CHANGES IN BONE METABOLISM AND STRUCTURE IN PRIMARY HYPERPARATHYROIDISM

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Abstract. *Parathyroid hormone (PTH) is a key regulator of bone turnover. Depending on the duration of action, the hormone causes catabolic and anabolic effects by binding with specific receptors (PTH1R) in the bone. Various cells expressing PTH1R on their surface are involved in the process – osteoblasts, osteocytes, bone marrow stromal cells, T-lymphocytes and macrophages. In physiological conditions PTH balances the bone metabolism. Intermittent pharmacological doses of PTH lead to the prevalence of bone formation and are used in the treatment of osteoporosis. Persistently elevated levels of PTH stimulate bone resorption by impacting mainly the cortical bone. New imaging and analysis techniques show that high PTH levels can also have an adverse effect on trabecular microarchitecture. Primary hyperparathyroidism (PHPT) is a disease characterized by increased bone metabolism, decreased bone mineral density (BMD), inadequate osteoid mineralization and an increased risk of fractures. Prolonged overproduction of PTH leads to stimulation of bone resorption and defects in bone formation, mainly causing loss of cortical bone mass, while in the trabecular bone predominate demineralization processes. One explanation of these findings is the enhanced stimulation of RANKL expression by osteoblasts with decreased OPG expression and bone formation at the same time.*

Key words: *Parathyroid hormone, primary hyperparathyroidism, bone turnover, Th17, osteoclasts, RANKL.*

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PHYSIOLOGICAL ACTION OF THE PARATHYROID HORMONE

Parathyroid hormone (PTH) is an 84 amino acids polypeptide, which is synthesized by the main cells of the parathyroid glands. It is one of the main regulators of calcium-phosphate homeostasis. Its synthesis and secretion are negatively controlled by calcium-sensitive receptors (CaSR) located on the membrane of parathyroid cells [1]. The low level of extracellular ionized calcium stimulates the production and release of PTH, while an increase in the calcium concentration leads to the receptor engagement and suppression of PTH secretion. Low

level of inorganic phosphate can also stimulate the synthesis and secretion of PTH directly or indirectly - by reducing the calcium levels [2].

PTH maintains the plasma levels of calcium and inorganic phosphate in the plasma within very narrow limits by various mechanisms. The hormone activates the 1-alpha hydroxylase enzyme in the kidney, which catalyzes the conversion of 25(OH) vitamin D to its active metabolite, calcitriol (1.25 (OH) 2D). Calcitriol is the main factor for calcium absorption in the intestine and to a less extent – for the absorption of inorganic phosphate. In addition, PTH increases the calcium reabsorption in the renal tubules and decreases the reabsorption of inorganic phosphate [2].

Furthermore, PTH regulates bone remodeling. It stimulates both osteoclast bone resorption and osteoblast bone formation, depending on the duration of its action [3]. PTH provides the balance between the two processes in physiological conditions and regulated the calcium and phosphate flux to and from the bone.

THE IMPACT OF PTH ON THE BONES

PTH impacts bones by binding with specific surface receptor – PTH receptor type 1 (PTHr1). Various cells in the bone microenvironment such as osteoblasts, osteocytes, bone marrow stromal cells, T cells and macrophages express PTHr1 on their surface.

Depending on the duration of exposure, PTH binding to PTHr1 on the surface of osteoblasts and their precursors causes either bone formation or bone resorption. Intermittent administration of PTH inhibits adipocyte differentiation of bone marrow stem cells from the osteoblast line, increases the number of osteoblast progenitors by suppressing their apoptosis and stimulates osteoblast proliferation and differentiation [3-5]. This happens through increasing the expression of osteoblast-specific genes such as Runx2, collagen type I α 1 in immature osteoblasts and alkaline phosphatase and osteocalcin in mature osteoblasts [6]. PTH reduces the expression of proapoptotic genes such as Bad, and enhances the expression of antiapoptotic proteins such as Bcl-2 [7]. In addition, PTH stimulates DNA repair, resulting in increase of osteoblast survival [8]. The anabolic effect of PTH requires the presence of insulin-like growth factor-I (IGF-I), fibroblast growth factor-2 (FGF-2) and Wnt-proteins [9].

