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REVIEWER PAPER

CALCIUM AND PHOSPHORUS IN MEDICINE AND TREATMENT

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Abstract

Calcium and phosphorus are essential for skeletal mineralization and perform a wide array of other biological functions. Calcium is a prime intracellular signalling molecule. It is also involved in muscle contractions (including the myocardium), digestion and blood coagulation. (Theobald 2005) Phosphorus is an intrinsic constituent of many organic substances such as nucleotides, nucleosides, phosphoamino acids and proteins, phospholipids, phosphoglycans and others (Huang, Moe 2011). In addition, it plays a critical role in cellular signalling through phosphorylation of proteins and other substances (Shaikh et al. 2008).

The present paper focuses on the role of these two elements in skeletal mineralization, and their use in treatment and medicine. First, it briefly discusses the calcium and phosphorus homeostasis, which occurs in three major organs: kidneys, intestines and bones, and involves an integrated hormonal system which maintains their normal serum levels. Moreover, disorders relevant to their abnormally high levels, hypercalcaemia and hyperphosphataemia, and excessively low levels, hypocalcaemia and hypophosphataemia, are described. Additionally, the physiology and pathology of bone as a prime store of both calcium and phosphorus are presented. Adequate intakes of these nutrients are essential for achieving the peak bone mass (PBM). A negative balance results from age-related bone loss, hence possible osteoporosis. This skeletal disorder is characterized by compromised bone strength, thus predisposing to an increased risk of fractures. One of the pharmacological interventions used in the treatment of osteoporosis is the administration of bisphosphonates. They inhibit the action of osteoclasts, prevent further bone losses and improve it strength (Ferrone, Geraci 2007). In the final part of the article, calcium phosphate based-ceramics, such as coralline hydroxyapatite, calcium hydroxyapatite (HAp), tri-calcium phosphate (TCP), bioglass, calcium phosphate cements (CPC) and their clinical applications are described.

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Key words: calcium, phosphorus, hypercalcaemia, hypocalcaemia, hyperphosphataemia, hypophosphataemia, osteoporosis, bisphosphonates, ceramics.

WAPŃ I FOSFOR W MEDYCYNIE I LECZNICTWIE

Abstrakt

Wapń i fosfor są niezbędne nie tylko dla mineralizacji kośćca, ale również w wielu procesach biologicznych. Wapń jest podstawowym przekaźnikiem informacji wewnątrzkomórkowej. Zaangażowany jest on również w skurcze mięśni (w tym mięśnia sercowego), trawienie oraz krzepnięcie krwi. Fosfor jest nieodłącznym składnikiem wielu substancji organicznych, takich jak: nukleotydy, nukleozydy, kwasy fosfoaminowe oraz białka, fosfolipidy, fosfoglikany itp. Ponadto wskutek fosforylacji białek i innych substancji odgrywa kluczową rolę w sygnalizacji komórkowej.

Praca skupia się na ich roli budulcowej oraz zastosowaniu w lecznictwie i medycynie. Na wstępie krótko przedstawiono homeostazę wapnia i fosforu, która dotyczy trzech głównych narządów: nerki, jelita i kości oraz obejmuje zintegrowany system hormonalny utrzymujący ich prawidłowe stężenie w osoczu. Ponadto opisano zaburzenia związane z ich nieprawidłowo wysokim – hiperkalcemia, hiperfosfatemia, oraz niskim – hipokalcemia, hipofosfatemia poziomem. Dodatkowo przedstawiono fizjologię i patologię kości, jako głównego magazynu zarówno wapnia, jak i fosforu. Odpowiednie spożycie tych substancji odżywczych jest niezbędne do osiągnięcia szczytowej masy kostnej (PBM). Ujemny bilans kostny wynika ze związanej z wiekiem utraty tkanki kostnej i może prowadzić do powstania osteoporozy. To zaburzenie funkcjonowania układu kostnego charakteryzuje się zmniejszoną wytrzymałością kości, co jest związane ze zwiększoną skłonnością do złamań. Bisfosfoniany są jedną z możliwości interwencji farmakologicznych stosowanych w leczeniu osteoporozy. Hamują one działanie osteoklastów, a tym samym zapobiegają dalszej utracie tkanki kostnej i wzmacniają jej wytrzymałość. Na zakończenie opisano wraz z klinicznym zastosowaniem ceramiki fosforanowo-wapniowe: hydroksyapatyt koralowy, hydroksyapatyt wapnia (HAp), fosforan trójwapniowy (TPC), bioszkło oraz cementy fosforanowo- wapniowe (CPC).

