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Phosphate toxicity: new insights into an old problem

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Abstract

Phosphorus is an essential nutrient required for critical biological reactions that maintain the normal homoeostatic control of the cell. This element is an important component of different cellular structures, including nucleic acids and cell membranes. Adequate phosphorus balance is vital for maintaining basic cellular functions, ranging from energy metabolism to cell signalling. In addition, many intracellular pathways utilize phosphate ions for important cellular reactions; therefore, homoeostatic control of phosphate is one of the most delicate biological regulations. Impaired phosphorus balance can affect the functionality of almost every human system, including musculoskeletal and cardiovascular systems, ultimately leading to an increase in morbidity and mortality of the affected patients. Human and experimental studies have found that delicate balance among circulating factors, like vitamin D, PTH (parathyroid hormone) and FGF23 (fibroblast growth factor 23), are essential for regulation of physiological phosphate balance. Dysregulation of these factors, either alone or in combination, can induce phosphorus imbalance. Recent studies have shown that suppression of the FGF23–klotho system can lead to hyperphosphataemia with extensive tissue damage caused by phosphate toxicity. The cause and consequences of phosphate toxicity will be briefly summarized in the present review.

Keywords

calcium; fibroblast growth factor 23 (FGF23); klotho; parathyroid hormone (PTH); sodium-dependent phosphate transporter; vitamin D

INTRODUCTION

Phosphorus is an essential nutrient for the body and is routinely consumed through food. After consumption, phosphorus is usually bound with oxygen and exists as phosphate in the body. Both organic and inorganic forms of phosphate are present in regularly consumed foods such as meats, fish, eggs, milk/dairy products and vegetables. The amount of total phosphate ingestion can be significantly influenced by processed food and/or beverage intake, as phosphate metabolites are used as additives in these items. Following a meal, inorganic phosphate can be rapidly absorbed across the small intestine and enter the blood stream causing an elevation in blood phosphate levels. The net efficiency of intestinal phosphate absorption is more than twice that of calcium absorption. An increase in serum levels of inorganic phosphate usually reduce serum levels of ionic calcium by forming a calcium—phosphate complex; such reduced ionic calcium concentration in turn stimulates release of PTH (parathyroid hormone) in an attempt to restore the serum calcium balance. In contrast, dietary phosphate deficiency, mostly due to malnutrition, not only induces hypophosphataemia, but can also impair the bone mineralization process and eventually lead

to the development of rickets [1,2]. Optimal phosphorus and calcium balance is important for skeletal growth, development and maintenance [3,4]. Despite the essential role of phosphate in living cells, the molecular regulation of intra- and extra-cellular phosphorus metabolism is poorly understood and is an active area of research.

PHOSPHATE METABOLISM

Phosphorus is widely distributed in the body. More than 80 % of total phosphate is present in the bone and teeth in the form of apatite. The remaining phosphate is mostly present in the viscera and skeletal muscle, with a very small amount in the extracellular fluids (<0.1 %) [5–8]. Intracellular phosphate ions are essential for oxidative phosphorylation and approx. 20 % of cellular phosphate is present in the mitochondria. Approx. 30 % of total cellular phosphate is stored in the ER (endoplasmic reticulum) and is used in phosphorylation of various proteins. The remaining cellular phosphate is present in the nucleus, Golgi complex and lysosomes. Transporting phosphate in and out of the cell according to the need of the body is a complex process and the exact molecular mechanisms of such delicate transport are not yet clear. It is necessary to mention that cells also use phosphate to transport cellular energy through the formation of ATP by oxidative phosphorylation. In addition, glucose and triacylglycerol (triglyceide) synthesis utilize phosphate to form glucose 6-phosphate and glycerol 3-phosphate respectively.

