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Review

Pathogenesis of secondary hyperparathyroidism

MARIO COZZOLINO, SABINA PASHO, GIUDITTA FALLABRINO, LAURA OLIVI, MAURIZIO GALLIENI, DIEGO BRANCACCIO

Renal Division, San Paolo Hospital, University of Milan, Milan - Italy

ABSTRACT: Chronic renal failure is the primary cause of secondary hyperparathyroidism (SHPT). Patients with mineral metabolism disorders commonly present with low serum calcium levels, hyperphosphatemia, and calcitriol deficiency. In normal renal function subjects, parathyroid cells have a low turnover and rarely undergo mitoses. In uremic conditions, however, parathyroid glands become hyperplasic and leave quiescence. During the last ten years, new molecular mechanisms have been investigated to better understand the pathogenesis of SHPT: the emerging role of the Calcium Sensing Receptor (CaSR); the importance of the parathyroid expression of the Vitamin D receptor (VDR); the growing evidence on the central role of the Fibroblast Growth Factor 23 (FGF-23). In contrast, the discovery of a parathyroid phosphate sensor or receptor has yet to be made. (Int J Artif Organs 2009; 32: 75-80)

KEY WORDS: PTH, Calcium, Phosphate, Vitamin D, Renal failure

INTRODUCTION

High phosphate (P), low calcium (Ca), and vitamin D deficiency represent the classical "triad" of the pathogenesis of secondary hyperparathyroidism (SHPT) in renal insufficiency, in which the down-regulation of the parathyroid Vitamin D Receptor (VDR) and the Calcium Sensing Receptor (CaSR) is a critical step (1). Unfortunately, thus far no data have supported the existence of a parathyroid P receptor/sensor. Disturbances in mineral and bone metabolism have traditionally been termed "renal osteodystrophy" and have been classified based on bone biopsy. Recently, however, KDIGO (Kidney Disease: Improving Global Outcomes) developed an internationally acceptable definition for renal osteodystrophy that should be used exclusively to define alterations in bone morphology associated with chronic kidney disease (CKD) (2). The term CKD-Mineral and Bone Disorder (CKD-MBD) can be used to describe bone and mineral metabolism abnormalities and/or ectopic calcification.

During the last decade, a new view of the molecular mechanisms of P homeostasis and SHPT pathogenesis has been opened up with the Fibroblast Growth Factor 23 (FGF-23) as a novel player in the field (3). Increased serum FGF-23 levels induce hypophosphatemia, calcitriol suppression, and hyperparathyroidism. In contrast, low FGF-23 is associated with high serum P levels, increased calcitriol levels, and hypoparathyroidism. Extensive research into several FGF-23 actions is still required (4).

The role of calcium

The Ca content in a healthy adult body is 1,000-2,000 grams (25,000-50,000 mmol). In particular, only less than 2% of Ca is present in the extracellular fluid (ECF), while >98% is part of the mineral component of bone. The Ca of the mineral phase at the surface of the crystals is in equilibrium with ECF Ca, even if only a minor fraction of the total pool (0.5%) is really exchangeable. Under normal conditions, both ECF Ca concentration and body Ca content are maintained at fixed values (5). Furthermore, the Ca in ECF is critical for different functions, and Ca ions inside the cell play a variety of cellular functions as well. Most intracellular Ca is found in insoluble complexes. In addition, intracellular Ca levels are very low (0.1mmol/L). The gradient between intracellular and plasma free Ca levels is constantly regulated, playing a critical role for the functional regulation of the single cell.

The normal plasma levels of Ca in healthy adults are in a range from 8.8 to 10.4 mg/dL (from 2.2 to 2.6 mmol/L). Plasma Ca is present in three forms: a) free ions; b) ions bound to plasmatic proteins; c) diffusible complexes. Importantly, free Ca ion concentrations might have a strong

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influence on many cellular functions, subject to tight control from the parathyroid hormone (PTH) and vitamin D ($1,25(OH)_2D_3$) (6). Because most Ca ions are bound to albumin, plasmatic protein concentration is a very important factor when the Ca ion concentration is investigated. The pH of plasma influences the percent of protein-bound Ca (7). In particular, to distinguish the ionized Ca from the Ca protein-bound fractions, the K/DOQI guidelines state that total Ca levels have to be adjusted for serum albumin concentration in order to better describe the free Ca (8). Usually, the following formula is used:

Corrected Ca (mg/dL, mmol/L) = Total Ca (mg/dL, mmol/L) + 0.02 x [40 – Serum Albumin (g/L)]

Extracellular Ca activates the extracellular CaSR, which is present in different tissues, such as the PT glands, thyroid, intestine, kidney, bone, bone marrow, brain, skin, lung, pancreas, and heart. Once CaSR is activated by Ca, it couples to a complex array of intracellular signal transduction cascades (7). Low serum Ca levels decrease the activation and the expression of the CaSR, a plasma membrane G-protein coupled molecule that allows PT cells to sense Ca ions in the extracellular fluid, thus greatly promoting PTH synthesis and secretion (9). In contrast, hypercalcemia activates the CaSR, rapidly suppressing SHPT. Recent evidence suggests that signaling through the CaSR plays an important role on PT hyperplasia (10). Moreover, Ca-dependent signaling through the CaSR may prevent PT hyperplasia even in tissues that are non-responsive to vitamin D (11). Clearly, serum Ca levels could also indirectly regulate PTH levels through a feedback of $1,25(OH)_2D$ on the PT glands. In SHPT, PT CaSR expression is reduced, but either vitamin D treatment or low phosphate intake are able to prevent PT CaSR content.

