

Inorganic phosphate in the development and treatment of cancer: A Janus Bifrons?

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Abstract

Inorganic phosphate (Pi) is an essential nutrient to living organisms. It is required as a component of the energy metabolism, kinase/phosphatase signaling and in the formation and function of lipids, carbohydrates

and nucleic acids and, at systemic level, it plays a key role for normal skeletal and dentin mineralization. Pi represents an abundant dietary element and its intestinal absorption is efficient, minimally regulated and typically extends to approximately 70%. Maintenance of proper Pi homeostasis is a critical event and serum Pi level is maintained within a narrow range through an elaborate network of humoral interactions and feedback loops involving intestine, kidney, parathyroid gland and bone, and depends on the activity of a number of hormones, including parathyroid hormone, 1,25-dihydroxy vitamin D, and fibroblast growth factor 23 as major regulators of Pi homeostasis. Notably, Pi intake seemingly continues to increase as a consequence of chronic high-phosphorus (P) diets deriving from the growing consumption of highly processed foods, especially restaurant meals, fast foods, and convenience foods. Several recent reports have generated significant associations between high-P intake or high-serum Pi concentration and morbidity and mortality. Many chronic diseases, including cardiovascular diseases, obesity and even cancer have been proposed to be associated with high-P intakes and high-serum Pi concentrations. On the other hand, there is also evidence that Pi can have antiproliferative effects on some cancer cell types, depending on cell status and genetic background and achieve additive cytotoxic effects when combined with doxorubicin, illustrating its potential for clinical applications and suggesting that up-regulating Pi levels at local sites for brief times, might contribute to the development of novel and cheap modalities for therapeutic intervention in some tumours. Overall, the influence of Pi on cell function and the possible relationship to cancer have to be fully understood and investigated further.

Key words: Calcium-phosphate nanoparticles; Inorganic phosphate; Cancer; High-phosphorus diets; Phosphorus intake; Doxorubicin; Combination therapy; Naturally occurring molecule; Osteosarcoma

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Core tip: Many chronic diseases, including cancer have been proposed to be associated with high-phosphorus intakes and high-serum inorganic phosphate (Pi) concentrations. On the other hand, there is also evidence that Pi can have antiproliferative effects on some cancer cell types, depending on cell status and genetic background and achieve additive cytotoxic effects when combined with doxorubicin, illustrating its potential for clinical applications and suggesting that up-regulating Pi levels at local sites for brief times, might contribute to the development of novel and cheap modalities for therapeutic intervention in some tumors, including triple-negative breast cancer and osteosarcoma.

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INORGANIC PHOSPHATE AND CANCER

One of most important nutrients to living organisms is Inorganic phosphate (Pi). It is required in the ATP formation, kinase/phosphatase signalling and in the synthesis of lipids, carbohydrates and nucleic acids. Furthermore, it plays a key role for normal skeletal and dentin mineralization^[1].

Diet represents the main source of Pi intakes, its intestinal absorption is minimally regulated and typically extends to approximately 70%. To maintain Pi levels within a proper range, an elaborate network, including intestine, kidney, parathyroid gland and bone, is involved in a feedback control in which hormones as parathyroid hormone (PTH), 1,25-dihydroxy vitamin D, and fibroblast growth factor 23 (FGF-23) are major regulators of Pi homeostasis^[2].

Diets always richer in phosphorus, due to a highly processed food, especially restaurant meals, fast foods, and cheap foods, have increased Pi intake^[3,4].

For example, in the United States the consume of phosphorus daily in meals is typically around 1400 mg, as inorganic phosphate (Pi) salts or as a part of organic molecules, that is almost doubled compared to the adult recommended dietary allowance.

The kidney is one of the major regulators of Pi homeostasis and can increase or decrease its capacity to reabsorb Pi; the increased cumulative use of ingredients containing Pi in food processing is now being shown to be potential toxic when it exceeds nutrient needs.

Several recent studies have underlined the relationship between high-Pi intake/high-Pi serum concentration and morbidity and mortality^[3,4].

A variety of conditions and diseases, especially

cardiovascular diseases, has been spotted in individuals with high-Pi intakes, resulting from chronic high-Pi diets. Other chronic diseases, including type 2 diabetes mellitus, obesity and even cancer have also been proposed to be associated with high-Pi intakes and high-Pi serum concentrations^[3-5].

As far as the mechanisms by which high Pi concentrations are linked to tissue damage and/or possibly to influence tumour growth, they are not completely understood and could very likely include a mixture of cell autonomous as well as autocrine, paracrine, and/or endocrine signals.

In particular, although both PTH and FGF-23 are stimulated to decrease the post-meal serum Pi concentration rise, approximately 1 h through the interruption of renal Pi reabsorption, it is hypothesized that if cells are exposed to even a brief high-serum Pi concentration there could be some signal alterations in cell functions leading to negative effects. Moreover, the increase of serum levels of FGF-23 or PTH might be toxic to particular cell types^[3,4,6].

