34] FGF-23 also inhibits CYP27B1 (25-hydroxyvitamin D 1α hydroxylase) decreasing the production of 1,25 $(OH)_2D$ and stimulates CYP24A1 (1,25-dihydroxyvitamin D 24-hydroxylase) increasing the conversion of 1,25 $(OH)_2D$ into inactive metabolites.[35]

The aim of the present study was to determine whether endogenous OPN has a protective role in renal and vascular calcification in the setting of a high phosphate load. To test this, OPN knockout mice were exposed to a high phosphate burden by placement on a high phosphate diet. We discovered that these mice developed both extensive renal and vascular calcification as well as aberrations in serum FGF-23 levels compared with wild-type mice. The data suggest that OPN has a major renal and vascular protective effect against ectopic calcification induced by high phosphate loading.

RESULTS

Renal insufficiency and altered mineral metabolism in high phosphate–fed OPN KO mice

Serum chemistries were determined at termination and are shown in Table 1. Blood urea nitrogen levels of the wild type (WT) + normal phosphate (NP) and knockout (KO) + NP groups were not significantly different. High phosphate (HP) feeding alone did not lead to significant increases in blood urea nitrogen in WT mice (compare WT+NP group with the WT+HP group). In contrast, the KO+HP group had elevated blood urea nitrogen (36 mg/dl), which was significantly higher when compared with all other groups, demonstrating renal insufficiency in these mice. The only difference in serum calcium levels was between the KO+NP and KO+HP groups, with the KO+HP group demonstrating lower calcium levels than the KO+NP group. Serum phosphorus levels were lower in both HP fed mice compared with their genotypic equivalent NP fed mice. There were no differences in phosphorus levels when comparing mice between genotypes given the identical dietary phosphate load. Parathyroid hormone (PTH) levels in the KO+HP group were significantly higher than in the WT+NP group, but there were no other differences between groups. FGF-23 levels were significantly elevated in the KO+HP group compared with all other groups. It is noteworthy that FGF-23 levels were significantly higher in the KO+HP group compared with the WT+HP group, and, although not reaching statistical significance, FGF-23 levels trended higher in the KO+NP group compared with the WT+NP group.

OPN-null mice on high phosphate diet develop renal and vascular calcification

Renal histology and immunofluorescence (IF) were performed to characterize renal morphology in all groups. Hematoxylin and eosin (H&E) staining of the kidneys was normal in both WT+NP and KO+NP groups. The WT+HP group demonstrated some abnormalities including mild interstitial inflammation and some subcapsular inflammation. This was more pronounced in the KO+HP group with marked presence of inflammatory cells surrounding tubular mineral deposits and some of the more cystic, dilated glomeruli (Figure 1a–d). Tubular mineral deposits were also frequently seen on the KO+HP group with H&E staining (Figure 1d, arrowhead). Periodic acid–Schiff staining demonstrated normal morphology in both NP groups. The WT+HP group showed occasional tubular dilatation, but this again was more prominent in the KO+HP group, which contained many dilated and degenerative tubules along with some cystic-appearing glomeruli (Figure 1e-h). Occasional tubular mineral deposits were also noted in the WT+HP