Słowa kluczowe: wapń, fosfor, hiperkalcemia, hipokalcemia, hiperfosfatemia, hipofosfatemia, osteoporoza

INTRODUCTION

Calcium is the fifth and phosphorus the sixth most abundant element in the human body (Huang, Moe 2011). An adult human body contains approximately 1200 g of calcium (Theobald 2005) and approximately 700 g of phosphorus in total (Moe 2008). Most of these elements (~99% of calcium and 80-90% of phosphorus) are found in bones, with smaller amounts stored in teeth. These two elements form an inorganic crystalline structure – hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. The remaining ~1% of calcium and 20-20% of phosphorus (10- 20%) are present in soft tissues and body fluids (Theobald 2005, Shaikh et al. 2008).

The recommended dietary intake of calcium is from 1,000 to 1,500 mg d^{-1} by healthy individuals, depending on the age. The key sources of dietary

calcium are milk and dairy products. In contrast to calcium, dietary phosphorus is present in abundance in most foods. It parallels dietary protein and is absorbed virtually twice as efficiently as dietary calcium. (BONJOUR 2009)

NORMAL PHYSIOLOGY

Calcium homeostasis

Calcium is metabolised mainly in kidneys, intestines and bones. It is regulated by the parathyroid hormone (PTH), its receptor PTHR, (POTTS, GARDELLA 2007), calcitriol (1,25 $(OH)_2$ vitamin D) and the vitamin D receptor (VDR). (JURUTKA et al. 2001)Extracellular ionized calcium levels are also maintained within the physiologic range owing to the calcium sensing receptor (CaR), which controls the PTH secretion. (THEMAN, COLLINS 2009) The reduction in plasma calcium inactivates CaR in the parathyroid cells, causing an increased PTH secretion, which acts on the PTHR. This results in an elevated calcium reabsorption in the kidney and net bone resorption. An increased secretion of calcitriol, which is also caused by the increased PTH, stimulates calcium absorption in the intestine through the activation of the VDR, decreased PTH secretion in the parathyroid glands and increased resorption in bones. An increase in serum calcium has contrary effects. (MOE 2008) This integrated hormonal response ensures the maintenance of normal serum calcium levels. It is strictly controlled within a narrow range, usually 8.5-10.5 mg dL^{-1} (2.12-2.55 mmol L^{-1}). (MAEDA et al. 2006)

Phosphorus homeostasis

Phosphorus metabolism is regulated by the fibroblast growth factor 23 (FGF-23) and the FGF receptor/Klotho complex (HUANG, MOE 2011) and, similarly to calcium homeostasis, by the PTH and PTHR. An increase in serum phosphorus causes FGF-23 secretion from the bone. The fibroblast growth factor 23 acts on the Na/Pi II co-transporters in renal cells, causing a decrease in phosphorus reabsorption. The FGF-23 also induces a decrease in the renal secretion of calcitriol, which is responsible for reducing the intestinal phosphorus absorption. The PTH has an opposite effect on 1,25 $(OH)_2$ vitamin D; its increase stimulates the secretion of calcitriol. A higher level of the PTH also decreases the renal phosphate reabsorption by acting on the renal Na/Pi II co-transporters. A decrease in serum phosphorus evokes contrary effects, leading to the restoration of normal levels of serum phosphorus (SHAIKH et al. 2008). Normal homeostasis maintains a serum phosphorus concentration between 2.5 to 4.5 mg dl^{-1} (0.81 to 1.45 mmol L^{-1}) (MOE 2008).

