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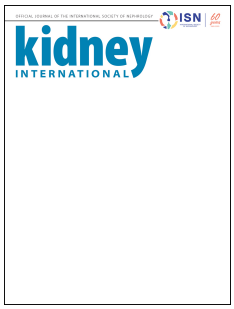

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Translational Science

Kidney toxicity of phosphate: is that crystal clear yet?

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Hyperphosphatemia is a well-known feature of advanced chronic kidney disease (CKD) and a major risk factor for cardiovascular events in patients with CKD, inducing calcium phosphate supersaturation and vascular calcifications.[1] Although the serum phosphate level is not recognized as an independent risk factor for CKD progression, increased urinary phosphate excretion has been associated with CKD progression in small cohort studies.[2,3] In experimental studies, exposure to a high phosphate diet has been shown to accelerate kidney damage in rats with reduced nephron number induced by partial nephrectomy.[4] Prior studies have not considered the role of fibroblast growth factor 23 (FGF23), which inhibits phosphate reabsorption by renal proximal tubules. FGF23 secretion is increased in CKD, which in turn increases phosphate concentration in the proximal tubular fluid. In a recent report in the *Journal of Clinical Investigation*, Shiizaki et al. hypothesized that increased phosphate concentration in the tubular fluid might be harmful for the kidney, and that patients with CKD might be particularly at risk due to the combination of reduced nephron number and increased FGF23 secretion.[5]

What did the study show?

Shiizaki et al. began their study with *in vitro* experiments, exposing HK-2 renal proximal tubular cells to increasing concentrations of extracellular phosphate, from 1.0 mM (control) to 7.0 mM, in the presence of 3.0 mM calcium. Increased phosphate concentrations induced a decrease in cell viability and the formation of calcium phosphate microparticles. Complementary experiments confirmed that cell toxicity was mediated by the particles and not by phosphate concentration itself. Next, they injected a fluorescent probe that binds to calcium phosphate crystallites in mice fed a high phosphate diet. They observed that the calcium phosphate particles adhered to the apical membrane of proximal tubular cells *in vivo*, and that this was associated with kidney inflammation and fibrosis. Acidification of urine decreased microparticle formation and kidney lesions, whereas urine alkalinization exacerbated kidney damage by increasing calcium phosphate precipitation. Shiizaki et al. further observed that Toll-like receptor-4 (TLR-4) expressed in tubular cells is involved in calcium phosphate microparticle tethering. *In vitro* exposure of HK-2 cells to calcium

phosphate particles induced endocytosis of these crystallites, disturbed endosomal trafficking, and activated inflammatory pathways.

The authors then exposed mice with or without unilateral nephrectomy to various phosphate-enriched diets to identify the threshold of tubular fluid phosphate concentration above which tubular damage would occur. Because the direct measurement of phosphate concentration in proximal tubular fluid is technically challenging, they used an estimation that was shown to correlate with direct measurement by micropuncture in a small number of animals. They defined a threshold of estimated tubular fluid phosphate concentration above which both circulating FGF23 and markers of tubular damage increased in parallel. In further *in vivo* experiments, the authors observed a decreasing number of nephrons (indirect estimation) with increasing phosphate excretion per nephron, suggesting that high phosphate load may cause nephron loss. Finally, the authors observed an independent relationship between FGF23 levels and CKD progression in a patient cohort, a finding compatible with the hypothesis that high FGF23-induced phosphate excretion promotes kidney damage.

Why is this study important?

This comprehensive set of experiments shows that an increase in the phosphate concentration of proximal tubular fluid accelerates nephron loss in various CKD models. Moreover, Shiizaki et al. provide original results regarding the role of calcium phosphate microcrystals that accumulate in proximal tubular cells, disturb endosomal trafficking, and eventually induce cell damage, inflammation, tubulo-interstitial fibrosis, and progression of CKD. Until recently, little attention has been paid to a possible effect of phosphate itself on tubular cells. Renal tubular calcium phosphate crystallization has been described in acute settings when phosphate delivery to the renal tubule is dramatically increased, for instance after phosphate-containing bowel prep for colonoscopy.[6] Calcium phosphate crystals have been previously shown to be involved in tubular lesions, and calcium phosphate tubular plugs may be associated with an unfavorable renal prognosis in kidney transplant patients.[7,8] In patients with CKD, prior studies have shown that urine phosphate excretion is associated with disease progression, but the potential contribution of FGF23 was not assessed.[2,3] The experimental

and clinical findings by Shiizaki et al. should prompt further clinical studies. Whether calcium and phosphate concentrations as high as those used in the *in vitro* experiments are reached in proximal tubule fluid *in vivo*, and whether intratubular calcium phosphate particles disrupt proximal tubular cell endosomal functions in patients with CKD remains to be seen. The demonstration that serum FGF23 levels are associated with progression of CKD is not definitive proof that the progression is causally linked to increased urine phosphate concentration. Serum FGF23 is tightly associated with glomerular filtration rate (GFR), which is the most potent risk factor for further decline in kidney function. FGF23 may also exert direct harmful effects on various tissues.[9]