Phosphorylation is an essential cellular reaction, where addition of a phosphate group to a molecule/protein (including enzymes or receptors), can determine the biological activity of that particular molecule. Phosphorylation is catalysed by specific protein kinases, whereas dephosphorylation is catalysed by phosphatases. More than 2000 chemical reactions in living cells use phosphate [9] and optimal intra- and extra-cellular phosphate balance is essential for efficacy of such biochemical reactions. Phosphate balance is tightly controlled by complex cross-organ communication among the kidney, intestine, bone and parathyroid gland (Figure 1) [6,10–14]. Our understanding of intestinal phosphate transport is mostly based on experimental studies that have shown that the bulk of phosphate absorption takes place in the duodenum and jejunum in rats, and in the ileum in mice [15]. NaPi (sodiumdependent phosphate) transport, involving NaPi-2b, is believed to actively regulate the intestinal phosphate absorption. Both 1,25-dihydroxyvitamin D and dietary phosphate can influence intestinal NaPi-2b activities [16]. For instance, low-phosphate diet, through inducing the renal expression of $1\alpha(OH)$ as can increase circulating 1,25-dihydroxyvitamin D levels, which in turn can enhance intestinal NaPi-2b protein expression to increase intestinal phosphate absorption. In a similar line of observation, the absorption ability of NaPi-2b knockout mice was 50 % less than that of wild-type animals following acute administration of phosphate [17]. However, the human relevance of the experimental studies need additional studies, as inactivating mutations of the human NaPi-2b gene did not show any considerable reduction in serum phosphate levels [18]. In general, the body's daily need of phosphate is covered by intestinal absorption from consumed food, and the serum level of phosphate is delicately maintained by renal excretion of phosphate.

Renal phosphate excretion and reabsorption is partly mediated by the NaPi system – NaPi-2a and NaPi-2c. There is a link between PTH activity and renal proximal tubular epithelial cell membrane integration and retrieval of NaPi-2a that is located in the luminal side of the tubular epithelial cells [19,20]. PTH is a strong inhibitor of NaPi-dependent phosphate reabsorption and thereby facilitates an increase in urinary phosphate excretion [19,20]. The lists of molecules that can regulate NaPi activities are growing and recent studies have shown that FGF23 (fibroblast growth factor 23) and klotho can suppress NaPi activities [21–24]. The amount of dietary phosphate content has a major regulatory effect on renal phosphate reabsorption [25]. Dietary phosphate restriction induces an adaptive

increase of intestinal phosphate uptake through a sodium gradient-dependent phosphate transport. Prolonged dietary phosphate restriction also increases NaPi-2a activity and, thereby, increases phosphate reabsorption in the kidney in an attempt to restore the balance [26]. Microtubules (tubulin) are believed to be involved in NaPi-2a activity, possibly by facilitating the rapid translocation of NaPi-2a from intracellular compartments to the cell membrane in response to a low phosphate diet [26]. The molecular events of systemic phosphate metabolism are not yet clearly defined and identification of the FGF23–klotho system as a potent phosphotonin has provided new mechanistic insights into homoeostatic control of phosphate [27,28].

FGF23-KLOTHO SYSTEM

Endocrine regulation of phosphate homoeostasis is a complex process and identification of the FGF23–klotho system has significantly enhanced our understanding of multi-organ interactions during systemic regulation of phosphate homoeostasis. FGF23 is an approx. 30-kDa protein that contains the FGFR (FGF receptor)-binding domain in the N-terminal and a potential klotho-binding site at the C-terminal [29]. FGF23 can bind to multiple FGFRs, including FGFR1c, FGFR3c and FGFR4 [21,30–33]. A recent *in vivo* study, however, did not show a significant response of FGF23 through FGFR3 or FGFR4 [34]. Furthermore, the interaction between FGF23-FGFRs and subsequent signalling activities required klotho as a cofactor. In the presence of klotho, FGF23 can bind to its receptor complex, with much higher affinity, to activate downstream signalling phosphoproteins, including FGFR substrate-2a, ERK (extracellular-signal-regulated kinase), p38, JNK (c-Jun N-terminal kinase) and AKT proteins [35,36].

