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Phosphate meeting cholesterol—consequences for cardiovascular disease in chronic kidney disease?

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Cardiovascular disease is highly prevalent in patients with chronic kidney disease. Hyperphosphatemia is associated with subclinical atheromatosis in chronic kidney disease. Phosphate-induced endothelial dysfunction and vascular calcification are thought to be key inducers of atherosclerosis in this condition. Zhou *et al.* now demonstrate that phosphate promotes *de novo* cholesterol synthesis in vascular smooth muscle and macrophages through increased 3-hydroxy-3-methylglutaryl coenzyme A reductase activation. This observation may change current concepts of atherosclerosis development and management in chronic kidney disease.

High serum phosphate levels are associated with an increased risk for cardiovascular diseases in patients with chronic kidney disease (CKD) as well as in the general population. This association appears to be linked, at least in part, to an increase in atherosclerotic risk. Studies have shown that hyperphosphatemia is independently associated with the prevalence of atheromatous plaques in patients with advanced CKD (stages 3, 4, and 5D)[1] and that a strong association exists between elevation of serum phosphate levels even within the normal range and atherosclerosis in subjects with normal kidney function.[2] In line with these observations, elevated phosphate levels have been demonstrated to correlate with plaque formation in a nonuremic, atherogenesis-prone animal model.[3] To date, the mechanisms involved in the pathophysiology of phosphate-induced atherosclerosis are not clearly understood. Following the observation that exposure of macrophages to calcium-phosphate crystals induces local inflammation, phosphate procalcific properties were initially thought to be responsible for the increased risk of atherosclerosis. However, the observation by Ellam *et al.*[3] that high dietary phosphate intake accelerated atherogenesis in apolipoprotein E (ApoE) knockout (KO) mice with normal kidney function independently of calcification raised the possibility that in addition phosphate may promote plaque development by mechanisms other than its well-known procalcific action. Interestingly, both experimental and clinical data have shown that hyperphosphatemia induces endothelial dysfunction, known to favor the development of atherosclerosis. Although the current view is that phosphate's proatherogenic properties are mainly due to its ability to induce vascular calcification and endothelial dysfunction, other more indirect actions also deserve consideration, including phosphate-induced changes in serum parathyroid hormone, FGF-23, and 1,25 diOH vitamin D3 levels, which have also been shown to be associated with cardiovascular disease in CKD.

In this context, the elegant demonstration by Zhou *et al.*[4] that phosphate is able to promote *de novo* cholesterol production in vascular smooth muscle cells (VSMCs) and macrophages through increased 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) activation invites reflection on current concepts of atherosclerosis development in CKD.

Effect of phosphate on *de novo* cholesterol synthesis

Increased biosynthesis of cholesterol mediated by HMGCR contributes to lipid accumulation in VSMCs, driving foam cell formation.[5] The activity of the *HMGCR* gene is under the control of transcription factor Sterol regulatory element binding protein 2 (SREBP2), whose activity is controlled by SREBP cleavage–activating protein (SCAP), a cholesterol sensor and chaperone of SREBP2. When the cell requires cholesterol, SCAP shuttles SREBP2 from the endoplasmic reticulum to the Golgi to activate proteolytic cleavage.