Wnt proteins induce the canonical Wnt/ β -catenin signaling pathway by pairing with a dimeric receptor complex composed of the Frizzled (Fzd) receptor and LDL co-receptor proteins (LRP 5/6). Activation of Wnt signaling stimulates the proliferation and differentiation of osteoblasts [10], prevents their apoptosis [11, 12] and increases production of osteoprotegerin (OPG) [13]. PTH is a canonical agonist of Wnt signaling, increasing β -catenin levels in osteoblast cells, thus stimulating bone formation [14]. In addition, PTH inhibits the production of sclerostin (osteocyte-secreted antagonist of Wnt signal) [15], Dickkopf-1 (LRP5 and LRP6 inhibitor) [14] and Sfrp-4 (antagonist of Wnt signaling) [16].

In contrast to osteoblasts, osteoclasts lack PTHr1. Their activation occurs indirectly with the participation of two essential cytokines – the macrophage colony-stimulating factor (M-CSF) and the receptor activator of nuclear factor kappa-B ligand (RANK) – RANKL. These cytokines stimulate osteoclast differentiation and activation, and subsequently bone resorption, by binding with their receptors on the osteoclasts. The role of PTH in these processes is to stimulate the expression and secretion of RANKL and M-CSF in the osteoblasts [17]. Osteoblasts also express another

mediator involved in bone remodeling – osteoprotegerin (OPG). OPG is a soluble decoy receptor for RANKL. By combining with RANKL, it prevents the formation of the RANKL-RANK complex and the subsequent bone resorption [18, 19]. Therefore, osteoclastogenesis is modulated by the balance in OPG and RANKL concentrations [20]. Prolonged infusion of PTH results in increased RANKL expression by osteoblasts, decreased OPG expression, increased RANKL/OPG ratio, and stimulation of osteoclastogenesis and bone resorption [21, 22].

Osteocytes also play a major role in the bone processes affected by PTH [23]. They participate in the regulation of bone homeostasis by coordinating the activity of osteoblasts and osteoclasts [24]. Osteocytes are the main source of RANKL [25] and the only source of sclerostin (Scl) – an inhibitor of Wnt signaling, which suppresses bone formation by reducing osteoblast differentiation, function and survival [26]. Intermittent doses of PTH have a direct inhibitory effect on Scl and stimulate bone formation as a result [27].

THE ROLE OF T CELLS IN PTH INDUCED BONE PROCESSES

Clinical studies with experimental animals demonstrated that PTH-induced bone loss was prevented in animals lacking T-lymphocytes. These observations have suggested that T cells are involved in the mechanisms through which PTH affects bone metabolism. In addition, T-lymphocytes express PTHr1 and disorders in these receptors suppress the catabolic effects of PTH [28]. A specific surface molecule expressed by activated T cells called CD40 ligand (CD40L) is associated with the lack of PTH-induced bone resorption in immunodeficient experimental animals. CD40L helps T-lymphocytes to pair with specific CD40 receptors on the surface of bone marrow stromal cells and sensitize them to the action of PTH. This enhances RANKL expression by stromal cells, decreases OPG expression, and increases osteoclastogenesis [29, 30]. Ablation of CD40L on T cells decreases the bone catabolic activity of PTH by reducing the number of stromal cells, lowering the RANKL/OPG ratio, and decreasing bone resorption [29].

There is evidence of the role of T lymphocytes in the anabolic action of PTH in the bones as well. M. Terachi et al. showed that intermittent administration of PTH to mice stimulates Wnt10b expression by T cells. Wnt10b activates the canonical Wnt signaling pathway in osteoblasts and stimulates the bone formation [31]. P. D'Amelio et al. studied the effect of intermittent and persistently high levels of PTH on Wnt10b production by T lymphocytes in humans [32]. They found increased expression of Wnt10b by T cells following intermittent PTH application, but not in subjects with PHPT.