The klotho gene encodes a type 1 membrane protein and the full-length 5.2 kb transcript encodes a 130-kDa membrane protein [37]. A disintegrin and metalloproteinases (ADAM-10 and ADAM-17) can cleave klotho from the plasma membrane [38]. Klotho expression is mostly detected in the distal convoluted tubules of the kidney, the parathyroid gland and the epithelium of the choroid plexus in the brain [39]; such restricted expression gives the tissue specificity for FGF23 action. Activation of the FGF23-klotho system can increase urinary phosphate excretion by reducing NaPi-2a and NaPi-2c co-transporter activity [21–23]. Whether the FGF23-klotho system directly reduces renal NaPi cotransporter activity, or such response is mediated through other FGF23 target molecules, is an unsolved issue. The reduction of serum phosphate levels following activation of the FGF23-klotho system is a universal phenomenon detected in both animal and human studies [28,40–45]. For instance, increased serum levels of FGF23 due to gain-of-function mutations of the human FGF23 gene are associated with hypophosphataemia, a condition triggered by excessive urinary phosphate wasting in patients with ADHR (autosomaldominant hypophosphataemic rickets) [28]. Similarly, hypophosphataemia in patients with ARHR (autosomal-recessive hypophosphataemic rickets), also known as osteomalacia, has been attributed to high serum levels of FGF23 [46]. Likewise, transgenic mice overexpressing human FGF23 develop hypophosphataemia due to severe urinary loss of phosphate, while Fgf23 knockout mice develop hyperphosphataemia due to increased renal uptake of filtrated phosphate. Genetic restoration of the systemic actions of human FGF23 in Fgf23 knockout mice can reverse hyperphosphataemia to hypophosphataemia [47]. As it happens, the phenotype of Fgf23 knockout mice mimics the clinical features of FTC (familial tumoral calcinosis) patients, in which hyperphosphataemia develops due to reduced activity of human FGF23 [44,47].

Recently, genetically modified animal models have convincingly demonstrated the *in vivo* requirement for klotho in FGF23-mediated regulation of phosphate metabolism. For example, bioactive FGF23 protein is unable to exert phosphate-lowering effects in mice

lacking klotho activities (either *klotho* – mice or *Fgf23* – *klotho* – double knockout mice) [45]. Similarly, the inactivation of the *klotho* function from *phex* mutant mice resulted in hyperphosphataemia in *phex/klotho* double mutant mice, even though double mutant mice have significantly high serum levels of FGF23 [43,48]. In a separate experiment, genetic inactivation of klotho from *FGF23* transgenic mice reversed a hypophosphataemic phenotype to a hyperphosphataemic phenotype [49]. In a similar study involving humans, a homozygous loss-of-function mutation in the *Klotho* gene induced severe hyperphosphataemia, despite high serum levels of FGF23 in the affected patient with tumoral calcinosis [42]. It is clear from previous studies that klotho is indispensable for FGF23-mediated regulation of systemic phosphate homoeostasis [11,50,51] and that suppression of the FGF23–klotho system can induce phosphate toxicity [52,53].

PHOSPHATE TOXICITY

Phosphate toxicity due to excessive retention of phosphate in the body can cause a wide range of cellular and tissue injuries (Figure 2). For instance, higher occurrence of vascular calcification, encountered in patients with CKD (chronic kidney disease), is related to the increased retention of phosphate in the body [1,48]. Genetic studies with mice have shown that phosphate toxicity is closely associated with cardiovascular calcification in *klotho* knockout mice [22]. More importantly, lowering serum phosphate levels in *klotho* knockout mice can markedly reduce vascular calcification, despite the presence of significantly higher serum calcium and 1,25-dihydroxyvitamin D levels [22,54]. A similar trend of phosphate toxicity and ectopic calcification is also noted in *Fgf23* knockout mice [55–58]. In humans, phosphate toxicity and low serum vitamin D levels have been implicated as independent risk factors for high mortality in CKD patients [59–61].

Complications of phosphate toxicity are also encountered in patients treated with phosphatecontaining laxatives or enemas. Serum analysis of 14 elderly patients exposed to a phosphate-containing enema has shown significantly elevated serum levels of phosphate within an hour and a significant decrease in serum calcium levels by 12 h [62]. In addition, administration of hypertonic phosphate-containing enemas to a paediatric group of patients resulted in a wide range of complications, including tetany, dehydration, hypotension, tachycardia, hyperpyrexia, cardiac arrest and coma [63,64]. Some of these complications can be attributed to abnormal mineral ion and electrolyte balance induced by phosphate toxicity, which can also serve to increase anion gaps. For instance, Domico et al. [65] found a total anion gap of 29 mmol/l in a patient with phosphate toxicity (38.3 mg/dl), which was normalized after therapeutically reducing serum phosphate [65]. In a separate study, rectally administered hypertonic phosphate solution to a 4-year-old chronically constipated girl with normal renal function was reported to have developed phosphate toxicity (23 mg/dl) with breathing difficulties and a depressed level of consciousness [66]. The girl experienced a generalized seizure 16 h following a phosphate-containing enema and was unresponsive to multiple doses of lorazepam. The patient only responded to 100 mg of intravenous calcium chloride, suggesting deleterious effects of phosphate toxicity on other mineral ions and electrolyte levels that provoked subsequent complications, even without the presence of predisposing risk factors [66]. In an animal study, phosphate toxicity (7-20-fold increase over control by 4 h) induced by a commercially available phosphate-containing enema (30-50 ml/kg of body weight) has been shown to induce 100 % mortality [67].