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a Risk factors for atherosclerosis and treatment approaches
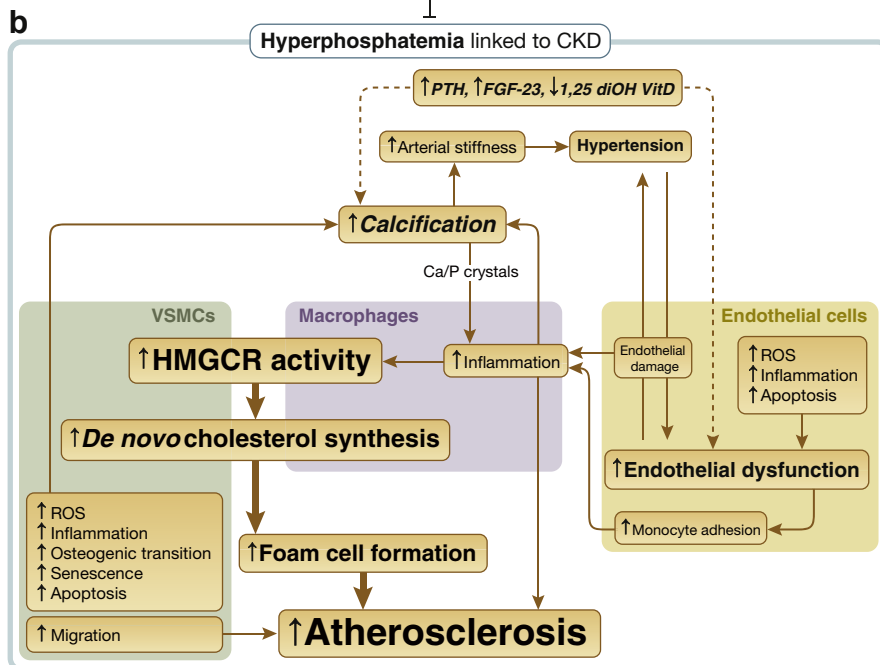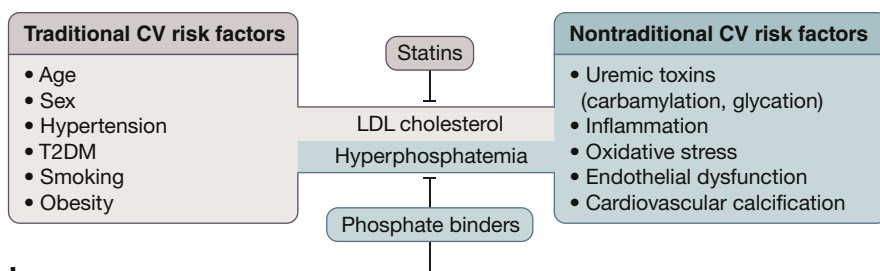
Figure 1 | Phosphate, atherogenesis, and cardiovascular risk: an updated overview. (a) Cardiovascular risk factors in chronic kidney disease (CKD), a new interplay between phosphate and cholesterol. (**b**) Schematic representation of the mechanisms by which phosphate is thought to promote atherogenesis. 1,25 diOH VitD, vitamin D3; CV, cardiovascular; FGF-23, fibroblast growth factor-23; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDL, low-density lipoprotein; PTH, parathyroid hormone; ROS, reactive oxygen species; T2DM, type-2 diabetes mellitus; VSMCs, vascular smooth muscle cells.

The cleaved N-terminal SREBP2 fragments (SREPB2-N) then enter the nucleus, bind to the sterol-regulatory elements in HMGCR and increase gene transcription, thereby promoting *de novo* cholesterol synthesis. Meanwhile, SCAP is glycosylated by the sequential action of Golgi enzymes α-mannosidase I (α-MAN I), α-mannosidase II (α-MAN II), and GlcNAc transferase I before returning to the endoplasmic reticulum. These modifications cause the N-glycans of SCAP to be heterogeneously converted from high-mannose glycans to complex glycans containing multiple branches (complex-type conversion), which makes SCAP less susceptible to degradation.

Zhou *et al.*[4] now demonstrate that phosphate enters VSMCs through a sodium-dependent phosphate cotransporter and promotes complex-type conversion of SCAP N-glycans by augmenting the activity of α-MAN II. As a result, SCAP modified with complex-type glycans cycles around the endoplasmic reticulum and Golgi and overactivates SREBP2, which in turn stimulates HMGCR activity and hence *de novo* synthesis of cholesterol. Confirming these *in vitro* data, Zhou *et al.*[4] observed a significant increase in SCAP, HMGCR, and SREBP2-N levels as well as in α-MAN II activity in aortas of ApoE KO mice fed a high-phosphate diet (HPD), whose increased atheroma

burden correlated positively with serum phosphate. Furthermore, they observed a correlation between hyperphosphatemia and increased SCAP protein levels and α-MAN II activity in radial arteries of Chinese patients who are uremic, as compared to no increase in SCAP protein levels and α-MAN II activity in radial artery of patients who are uremic with normal serum phosphate levels. They thereby provided further evidence for the claim that the association of hyperphosphatemia with atherosclerosis in patients with CKD is linked to phosphate-enhanced cholesterol synthesis.