DISORDERS OF MINERAL METABOLISM

Hypercalcaemia

Hypercalcaemia occurs when the concentration of serum ionized calcium, which represents the biologically active fraction of total calcium, is abnormally increased. The most common symptoms caused by high serum calcium levels are gastrointestinal disorders, e.g. nausea, vomiting, constipation and abdominal pain. Hypertension and the shortening of the QT interval on an electrocardiogram are the cardiovascular effects. Hypercalcaemia can cause nephrogenic diabetes insipidus and lead to acute renal failure. In most cases, such disorders in calcium concentrations are caused by malignancy and hyperparathyroidism (Renkema et al. 2008). One of the pharmacological options used in the therapy of malignancy associated with hypercalcaemia is the intravenous administration of bisphosphonates. There are two such agents which have been approved in the United States, pamidronate and zoledronate. They induce osteoclast apoptosis. preventing bone resorption and therefore reducing the level of serum calcium (Drake et al. 2008).

Hypocalcaemia

Hypocalcemia results from a low ionized calcium concentration. Mild hypocalcaemia is asymptomatic. The periodontal numbness and carpopedal spasms are typical of large or abrupt changes in ionized calcium. In some cases, tetany might develop. Abnormally low calcium levels may be caused by precipitation into the extraskeletal tissue as well as some malignancies with blastic bone metastases. The hungry bone syndrome, which follows parathyroidectomy, induces a reduction in both ionized calcium and phosphorus concentration due to a sudden decrease in the PTH. Ionized calcium is also bound by phosphorus in acute hyperphosphataemia. There are two forms of intravenous calcium infusions which are recommended in symptomatic hypocalcaemia, calcium gluconate (10 ml vial = 94 mg elemental calcium) and calcium chloride (10 ml vial = 273 mg elemental calcium). Due to the risk of precipitation, this kind of treatment is not indicated for severe hyperphosphataemia. (Moe 2008) In asymptomatic hypocalcaemia, oral supplementation of calcium carbonate (which has 40% of calcium per tablet) is recommended (Nieves 2005).

Hyperphosphataemia

Hyperphosphataemia can be induced by increased intestinal absorption, cellular release or sudden intracellular to extracellular dislocations and reduced renal excretion. Persistent hyperphosphataemia is solely caused by acute or chronic kidney disease. High oral intake of phosphate-containing laxatives or enemas and vitamin D overdose induce an increase in the in-

testinal absorption. An acute tumour lysis syndrome, leukaemia as well as hyperthermia, haemolysis, rhabdomyolysis and profound catabolic stress increase the tissue release of phosphorus. Acute hyperphosphataemia typically does not cause symptoms, although the precipitation of phosphorus with calcium induces the symptoms of hypocalcaemia (Moe 2008). The treatment of hyperphosphataemia involves dietary phosphate limitation and oral phosphate binders such as calcium carbonate or calcium acetate (Rees, Shroff 2010).

Hypophosphataemia

Hypophosphataemia generally results from reduced intestinal absorption or increased gastrointestinal losses as well as excess renal wasting from renal tubular defects, extracellular to intracellular displacements and hyperparathyroidism. It is often classified as mild (<3.5 mg dl^{-1}), moderate (<2.5 mg dl^{-1}) and severe (<1.0 mg dl^{-1}). The haemolysis, rhabdomyolysis, impaired platelet and WBC function, muscle weakness and rarely neurologic disorders are the characteristic symptoms of moderate and severe hypophosphataemia. The most common causes of hypophosphataemia are antacid abuse, chronic diarrhoea, vitamin D deficiency, alcoholism, extracellular fluid volume expansion and primary hyperparathyroidism. In the treatment of moderate and severe hypophosphataemia, oral supplementation is more appropriate than intravenous phosphate preparations. After intravenous administration, phosphorus can complex calcium and induce extraskeletal calcification. Neutraphosph K capsules® or Neutraphosph® solution are the pharmacological oral intake options. (Moe 2008)

