Countering vascular calcification through mitochondrial phosphate transport inhibition.

Hyperphosphatemia is a condition characterized by abnormally high serum phosphate levels. It is common among individuals with chronic kidney disease (CKD), especially in those with severely reduced kidney function. In the earlier stages of CKD, blood phosphate levels can fluctuate but typically stay within a normal range, likely due to hormonal mechanisms mediating phosphate reabsorption. However, as kidney function declines these regulatory mechanisms become inadequate, resulting in elevated serum phosphate levels. This excess phosphate can trigger a cascade of events, including the secretion of substances by vascular smooth muscle cells (VSMCs) that leads to calcification. Calcification generally occurs in the blood vessels, heart valves, myocardium, and other soft tissues, contributing to the high prevalence of cardiovascular diseases (CVD) in individuals with CKD.

Mitochondria play a pivotal role in this process. Excessive phosphate uptake by mitochondria can disrupt their dysfunction, leading to oxidative stress and further exacerbating vascular calcification and damage to tissues and cells. Understanding how phosphate uptake and metabolism within mitochondria contribute to these processes is therefore essential to understanding the cellular mechanisms underlying CKD progression.

The role of mitochondrial phosphate transport in the pathogenesis of mitochondrial oxidative stress and vascular calcification is currently unclear. To explore this concept further, Nguyen and colleagues set out to investigate whether inhibiting mitochondrial phosphate transport could mitigate the detrimental intracellular signaling and vascular calcification induced by elevated phosphate levels.[1] The team employed an array of methodologies, including quantitative PCR, RNAi-mediated gene silencing, and micro-CT imaging, to shed light on potential therapeutic targets for preventing or managing complications associated with CKD, such as CVD and vascular calcification. These experiments involved the use of primary rat VSMCs and cultured aortic rings, and a murine model of chronic renal failure induced by a high-phosphate diet.

Methods

The researchers first used PCR analysis to investigate how the expression profiles of mitochondrial phosphate transporters in primary VSMCs were affected when exposed to high levels of phosphate. One particular transporter, phosphate carrier (PiC), was found to be the most abundant, while others such as uncoupling protein 2 (UCP2), dicarboxylate carrier (DIC), slc25a24, and slc25a25 were less abundant. Notably, when the cells were exposed to high phosphate levels, the amount of PiC protein increased. This increase was observed as early as 15 minutes after exposure to high phosphate levels and continued for up to 24 hours.

After establishing that PiC showed the highest expression levels among mitochondrial phosphate transporters in VSMCs, the research team set out to investigate its contribution to vascular calcification. To achieve this, they employed RNAi-mediated gene silencing techniques to suppress PiC expression. Utilizing Dharmacon siRNA reagents, they achieved an impressive 80% reduction in both PiC mRNA and protein levels. This suppression resulted in a decrease in superoxide production and inhibited the activation of ERK1/2, which are signaling molecules associated with cell growth and differentiation. PiC knockdown both prevented the upregulation of osteogenic genes and diminished the calcific changes in VSMCs triggered by elevated phosphate levels. To confirm the preventive effect of PiC knockdown on phosphate-triggered vascular calcification, the team conducted additional experiments on cultured rat aortic rings *ex vivo*, as illustrated in Figure 1.

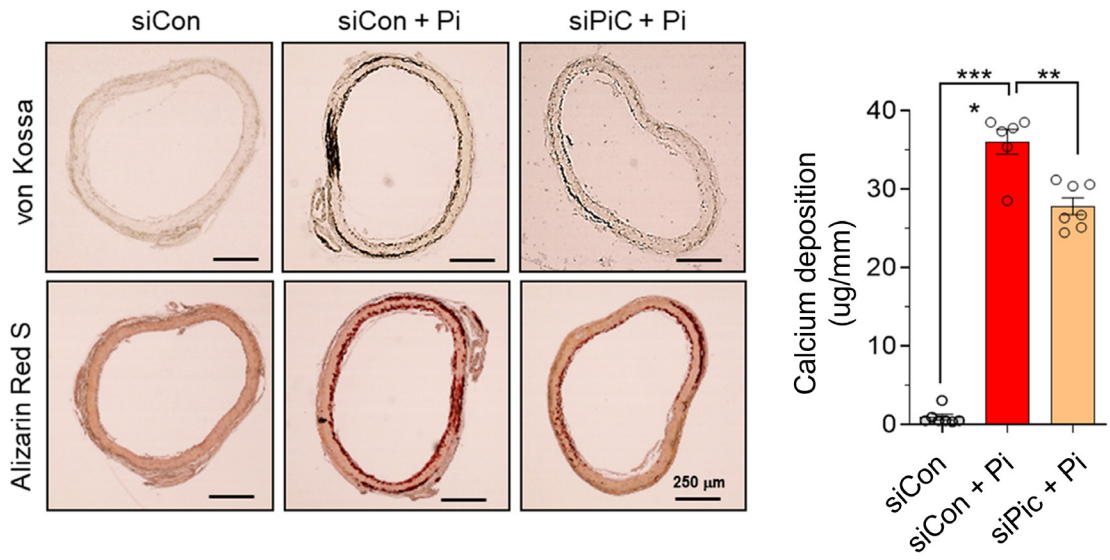

Figure 1: Calcification of rat aortic rings after incubation ex vivo with a high Pi-containing medium for 7 days as visualized by Alizarin Red S. siCon = control siRNA, siPiC = PiC silencing siRNA, Pi = phosphate. Image credit: Thi Nguyen, N., Thi Nguyen, T., Nguyen, H.T. et al. Inhibition of mitochondrial phosphate carrier prevents high phosphate-induced superoxide generation and vascular calcification. Exp Mol Med 55, 532-540 (2023). https://doi.org/10.1038/s12276-023-00950-0 licensed under Creative Commons Attribution 4.0 International License.