Numerous recent studies have reinforced a long-standing hypothesis that there could be a phosphate-sensing mechanism capable of detecting serum and local phosphate variations and of informing the body, the local environment or the individual cell^[7,8]. Because of the fact that the intracellular environment is electronegative compared to the extracellular one, the Pi transit into the cell does not happen by simple diffusion, but is mediated by Na⁺-coupled Pi cotransporters, which is a regulated event^[9]. In addition, Pi is coming out as an essential signalling molecule capable of modifying a lot of cellular functions by varying signal transduction pathways, gene expression and protein levels in many cell types^[8,10-12].

It has been shown that high tissue phosphate concentrations increase oxidative stress in endothelial cells^[13]. In human vascular smooth muscle cells it has been demonstrated that inorganic phosphate has effects on cell cycle and apoptosis, as well as, in the same cells, the increase of phosphate levels influences cellular and matrix elements promoting calcification^[14,15].

Moreover, it has also been supposed that high inorganic phosphate value speeds up senescence process in mouse models^[16]. Recent data have confirmed that diets with a high intake of Pi enlarge tumorigenesis in the two-stage skin carcinogenesis model and K-ras lung cancer model in mice^[17,18].

In addition, inorganic phosphate has been demonstrated to promote the activation of distinct pathways like ERK1/2 and Akt kinases, as well as it stimulates cell growth in specific cell types, such as preosteoblastic MC3T3-E1 cells, human lung cells, epidermal JB6 cells, proposing Pi as a mitogenic molecule in these cells^[17-21].

Recently, a large scale transcriptomics and proteomics research has evidenced that many pro-angiogenic genes and proteins are upregulated by raised Pi levels in preosteoblasts cells^[22] as osteopontin (OPN), a secreted cytokine, and forkhead box protein C2 (FOXC2), a

forkhead box transcription factor, both proteins recently associated with tumour angiogenesis. Lately, it has been demonstrated that in cancer cells Pi encourages tube formation and endothelial cells migration in vitro if exposed to elevated extracellular Pi levels, with FOXC2 and OPN as possible proteins involved in this mechanism^[23].

Notably, the pro-tumors and proliferative effects of Pi are not possible to extend to all cell types, in fact, it has been related that in MO6-G3 odontoblast-like cells Pi induces apoptosis^[23,24].

Previously, in the last years, we published a succession of articles, in which the aim has been to study the effects of elevated Pi on human osteosarcoma cell line U2OS and to know possible molecular mechanisms involved^[25-28].

Initially, we demonstrated that inorganic phosphate inhibits cell growth and reduces aggressiveness of human osteosarcoma cell line U2OS, identifying adenylate cyclase, beta3 integrin, Rap1, ERK1/2 as proteins whose expression and function are influenced by Pi^[25,26].

Later on, we proved also that Pi is capable of increasing the sensibility of osteosarcoma cells to doxorubicin in a p53-dependent manner and through down-regulation of ERK1/2 pathways^[27,28].

More recently, we described initial evidence of a strong antiproliferative action of Pi in MDA-MB-231 cell line, an extremely aggressive human triple negative breast cancer model, enlarging the hypothesis of Pi as a novel signalling molecule capable of modifying the function and survival of specific cell types^[11].

As part of our continuing effort to extend the knowledge on the role of inorganic phosphate as a "naturally occurring molecule" acting also as a "sensitizer" to increase the therapeutic index of clinical antitumor drugs, in a current study we describe that Pi induces strongly sensitization to doxorubicin by apoptosis induction in MDA-MB-231. We also show that Pi increases doxorubicin-induced cytotoxicity and that this mechanism involves ERK1/2 and STAT3 down-regulation^[29].

It is important to underline that in our studies we use a very low doxorubicin dose (until 0.1 μmol/L) that it is known to be a bearable dose because related to minimal side effects in patients, thus suggesting the possible clinical relevance of this positive pharmacological interaction^[30,31].

Latterly, new drug delivery system, called Calcium-phosphate nanoparticles, has been built up. Moreover, it is important to remember that hydroxyapatite nanoparticles release inorganic phosphate and that its retention, most likely, modifies Pi concentration at local sites^[32,33].

Furthermore, phosphate is the richest anion in the intracellular environment, with a concentration of 100 mmol/L, so it is easy to find an increase of extracellular Pi as a consequence of cell death induced by chemotherapy.

Maintenance of Pi systemic levels remains a crucial point, because an increase of serum values, even if moderate, and polymorphisms in genes implicated in Pi

homeostasis may have effects on ageing process and lifetime^[2].

The quantities of inorganic phosphate continue to rise in the diet, in particular way in the western countries, and an increase of the morbidity and mortality in the exposed population has been linked to this habit^[3,4].

In Particular, it is known that diet is an environmental element which can be manipulated; it has important consequences on genomics and proteomics functions and it is strongly connected to cancer^[34,35].

Inorganic phosphate, as a common dietary element, might modify cells behaviour. However, the possibility that Pi can modify cell functions and its relationship to cancer have to be fully understood and investigated further^[36,37].

By the way, the findings that inorganic phosphate, a simple "naturally occurring molecule", can have antiproliferative actions on some cancer cell types, depending on cell status and genetic background (p53, estrogen receptors, caspases expression, etc.) and can increase cytotoxic effects when combined with doxorubicin, show its potential for clinical applications, suggesting that up-regulating Pi levels at local sites for brief times might contribute to the development of novel and cheap modalities for therapeutic intervention in some tumors, including triple-negative breast cancer and osteosarcoma.

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