BONE PHYSIOLOGY AND PATHOLOGY

The skeleton is a dynamic organ which consists of long bones, vertebrae and the skull. The prime store for both calcium and phosphorus is bone. It contains a number of specialized cells and collagen fibres encrusted with crystalline material. Osteoblasts are responsible for the production of bone collagen fibres and other organic constituents of the matrix. These forms of fibroblast are engaged in the process of bone mineralisation and partially in the regulation of bone resorption. Osteocytes, which are mature osteoblasts, cannot create new bone because they are encased in mineralized bone. The third kind of bone cells are osteoclasts, i.e. macrophages derived from stem cells of bone marrow. They are situated on the surface of bone and induce bone resorption.

Each bone consists of two forms of bone tissue: compact and trabecular. The exterior parts of bones, especially the shafts of long bones, are built of compact bone, which is thick and dense. It plays the structural role and

forms cortices. The interior parts of long bones are made up of a network of trabeculae, which have a metabolic function. This spongy trabecular bone tissue occurs primarily in vertebrae of the spine and in the pelvis. Compact bone constitutes about 80% of bone; the remaining 20% is trabecular (THEOBALD 2005).

All bones in the human body are continually remoulded throughout the lifespan. The bone remodelling process repairs the bones, hence strengthens the skeleton. This bone turnover consists of the balanced activity of osteoclasts, which induce bone resorption, and osteoblasts, which result in bone formation. Approximately 4% of cortical bone and 28% of trabecular bone undergo modification each year (FERRONE, GERACI 2007). Healthy skeletal growth of children ensures positive bone balance (formation > resorption). The peak bone mass (PBM) reached by healthy adults indicates neutral bone balance (formation = resorption). Negative bone balance (formation < resorption) is characteristic of elderly individuals and results in aged-related bone loss.

Osteoporosis

Osteoporosis is described as a skeletal disorder characterized by low bone mass and compromised bone strength, causing an increase in bone fragility and susceptibility (HONIG 2010), The diagnostic criteria for osteoporosis are based on the bone mineral content (BMC) or bone mineral density (BMD). BMD within 1 standard deviation (SD) of a young adult is considered normal, between -1 and -2.5 SD is diagnosed as osteopenia and more than -2.5 SD is considered as osteoporosis or established osteoporosis if, at the same mass definition, an osteoporosis-related fracture occurs. Fragility fractures, which are specific to osteoporosis, involve the vertebral body, distal forearm and proximal femur, although they can occur in various parts of the skeleton (CASHMAN 2007). The fractures resulting from osteoporosis induce an increase in morbidity and mortality. After the age of 50 years, the risk of such fracture in women is 40% and in men 15% (EASTELL 2005).

Adequate nutrition plays a crucial role in prevention and treatment of osteoporosis. Calcium, often in combination with vitamin D, and phosphorus are the nutrients of greatest significance.

Calcium in osteoporosis

A number of studies have demonstrated that higher calcium intake at every stage of life is associated with higher bone mineral density. Increased calcium intake can maximize the peak bone mass and optimize the rate of bone loss associated with aging (North American Menopause Society 2006, TANG et al. 2007, HUNCHAREK 2008).