Persistently elevated PTH levels stimulate the differentiation of CD4 lymphocytes into specific CD17 positive T-helpers (Th17). These cells potentially induce osteoclastogenesis through IL-17A secretion

[29, 33]. IL-17A is a proinflammatory cytokine, part of the IL-17 family of six members (IL-17A to IL-17F) involved in chronic inflammation and tissue destruction. IL-17A binds with specific membrane receptors - IL-17R. Many cells such as osteoblasts, osteocytes, fibroblasts, epithelial and endothelial cells express these receptors. The IL-17/IL-17R complex stimulates the secretion of RANKL by osteocytes and increases osteoclastogenesis. Jau-Yi Li et al. demonstrated that there is no PTH induced bone resorption and loss in mice without IL-17R [34]. In the same study, treatment of mice with neutralizing anti-IL-17A antibodies blocked PTH-induced bone loss and prevented RANKL production by osteoblast cells [34].

PTH stimulates the T-cell production of another inflammatory cytokine – tumor necrosis factor alpha (TNF α), which enhances RANKL activity and osteoclastogenesis [35]. In addition, TNF α induces the differentiation of CD4-T cells into Th17 cells and the subsequent secretion of IL-17A.

PRIMARY HYPERPARATHYROIDISM AND CHANGES IN BONE TURNOVER

Primary hyperparathyroidism (PHPT) is a condition characterized by chronic overproduction of PTH by the parathyroid glands. It occurs as a result of parathyroid adenoma, hyperplasia and, in very rare cases, parathyroid carcinoma. Highest incidence of PHPT was recorded in the seventh decade, affecting mostly women (74%). Prior to age 45, the incidence of the disease was similar in both sexes [36].

Prolonged overproduction of PTH, which is observed in PHPT, leads to stimulation of bone resorption and a defect in bone formation, causing loss of cortical and often trabecular bone mass. This is due to enhanced stimulation of RANKL expression by osteoblasts, in parallel with decreased OPG expression and bone formation. As a result, bone turnover is increased, bone mineral density (BMD), especially of cortical bone, is decreased and the risk of fractures increases.

Persistent high levels of PTH in the bone microenvironment alter the formation/resorption balance through the mechanisms described in the previous section. It is assumed that bone marrow lymphocytes play a key role in this process. Jau-Yi Li et al. investigated the effect of PHPT on the production of inflammatory cytokines in humans by comparing the IL-17A levels in healthy individuals and patients with PHPT before and 1 month after surgery. The study found three-fold higher IL-17A in patients with PHPT compared to controls. In addition, normalization of the studied proinflammatory cytokines in the PHPT group was observed after successful parathyroidectomy. The authors also found an association of PHPT with increased levels of TNF α and IL-23 [34]. These data suggest that the increased expression of IL-17A in patients with PHPT is due to the elevated levels of circulating PTH. However, the

possibility of participation of other factors regulated by PTH cannot be ruled out.

A typical manifestation of PHPT is the diffuse demineralization of the skeleton with subperiosteal bone resorption, affecting mainly the cortical bone. The impaired mineralization is the result of the increased bone turnover and osteoid formation and the PTH-induced phosphate loss. The demineralization of cranial bones leads to a typical thinning giving it a “salt and pepper” appearance. Increased osteoclast activity predisposes to induction of the inflammatory cascade with infiltration of macrophages and growth of fibrous tissue in the medullary spaces. The result is the so-called “osteitis fibrosa cystica”, with typical bone cysts and brown bone tumors [37-39]. Brown bone tumors are multifocal bone lesions formed in areas with rapid bone resorption. Their color is due to the tendency to hemorrhage and hemosiderin deposits formation. They are more often localized in the jaw, skull, pelvis, clavicle, ribs, femurs and spine. Bone lesions are clinically manifested by pain, skeletal deformities and pathological fractures [40-42].

The number of diagnosed asymptomatic forms of PHPT has increased in recent decades after the expanded routine measurement of serum calcium. The severe bone manifestations of the disease have become less common. However, evidence of bone involvement can be found in a large number of asymptomatic patients through highly sensitive methods of assessing bone changes.

PHPT AND BONE MINERAL DENSITY

The gold standard for determination of bone loss is Dual-energy X-ray absorptiometry (DXA). This is a non-invasive method that can detect decrease in BMD as low as 2%. Low-dose X-rays are used to measure the degree of absorption of X-rays by bone and soft tissue.