Zhou *et al.*[4] did not observe an increase in low-density lipoprotein receptor protein expression in VSMCs exposed to high phosphate concentrations or in the aortas of ApoE mice fed on an HPD. This demonstrates that the atherogenic properties displayed by phosphate in cultured VSMCs and mouse aorta roots were not the consequence of increased cholesterol uptake. The fact that there was no change in serum total cholesterol levels in response to hyperphosphatemia in mice in both the study of Zhou *et al.*[4] and that of Ellam *et al.*[3] reinforced the hypothesis of a direct proatherogenic effect of phosphate. However, one should keep in mind that a link may exist between serum phosphate and serum cholesterol given that a previous report by Tanaka *et al.*[6] showed that dietary phosphate restriction reduced the susceptibility of mice to develop hyperlipidemia in response to a high-cholesterol diet.

The report by Zhou *et al.*[4] constitutes the first evidence that, beyond vascular calcification and endothelial dysfunction, phosphate can promote atherogenesis by stimulating cholesterol synthesis directly. This could explain the strong association between hyperphosphatemia and atherosclerosis observed in patients with CKD and even in the general population. Given this association, the hitherto unknown effect of phosphate on HMGCR-mediated cholesterol synthesis together with its already known effects favoring endothelial dysfunction and vascular calcification, the prescription of phosphate-lowering therapies appears to

be all the more indicated for the prevention of atheromatous cardiovascular disease (CVD). Ten years ago, our group studied the impact of phosphate binders on atherogenesis in uremic ApoE KO mice. We observed that the administration of lanthanum carbonate and sevelamer-HCl, respectively, retarded the progression of both atherosclerosis and vascular calcification in this animal model.[7,8] These beneficial effects were observed along with a decrease of serum phosphate and CaxP product, in the absence of changes in circulating levels of inflammatory markers or uremic toxins. The fact that either phosphate binder blocked vascular collagen type I expression suggested that a better control of the calcification process through reduced VSMC osteogenic transition might have reduced calcification-induced atheromatosis. The fact that sevelamer-HCl, but not lanthanum carbonate, reduced the expression of nitrotyrosine suggested further that its beneficial effects on atherogenesis might be linked, at least in part, to a reduction of vascular oxidative stress. In our studies, like in that of Zhou et al.,[4] the proatherogenic effects of phosphate were not linked to a better control of serum cholesterol levels, because the degree of hypercholesterolemia induced by CKD in the animal model remained unchanged in response to sevelamer-HCl.[7] The possibility that the phosphate binder treatment may have reduced atherogenesis by decreasing phosphate-enhanced vascular cholesterol synthesis is not ruled out but was not envisaged at that time.

Clinical implication

The data obtained by Zhou et al.[4] and Ellam et al.,[3] combined with the observations made by our group[7,8] that sevelamer and lanthanum carbonate can efficiently reduce the progression of atherosclerosis in mice, shed new light on the concept proposed by Ellam et al.[9] in 2012 that phosphate binders could become the new statins. Moreover, the study by Zhou et al.[4] underlines for the first time the complex relationship of the classical risk factors for atheroscle-

rosis including age, smoking, and hyperlipidemia, with nonclassical risk factors such as hyperphosphatemia in patients with CKD. This opens new ways for the management of CVD in such patients (Figure 1). Well-designed clinical trials will be needed to address the impact of phosphate-lowering therapies beyond statins in this disease condition.

If statins have been demonstrated to be efficient in preventing cardiovascular events in patients with normal kidney function, several studies showed that they are less efficacious in reducing CVD risk in the presence of CKD. There is experimental evidence that the HMGCR activity of VSMCs induced by inflammatory cytokines weakens statins' inhibitory effect on HMGCR, inducing a state of statin resistance. In keeping with this observation, the amount of statin required to lower serum cholesterol and decrease aorta lipid accumulation in experimental animals *in vivo* was shown to rise from 2 to 10 mg/kg/d in the presence of inflammatory stress.[5] Future studies should investigate whether the newly described mechanism of phosphate-induced HMGCR activation may also account for VSMC resistance to statins in CKD, and whether the use of phosphate binders in patients with CKD treated with statins may enhance the efficacy of statins in preventing cardiovascular events in such patients. Interestingly, serum phosphate independently correlates with inflammation in CKD, and phosphate increases the release of proinflammatory cytokines by VSMCs. In the study by Zhou et al.,[4] hyperphosphatemia was not linked to an increase of systemic inflammation in patients with CKD and nonuremic mice, respectively. However, the possibility cannot be ruled out that part of the observed stimulatory effect of phosphate on HMGCR activity in VSMCs is linked to increased vascular inflammation.