An inadequate intake of calcium results in an increase in the PTH levels, which in turn causes an increase in bone remodelling, leading to

a significant loss of bone and increased fracture risk. Elevations in the serum PTH and larger bone losses are usually associated with lower levels of vitamin D. Calcium given in sufficiently large doses may reduce elevated PTH levels and lower the rate of bone remodelling. Thus, when adequate quantities of dietary calcium cannot be provided, calcium supplementation is required. Calcium carbonate and calcium citrate should be used, optimally in doses <500 mg a day, because calcium absorption decreases with larger loads. Calcium is also better absorbed with food, hence most supplements should be taken with meals (NIEVES 2005). Calcium in a dose of 1,000 mg day^{-1} in combination with vitamin D in a dose of 500 IU day^{-1} has been demonstrated to prevent hip fractures in elderly housebound patients (EASTELL 2005). Moreover, calcium supplementation also delays bone losses in postmenopausal women. This effect on bone mass may be altered by factors such as the age, number of years since the menopause, baseline calcium intake before supplementation and physical activity. The benefits of taking calcium supplements may be greater in elderly and late postmenopausal women as well as in women with low baseline calcium intakes. Hormonal replacement therapy (HRT), which is one of the ways to prevent osteoporosis, appears to be more effective with calcium supplementation (NIEVES 2005, CASHMAN 2007).

Phosphorus in osteoporosis

An adequate phosphorus intake is crucial for bone health. Hence, a decrease in serum phosphorus will restrict bone formation and mineralization. (HEANEY 2004) On the other hand, an excessive intake of phosphorus, especially when combined with a low calcium intake, may be deleterious. In such cases, it may also lead to secondary hyperparathyroidism and bone loss. Phosphorus deficiency resulting from a low phosphorus intake or negative phosphorus balance due to food phosphorus bound to supplemental calcium can restrict the activity of osteoblasts and increase the bone resorption. Higher bone density is relevant to a diet adequate in calcium, moderate protein and sufficient phosphorus, hence the ratio of phosphorus to calcium is more significant than the intake of phosphorus alone. (HEANEY, NORDIN 2002)

Bisphosphonates

Phosphorus is a basic component of primary pharmacologic agents currently used against bone loss due to osteoporosis. Bisphosphonates are synthetic analogues of natural pyrophosphate, in which oxygen occupies the place of a carbon atom making the compounds resistant to hydrolysis. Their crucial feature is a very high affinity for bone mineral, especially at sites of active bone remodelling and consequent deposition. Bisphosphonates inhibit bone resorption by decreasing the recruitment and function of osteoclasts and increasing apoptosis (GASS, DAWSON-HUGHES 2006), Their anti-resorption

activity peaks within approximately 3 months of a course of oral bisphosphonate therapy and remains fairly constant with the continuation of treatment. Intravenous (IV) bisphosphonate administration causes a more rapid decreasein bone resorption than oral bisphosphonate therapy. The duration of the inhibition mostly depends potency of bisphosphonates for binding hydroxyapatite crystals. All bisphosphonates are poorly absorbed in the gastrointestinal tract, which is due to their hydrophilic properties. Additionally, about 50% of the uptaken quantity is retained in the skeleton and the remaining amount is cleared from the circulation by renal excretion. Importantly, when oral bisphosphonate is administered, patients should remain upright for 30 minutes and avoid eating for 2 hours before and at least 30 minutes after taking a pill. Maintenaning an adequate calcium and vitamin D supply is also a key issue in the bisphosphonate therapy.

Possible pharmacological options include alendronate and risedronate, which can be administered once a week, and ibandronate and risedronate taken once a month. Less frequent dosing requires intravenous preparations such as pamidronate, ibandronate and zoledronic acid (Drake et al. 2008). Recently, bisphosphonates have become the major therapeutic solution, commonly chosen to treat not only osteoporosis but also Paget's disease (Reid et al. 2005), osteogenesis imperfecta (OI) (Akcay et al. 2008), hypercalcaemia and malignancy metastatic to bone (Clemons et al. 2006). However, the latest reports indicate that the use of bisphosphonate therapy is implied in pathologic conditions, including osteonecrosis of the jaw (Kramer, Fantasia 2011).