Calcium phosphate supersaturation and crystallization depend not only on urine phosphate concentration, but also on urinary calcium concentration, pH, and various calcification inhibitors. Increases in serum parathyroid hormone levels can modify these parameters, an issue not addressed in the present paper. The dramatic decrease in urine calcium excretion observed in advanced stages of CKD could in theory counteract, at least partly, the risk of calcium phosphate microparticle formation in tubular fluid. The findings by Shiizaki et al. advocate for further studies, including detailed assessment of phosphate concentration and calcium phosphate supersaturation in the tubular fluid, together with FGF23 and parathyroid hormone assays, in longitudinal studies dedicated to CKD progression.

Modifications in urine pH and electrolyte concentrations in distal parts of the tubule may also play an important role. In the experimental models developed by Shiizaki et al., urine alkalinization worsened kidney function. This observation raises questions regarding the current clinical practice of bicarbonate administration to manage metabolic acidosis, particularly in patients with high phosphate load. Secondary analyses of prior trials of bicarbonate administration could potentially explore the association between urinary phosphate excretion and eGFR decline. Future studies should consider the relevance of these findings to other conditions with high urinary phosphate and bicarbonate excretion, such as proximal tubulopathy induced by light chain deposition or medication toxicity. Finally, it might also be interesting to use imaging techniques in order to directly visualize the induction of microcrystal formation in the proximal renal tubule by phosphate loading *in vivo*.

References

1. Adeney KL, Siscovick DS, Ix JH, et al. Association of Serum Phosphate with Vascular and Valvular Calcification in Moderate CKD. *JASN*. 2009;20(2):381-387. doi:10.1681/ASN.2008040349

2. Kawasaki T, Maeda Y, Matsuki H, Matsumoto Y, Akazawa M, Kuyama T. Urinary phosphorus excretion per creatinine clearance as a prognostic marker for progression of chronic kidney disease: a retrospective cohort study. *BMC Nephrol*. 2015;16:116. doi:10.1186/s12882-015-0118-1

3. Santamaría R, Díaz-Tocados JM, Pendón-Ruiz de Mier MV, et al. Increased Phosphaturia Accelerates The Decline in Renal Function: A Search for Mechanisms. *Sci Rep*. 2018;8(1):13701. doi:10.1038/s41598-018-32065-2

4. Haut LL, Alfrey AC, Guggenheim S, Buddington B, Schrier N. Renal toxicity of phosphate in rats. *Kidney Int*. 1980;17(6):722-731. doi:10.1038/ki.1980.85

5. Shiizaki K, Tsubouchi A, Miura Y, et al. Calcium phosphate microcrystals in the renal tubular fluid accelerate chronic kidney disease progression. *J Clin Invest*. Published online June 29, 2021:145693. doi:10.1172/JCI145693

6. Ori Y, Herman M, Tobar A, et al. Acute phosphate nephropathy-an emerging threat. *Am J Med Sci*. 2008;336(4):309-314. doi:10.1097/MAJ.0b013e318167410c

7. Aihara K, Byer KJ, Khan SR. Calcium phosphate-induced renal epithelial injury and stone formation: involvement of reactive oxygen species. *Kidney Int*. 2003;64(4):1283-1291. doi:10.1046/j.1523-1755.2003.00226.x

8. Lionet A, Haeck M, Garstka A, et al. First investigation on microcrystalline pathologies of kidney allografts through cellular scale physicochemical techniques. *Comptes Rendus Chimie*. 2016;19(11):1542-1547. doi:10.1016/j.crci.2015.09.016

9. Musgrove J, Wolf M. Regulation and Effects of FGF23 in Chronic Kidney Disease. *Annu Rev Physiol*. 2020;82:365-390. doi:10.1146/annurev-physiol-021119-034650

Disclosure

The authors declared no competing interests.

Figure legend

Figure 1. Summary of the mechanisms potentially responsible for calcium phosphate microcrystal nephrotoxicity in chronic kidney disease (CKD). High-phosphate diet, reduced number of nephrons (CKD) and secretion of fibroblast growth factor 23 (FGF23) increase the concentration of inorganic phosphate (Pi) in primitive urine. Calcium phosphate supersaturation results in microcrystal formation. At the surface of proximal tubular cells, toll like receptor 4 (TLR4) binds calcium phosphate microparticles whose endocytosis may induce tubular damage, inflammation, fibrosis and eventually aggravate CKD.