Bone damage in PHPT is characterized by greater involvement of cortical bone than trabecular bone [43], in contrast to postmenopausal osteoporosis, where trabecular bone loss predominates [44]. Hence, in PHPT, lower BMD is usually seen in bones with a predominantly cortical fraction, such as the distal radius (the border between the middle and distal thirds of the radius) [45]. No change in lumbar spine BMD has been observed in a cohort study that monitored changes in BMD in patients with PHPT over a period of 15 years [46]. However, the authors of the same study found a decrease in the proximal femur BMD and even more in the distal radius BMD. These results suggest that lumbar spine BMD is not a useful predictor of fracture risk in PHPT. The study also suggested the measurement of distal radius BMD might be mandatory in all patients with PHPT [47]. Due to the fact that PHPT affects menopausal women more often [36] many cases demonstrate a mixed densitometric

pattern with simultaneous or predominantly reduced lumbar vertebral BMD. In addition, data from new imaging and analysis techniques such as high-resolution quantitative computed tomography (HRpQCT) and trabecular bone score (TBS) suggest that high PTH may also adversely impact the trabecular microarchitecture [48, 49]. Therefore, the measurement of BMD at all skeletal sites is recommended (lumbar spine, proximal femur and distal radius) in PHPT [50].

PHPT AND BONE TURNOVER MARKERS

Bone turnover markers provide another instrument for bone loss assessment. They reflect the secretory and breakdown products of bone cells and bone collagen and are used as indirect indicators to assess bone metabolism. Bone formation markers are products of osteoblast activity. Bone formation markers that have been more extensively studied are total alkaline phosphatase (ALP) and its bone fraction (BALP), procollagen type 1 N-terminal propeptide (P1NP), procollagen type I C-terminal propeptide (PICP) and osteocalcin. Bone resorption markers like deoxypyridinoline (DPD), C-terminal telopeptide of type 1 collagen (CTX), and hydroxylysine glycosides are products of collagen degradation.

These markers can be useful indicators of the degree of bone involvement in PHPT. Being a disease of increased bone metabolism, PHPT is characterized by elevated markers of both bone formation and bone resorption [50]. There is a direct relationship between their level and the severity of PHPT [51, 52].

EFFECT OF PHPT TREATMENT ON BONE REMODELING

Parathyroidectomy is the most effective method for curative treatment of PHPT. It is indicated in all cases of symptomatic PHPT and parathyroid carcinoma. Parathyroidectomy is also recommended in some patients with asymptomatic PHPT, including those with a BMD below -2.5 SD or a previous low-energy fracture as recommended by the American Association of Endocrine Surgeons (AAES) criteria for surgical treatment [47].

The level of PTH in the body drops rapidly after the removal of the abnormal parathyroid gland with subsequent normalization of serum calcium levels. This leads to a rapid decrease in bone resorption [53] and slower increase in BMD (up to 10-12%) in areas with trabecular bone (spine and proximal femur). Those changes are the result of re-mineralization occurring in the trabecular bone after parathyroidectomy. Although PTH exerts its catabolic effect mainly in the cortical bone, there is no significant postoperative increase in BMD in these areas [46, 53, 54].

The postoperative period is also associated with a rapid reduction in the markers of bone resorption, followed by a gradual decrease in those of bone for-

mation, most significantly during the first year. The degree of postoperative increase in BMD is higher in patients with more severe disease and depends proportionally on preoperative calcium and PTH levels [55]. A positive association was found between BMD and the levels of bone turnover markers after parathyroidectomy [52, 56-60].

Medical treatment is applied in case of contraindications for surgery, patient refusal, failed parathyroidectomy or lack of surgical criteria for operation. The aim is to suppress the increased secretion of PTH and normalize the levels of calcium and inorganic phosphate, as well as to prevent or alleviate the complications. Two groups of drugs are mainly used – antiresorptive drugs, which reduce bone metabolism, and calcimimetics, which impact the secretion of PTH.

Antiresorptive drugs inhibit osteoclast activity and reduce bone resorption, leading to a decrease in serum and urinary calcium levels. However, there is a tendency for reduction of this effect with time, as a result of the remodeled space filling [61].