Phosphate toxicity has recently been found to accelerate the mammalian aging process by inflicting tissue damage and reducing survival [40]. Genetically engineered *klotho*-null mice developed phosphate toxicity as early as 3 weeks of age that induced premature aging. The effects of premature aging in genetically engineered mice included, but were not limited to, loss of body weight, kyphosis, hypogonadism, infertility, generalized tissue atrophy and

reduced life span [40,41,43,68–70]. Some of these changes in klotho knockout mice bear similarities to human aging. Molecular and biochemical analysis suggests that increased renal activity of NaPi-2a leads to phosphate toxicity in klotho knockout mice. In fact, the extensive aging phenotypes in short-lived klotho knockout mice can be suppressed by genetically reducing phosphate toxicity in NaPi2a/klotho double knockout mice to extend their survival [40]. The genital organs of hyperphosphataemic klotho knockout mice of both sexes are severely atrophic and such hypogonadism is associated with premature infertility, which is a major consequence of accelerated aging in both humans and experimental animals [68,69]. Notably, the hyperphosphataemic klotho knockout mice regained fertility by genetically reducing serum phosphate levels, as evidenced in the NaPi2a/klotho double knockout mice. More importantly, the NaPi2a/klotho double knockout mice lost their fertility when fed a high-phosphate diet, clearly suggesting that phosphate toxicity can affect fertility and thereby influence the aging process. A similar premature aging trend is exhibited in klotho knockout mice where widespread tissue atrophy in the spleen, skeletal muscle, intestine and skin is present [71]. However, reducing serum phosphate levels in klotho knockout mice rescues tissue atrophy in NaPi2a/klotho double knockout mice. When phosphate toxicity is induced in NaPi2a/klotho double knockout mice by feeding on a high-+phosphate diet (1.2 %), premature aging, including generalized tissue atrophy, reappeared and all the mice died by 15 weeks. Of relevance, none of the NaPi2a/klotho double knockout mice fed on a normal-phosphate diet (0.6 %) died until 20 weeks of the observational period [40]. These in vivo genetic and dietary observations clearly suggest that phosphate toxicity can accelerate the mammalian aging process.

In addition to accelerating the aging process, increased dietary phosphate intake has been shown to stimulate the growth and size of lung tumours [72]. A large number of processed foods, including meats, cheeses, beverages and bakery products, use phosphate-containing food additives. Consumption of these items could significantly increase phosphorus intake and impair the normal homoeostatic balance of calcium and phosphate. For instance, rats fed with phosphoric-acid-containing soft drinks developed significant hypercalciuria and hyperphosphaturia, along with dysregulation of serum PTH and vitamin D, provoking reduced bone mineralization [73,74]. In a related human study, consumption of phosphoric acid-containing soft drinks was found to be associated with hypocalcaemia in postmenopausal women [75].

The mechanism by which phosphate toxicity accelerates the aging process is not clear. Phosphate toxicity can exert cytotoxic effects to compromise the functional ability of various organ systems. Phosphate toxicity can induce an increased rate of apoptosis in various tissues that can be suppressed by reducing the phosphate burden [40]. In an experimental study, when rabbits were injected intradermally with hypertonic phosphate solution, a pronounced erythema and indurations were noted by 24 h, which progressed to central necrosis and full thickness tissue loss by 5–7 days [76]. In fact, patients exposed to a phosphate-containing enema developed necrotic changes of the abdominal tissues, including loss of internal and external sphincters due to extensive tissue necrosis [76]. As mentioned above, phosphate is essential for cell signalling activities and altered phosphate balance may impair the homoeostatic control of signalling activities leading to cellular and tissue damage. Phosphorylation of inositol to phosphatidylinositol and cleavage of inositol triphosphate represent a major intracellular regulation of phosphate metabolism that also affects intracellular calcium metabolism. Recently, dietary phosphate has been shown to stimulate the Akt-mediated signalling network and provoke an increase in lung tumorigenesis [72].