In the studies by Zhou et al.[4] and Ellam et al.,[3] a correlation between hyperphosphatemia and atheroma burden was also observed in oral phosphate loaded, nonuremic ApoE KO

mice. Because serum phosphate levels within the normal range were found to be associated with an increase in cardiovascular events in the general population, further studies should be undertaken to examine the hypothesis that even a mild increase of serum phosphate could represent a nontraditional risk factor for atherosclerosis in people with normal kidney function.

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Hypoxia signaling in renal pericytes—is it safe to activate?

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While excitement has grown for the use of hypoxia-inducible factor (HIF) prolyl hydroxylase inhibitors for treating renal anemia, multiple preclinical studies have shown the complex and cell-type–dependent roles of HIFs in kidney disease pathogenesis, including renal fibrosis. Pan *et al.* now clearly show that activating the HIF signaling in the *Gli1*-lineage myofibroblasts restores erythropoietin production while not adversely affecting matrix production, mitigating the concerns of exacerbated fibrosis by HIF prolyl hydroxylase inhibitors.

see basic research on page 1354

Renal fibroblasts and pericytes are the major source of erythropoietin (EPO), which regulates erythropoiesis. Dysfunction of renal EPO-producing cells (REPCs) and their phenotypic transformation to myofibroblasts underlie the pathogenesis of renal anemia.[1,2] REPCs are located juxtaposed to peritubular capillaries in corticomedullary junctions. They extend their long processes, which tightly wrap peritubular capillaries, and create interconnected networks.[1] In anemic or hypoxic conditions, cortical $CD73^+$

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$PDGFRb^+$ interstitial cells (physiological reserve of EPO production) start to produce EPO to meet the demand of erythropoiesis. However, as chronic kidney disease (CKD) worsens, these EPO-producing interstitial cells become matrix-producing myofibroblasts and lose their EPO-producing ability.[1,2] Therefore, myofibroblast transition of REPCs underlies the pathogenesis of both relative insufficiencies of EPO in renal anemia and fibrosis—2 common pathologies of advanced CKD.

Erythropoiesis-stimulating agents (ESAs; recombinant human EPO and engineered preparations of recombinant human EPO) have been safely and efficiently used to treat renal anemia in patients. However, this common therapy has some clinical challenges, such as cardiovascular adverse events linked to the supra-physiological concentration of supplemented EPO and EPO hypo-responsiveness.[3] Recently, hypoxia-inducible factor (HIF) prolyl hydroxylase (PH) inhibitors have emerged as an alternative therapeutic approach to renal anemia in patients.[3] HIF-PHs (PHD1, PHD2, and PHD3) are the key enzymes for cells to control cellular hypoxia adaptation mediated by HIFs (Figure 1a).[3] HIF-PH inhibitors prevent the proteasomal degradation of HIF-α subunits, thereby stabilizing this transcription factor and promoting heterodimerization with HIF-β to activate adaptive responses to hypoxia, including the direct activation of *Epo* gene transcription.[2,3]

Because HIF-PH inhibitors increase EPO production from native kidneys without exogenous bolus, excitement has grown for the use of HIF-PH inhibitors to treat anemia in CKD and end-stage kidney disease (ESKD). These oral small molecule inhibitors are already in clinical use in China and Japan for the treatment of anemia in both CKD and ESKD. While the drugs have theoretical and observed benefits (such as improving iron metabolism) beyond EPO induction,[3] the failure of 1 HIF-PH inhibitor, vadadustat, to reach the noninferiority margin for the primary cardiovascular safety composite endpoint (all-cause mortality, nonfatal myocardial infarction, and nonfatal stroke) compared with darbepoetin in patients with CKD not on dialysis has raised some safety concerns (Akebia press release, September 3, 2020, PRO_2TECT trial), despite meeting noninferiority for this endpoint in the prespecified US subgroup (Akebia press release, October 23, 2020, PRO_2TECT trial) and in the analogous phase 3 trial in ESKD.[3] The US Food and Drug Administration and European Medicines Agency have yet to approve any HIF-PH inhibitor for the treatment of renal anemia as of early 2021.

Multiple preclinical studies have shown the complex and cell-type–dependent roles of HIF in the pathogenesis of kidney diseases.[2] While there are studies favoring HIF activation to prevent kidney disease progression such as protecting cells from ischemic injury (ischemic preconditioning), mouse genetic studies point out some concerns in the long-term use of HIF activators, spanning from worsening