Calcium phosphate as ceramics

Calcium and phosphorus in a form of calcium phosphate-based ceramics have been used in dentistry and in orthopaedics since 1980s. These synthetic scaffolds have been proven to induce a biologic response similar to that of bone. Alone, the ceramics show minimal immediate structural support and do not have any osteogenic or osteoinductive properties. Once attached to healthy bone, the osteoid is produced directly onto the surfaces of the ceramic, thus it mineralizes and causes new bone remodelling.

Coralline hydroxyapatite

The naturally occurring porous structure made by calcium phosphate (coralline), which is similar to human cancellous bone, was found in certain coral species. These sources of hydroxyapatite of natural origin are transformed, yet maintaining their macroporous and interconnected structure. Hence, they provide an in-growth of host tissue upon implantation and diffusion of a nutrient throughout the graft material. These coralline-derived ceramics are known as Pro Osteon ® (Interpose Cross International, USA) and Biocoral ® (Biocoral, USA) (Kretlow et al. 2009).

Calcium hydroxyapatite (HAp)

Synthetically obtained hydroxyapatite (HA) is typically sintered at temperatures above 1,000°C and is a highly crystalline form of calcium phosphate. It has the formula $Ca_{10}(PO_4)_6(OH)_2$ with a calcium-to-phosphate atomic ratio of 1.67. Its chemical similarity to the mineralized phase of bone explains the osteoconductive and biocompatible properties. Synthetic hydroxyapatite is typically used as a coating for dental and orthopaedic implants (Nandi et al. 2010). Plasma spray HA has been applied to a metallic femoral stem and cup. It prevents complications caused by the use of the poly(methyl methacrylate -PMMA (Moroni et al. 2005). Hydroxyapatite-coated pins improve pin attachment regardless of the type of bone and loading conditions. They also depress the rate of infection and losses during external fixation (Nguyen et al. 2004).

Tri-calcium phosphate (TCP)

This synthetic material, $Ca_3(PO_4)_2$, is similar in chemical composition and crystallinity to the mineral phase of bone. Similarly to hydroxyapatite, the bioabsorbility and biocompatibility are also characteristic of TCP. There are two forms of tri-calcium phosphate, α and β-crystalline. In contrast to hydroxyapatite, which is non-resorbable under physiological conditions, β-TPC has been demonstrated to degrade within 6 weeks after implantation (Kretlow et al. 2009). Tri-calcium phosphate implants have been used for two decades in dental applications and in orthopaedics as synthetic bone void fillers (Shigaku, Katsuyuki 2005).

Bioglass

Bioactive glass ceramics are composed primarily of silica, sodium oxide, calcium oxide and phosphates. They bind to bone without an intervening fibrous connective tissue interface. This material is also biocompatible and osteoconductive (Dorozhkin 2009). Bioglasses have been used in clinical applications as filling material in benign tumour surgery, in obliteration of frontal sinuses, for tympanoplastic reconstruction, repair of orbital floor fractures, treatment of periodontal bone defects, lumbar fusion, reconstruction of facial bone defects as well as for reconstruction of iliac crest defects after bone graft harvesting (Nandi et al. 2010).

Calcium phosphate cements (CPC)

Generally, calcium phosphate cements are formed by combining dry powder (CaP) and a liquid component in a reaction which occurs under physiologic pH and temperatures. Dicalcium phosphate, dicalcium phosphate dihydrate, calcium-deficient hydroxyapatite and amorphous calcium phosphate have been used as the solid phase. As a liquid phase, an inorganic or organic acid, or else sodium phosphate solutions can be used. The injectability

and ability to mould for variable periods before hardening are specific to calcium phosphate cements. At least three formulations of CPC have been approved by the Federal Drug Administration for clinical use: Norian ® (Synthes Craniomaxillofacial, USA), Mimix ® (Walter Lorenz Surgical, USA) and BoneSource ® (Stryker Leibinger, Germany) (KRETLOW et al. 2009) They have been successfully used for clinical applications such as cranial defect repair (JI, AHN 2010).

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