CONCLUSIONS

Common causes of phosphate toxicity in humans include impaired renal function, rhabdomyolysis and tumour lysis syndrome. In addition, exogenous phosphate toxicity is also documented in patients with Hirschsprung disease when exposed to hypertonic phosphate enemas [77]. Of relevance, phosphate toxicity induced by excessive exogenous phosphate administration can be fatal [66,67,78,79]. Although the lethal dose of phosphate in humans is unknown, Martin et al. [67] reported that the lethal dose of phosphate in pigs was 35 mmol/kg of body weight. As for mice, 14-16 mg/dl of phosphate serum level could cause 100 % mortality by 15 weeks of age [40]. Overall, human and animal studies have convincingly demonstrated the toxic effects of phosphate in accelerating various pathologies, ranging from vascular calcification to tumour formation and aging. Acute phosphate toxicity can provoke hypocalcaemia and associated symptoms including tetany, hypotension and tachycardia. Moderate phosphate toxicity that takes longer to develop can lead to the deposition of calcium phosphate crystals in various tissues, including often fatal cardiovascular calcification. An abnormal deposit of calcium phosphate crystals due to phosphate toxicity is usually an irreversible process. Although, without pre-existing renal or gastrointestinal diseases, acute phosphate toxicity is relatively rare, the deleterious effects of chronic ingestion of unrestricted amounts of phosphate in individuals is not clear, and needs to be studied in more depth. Particularly, the effects of chronic unrestricted ingestion of high phosphate-containing processed foods and soft drinks on functionality of various organ systems require careful analysis. Finally, maintaining the phosphate balance in the diet may be important for a healthy life and longevity, as phosphate imbalance can induce serious debilitating complications.

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Abbreviations

ADAM a disintegrin and metalloproteinase

ADHR autosomal-dominant hypophosphataemic rickets

CKD chronic kidney disease

FGF23 fibroblast growth factor 23

FGFR fibroblast growth factor receptor

NaPi sodium-dependent phosphate transporter

PTH parathyroid hormone

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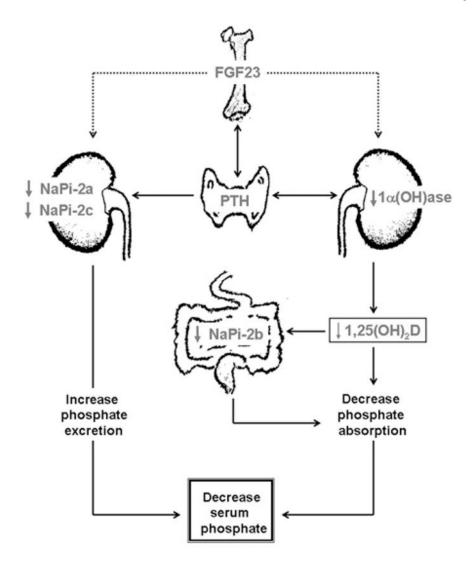
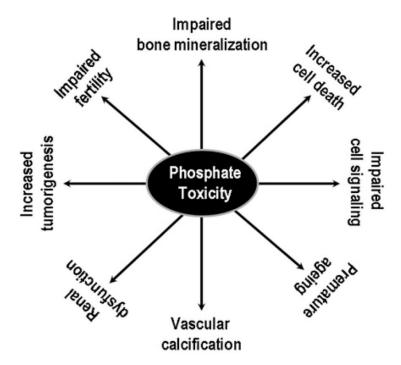


Figure 1. Simplified diagram showing muti-organ interactions in regulation of phosphate homoeostasis

FGF23 produced in the bone cells can suppress renal NaPi-2a and NaPi-2c co-transporter activities to increase the urinary excretion of phosphate. Likewise, FGF23 can also suppress renal expression of $1\alpha(OH)$ as to reduce production of $1,25(OH)_2D$ (1,25-dihydroxyvitamin D), which can suppress intestinal NaPi-2b activities to reduce phosphate absorption, resulting in decreased serum phosphate levels [6].

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 $Figure \ 2. \ Partial \ list \ of \ pathological \ events \ related \ to \ phosphate \ toxicity \ as \ documented \ in \ both \ human \ and \ animal \ studies$

Summarized from [40,66,67,72,78,79].