Recognizing the crucial role of PiC in mediating the harmful effects of high phosphate levels on VSMCs, the team extended their investigation to explore the impact of inhibiting mitochondrial phosphate uptake in a murine model of chronic renal failure. They utilized male C57BL/6 mice, which were fed a high-phosphate diet for 12 weeks to induce renal failure. During this period, the mice were randomized to different groups, including a group given nonselective pharmacological blockers of mitochondrial phosphate transporters, butylmalonate (BMA) and mersalyl.

To assess the extent of vascular calcification, the entire aorta was imaged using a Quantum™ microCT system. This imaging technique allowed the researchers to observe calcification signals throughout the length of the aorta. As illustrated in Figure 2, treatment with BMA or mersalyl reduced the calcification signal intensity from the thoracic aorta in the CKD model. These findings suggest that pharmacologic inhibition of mitochondrial phosphate uptake could be a potential strategy to reduce CKD-related vascular calcification.

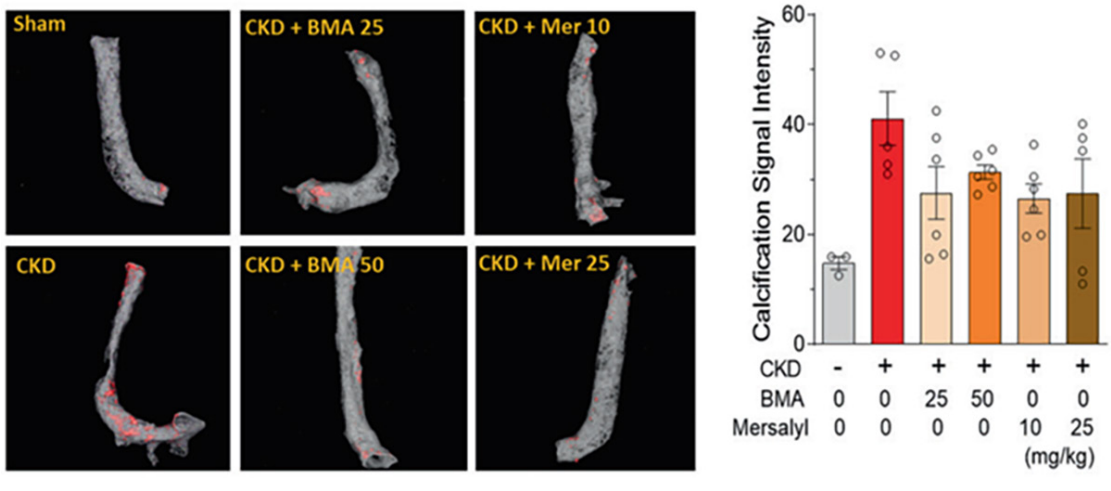

Figure 2: Calcified areas in the aortic rings of 5/6 nephrectomy mice were visualized by micro-CT imaging (mice; n = 3-6). Image credit: Thi Nguyen, N., Thi Nguyen, T., Nguyen, H.T. et al. Inhibition of mitochondrial phosphate carrier prevents high phosphate-induced superoxide generation and vascular calcification. Exp Mol Med 55, 532-540 (2023). https://doi.org/10.1038/s12276-023-00950-0 licensed under Creative Commons Attribution 4.0 International License.

Conclusion

Hyperphosphatemia is a complication of reduced kidney function in individuals with CKD and is associated with cardiovascular tissue calcification, which can lead to cardiovascular morbidity and mortality. Given the significant clinical consequences of hyperphosphatemia in CKD, Nguyen and colleagues investigated the role of mitochondrial phosphate transport in vascular calcification. Through targeted genetic suppression of PiC and pharmacological inhibition of mitochondrial phosphate uptake, the team were able to uncover a pivotal role of PiC in this process and demonstrate the potential efficacy of pharmacological inhibitors in reducing vascular calcification. These observations underscore the importance of further studies on mitochondrial phosphate transport inhibition as a novel therapeutic strategy for addressing vascular calcification related to chronic renal diseases.

Reference

1. Thi Nguyen, N., Thi Nguyen, T., Nguyen, H.T. et al. Inhibition of mitochondrial phosphate carrier prevents high phosphate-induced superoxide generation and vascular calcification. *Exp Mol Med* 55, 532–540 (2023). https://doi.org/10.1038/s12276-023-00950-0

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com