Most studies with PHPT patients have been performed with bisphosphonates and mainly alendronate. A rapid decrease in the bone resorption markers was observed during the treatment course, with a subsequent decrease in the bone formation markers [62], i.e. alendronate inhibits bone resorption and, at a later stage, bone formation, leading to an increase in BMD. Several studies have shown a tendency towards increase in PTH levels during alendronate treatment [62]. Denosumab is a monoclonal anti-RANKL antibody that “switches off” the RANKL/RANK activation of osteoclast formation, recruitment and activation. As such it is currently indicated for treatment of postmenopausal osteoporosis and also reduces serum calcium levels in hypercalcemia of variable origin including PHPT. Several research groups have reported increase in BMD and decrease in bone turnover in subjects with PHPT treated with denosumab.

Other anti-osteoporosis drugs, such as estrogens, oral contraceptives, and selective estrogen receptor modulators (SERMs), have similar effects in patients with PHPT. These treatments lead to a decrease in serum calcium levels, reduction in bone turnover markers and increase in BMD, without changes in PTH plasma levels. Their clinical use however is limited.

Calcimimetics are a group of drugs that interact with the membrane calcium receptor (CaSR) on the parathyroid cells and the kidneys induce conformational changes in the receptor, sensitize it to the calcium ions and suppressing PTH secretion. Cinacalcet has been shown to reduce plasma calcium and PTH levels and urinary calcium excretion. However, with regard to bone, an increase in the markers of bone remodeling has been reported with no effect on BMD. It was proposed that the cause is an interaction of calcimimetics with the CaSRs expressed on the os-

teoclasts, with subsequent stimulation of osteoclastogenesis and bone resorption.

Vitamin D deficiency is common in PHPT and exacerbates the risk of bone damage. A number of studies have demonstrated that vitamin D replacement therapy in PHPT reduces serum PTH and improves bone mass without increasing blood and urine calcium levels.

SUMMARY

Primary hyperparathyroidism (PHPT) is an endocrine disorder characterized by elevated serum calcium levels, decreased inorganic phosphate levels, and inadequately high PTH-to-calcium ratio. The number of diagnosed asymptomatic forms of the disease has increased in the recent decades with the introduction of routine measurement of serum calcium. The severe classical clinical manifestations associated with bone and kidney involvement, such as bone cysts, brown bone tumors and nephrocalcinosis, have become less common. However, high PTH leads to bone damage, resulting in increased bone metabolism and bone loss, even in asymptomatic cases.

PTH is a key regulator of bone turnover. Various cells expressing PTHR1 on their surface such as osteoblasts, osteocytes, bone marrow stromal cells, T-lymphocytes and macrophages, are involved in PTH-induced bone processes. Osteoclasts do not have PTHR1, but they are activated indirectly with the involvement of osteoblasts and the RANKL-RANK-OPG system. PTH has both catabolic and anabolic effects in the bone by pairing with specific receptors (PTHR1), and the balance between them depends on the duration of PTH action. In physiologic conditions the hormone modulates bone metabolism. Intermittent exogenous doses of PTH stimulate bone formation, while persistently high concentrations of PTH are responsible for increased bone resorption and subsequent bone loss.

In PHPT the balance between bone formation and bone resorption is skewed towards the latter with subsequent deterioration of bone quality and increased fracture risk. A characteristic feature of the disease is the adverse impact on the cortical bone compared to the trabecular bone. New imaging and analysis techniques, such as high-resolution quantitative computed tomography (HRpQCT) and trabecular bone score (TBS), show that high PTH can also affect the trabecular microarchitecture.

A number of studies with experimental animals in recent years have suggested that T cells are involved in the mechanisms by which PTH affects bone metabolism. T cells synthesize proinflammatory cytokines (TNF α and IL-17) in response of PTH action in PHPT. These cytokines stimulate RANKL expression, inhibit OPG expression and induce osteoclastogenesis and bone resorption.

Recent advances in medical knowledge have just started to unravel the complexity of the interactions of PTH

with the bone in PHPT. Further research is necessary to evaluate the connection between bone marrow cells, the immune system and PTH-induced phenomena in the bone. Clarifying the clinical significance of these mechanisms would help also identify new therapeutic targets for the treatment of bone disease in PHPT.

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