Further support for the pathophysiological relevance of changes in the expression of PT Transforming Growth Factor alpha (TGFα and, its receptor, Epidermal Growth Factor Receptor (EGFR) in controlling proliferative activity came from studies which evaluated the expression of these 2 proteins after the suppression of PT hyperplasia by high-Ca intake or its further enhancement by low dietary Ca. A diet high in Ca controlled PT hyperplasia in 5/6 nephrectomized rats, decreasing PT cell growth and the expression of both TGFα and EGFR (12). In contrast low-Ca intake induces PT hyperplasia, preventing the increases in TGFα and EGFR in the PT glands.

The role of phosphate

Since phosphate (P) is involved in many cellular processes, it is a key component not only of bone tissue, but also of many other tissues in healthy adult subjects. The total body amount of P in a healthy adult is about 1,000 grams (32 moL), 850 grams of which is usually stored in the bone tissue (13).

In fasting plasma, most of the P is present as inorganic orthophosphate in concentrations from 2.8 to 4.0 mg/dL (0.9 to 2.3 mmol/L). Contrary to Ca, approximately 50% of which is bound, only about 12% of the P is bound to plasmatic proteins. A low P intake promotes a reduction in renal P excretion, preventing hypophosphatemia. Clearly, renal tubular cells retain the ability to increase P tubular transport, with a variability among different portions of proximal tubules. Hypophosphatemia stimulates 25(OH)D-1α-hydroxylase critically modulated by renal tubular P fluxes (14). Conversely, hyperphosphatemia and increased renal tubular fluxes result in reduced P reabsorption, increased clearance of P, and suppressed activity of 25(OH)D-1α-hydro-xylase.

The kidney is the major organ in charge of controlling P losses. P filtered through the glomerulus is reabsorbed to a large degree in the proximal tubule, resulting only in a 10% to15% excretion of the filtered load. Physiologically, the proximal tubular reabsorption increases if the filtered P load decreases. In contrast, the P clearance increases and renal tubular reabsorption increases if the filtered P load increases (15). Furthermore, P is a key regulatory factor of PT function. Indeed, elevated serum P levels induce SHPT through both direct mechanisms (inhibition of $1,25(OH)_2D_3$ production) and indirect ones (subsequent reduction of Ca levels) (16).

Hyperphosphatemia due to decreased glomerular filtration rate is an important factor in the pathogenesis of SHPT (17-18). P may also regulate PT function at post-transcriptional level, as it improves PTH mRNA stability (19). In contrast to the mitogenic effects of hyperphosphatemia, dietary P restriction appears to counteract the proliferative signals induced by uremia, thus preventing PT cell replication and the increase in PT gland size (20).

Recently, the opposing effects of high and low dietary P on PT hyperplasia have been demonstrated in 5/6 nephrectomized rats (21). A diet high in P worsens

PT hyperplasia induced by uremia by enhancing PT expression of the growth factor, TGFα. Moreover, the rapid return of PT TGFα expression to normal levels by P restriction may suggest that low P counteracts uremia-induced PT cell growth by preventing a PT TGFα increase (21). Similar to the changes in TGFα expression, a diet high in P increased PT EGFR content to levels above normal, while P restriction reduced PT EGFR content (21).

In addition, administration of highly specific EGFR-tyrosine kinase inhibitors (TKI), which block downstream signaling from TGFα-activated EGFR, completely prevented high P- and low Ca-induced PT cell growth (22). More importantly, the suppression of signals downstream from TGFα binding to EGFR with EGFR-TKI treatment also revealed that TGFα self-upregulation in the PT glands is a main determinant of hyperplastic growth; and that enhanced TGFα- activation of EGFR mediates the reduction in PT vitamin D receptor levels, thereby causing resistance to both the antiproliferative and PTH-suppressive properties of calcitriol therapy (23).

Very recent studies (24) examined the role of activator protein 2alpha (AP2), an inducer of TGFα gene transcription, in the upregulation of PT TGFα in SHPT. Both in rats and in humans, SHPT PT AP2 expression strongly correlated with TGFα levels and with the rate of PT cell hyperplasia. Therefore, increased AP2 expression and transcriptional activity at the TGFα promoter determine the severity of the growth driven by PT TGFα self-upregulation in SHPT (24).

The role of vitamin D

Vitamin D is not a "vitamin", it is a hormone. Classically, the metabolic control for activation of vitamin D is regulated by the liver and kidneys, while the target tissues for vitamin D are the bone and the gut. Ca, P, PTH, and other peptides regulate renal vitamin D handling.

1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$), is the active metabolite of vitamin D, although its serum concentration does not correlate with vitamin D stores. It has been demonstrated that $1,25(OH)_2D_3$ promotes active and passive intestinal absorption of Ca and P, and consequently bone mineralization. Conversely, $1,25(OH)_2D_3$ suppresses PTH synthesis and PT cell proliferation through a genomic activity (25). The genomic effect of $1,25(OH)_2D_3$ is modulated by specific cytosolic receptors for vitamin D (VDR) in target cells. VDR forms a heterodimer with the retinoid X receptor that enables the complex $1,25(OH)_2D_3$-VDR to bind with high affinity to the vitamin D response element (VDRE) on the transcription promoters of vitamin D-sensitive genes. VDR has been detected in vitamin D-sensitive tissues (bone, intestine, kidney and parathyroid glands) and even in tissues where vitamin D activity is still unclear (myocardium, brain, pancreas and testis). In addition to the genomic effect, a rapid non-genomic effect of $1,25(OH)_2D_3$ was found in intestinal cells (14). PT VDR content is reduced in SHPT and vitamin D therapy prevents PT cell growth by inducing VDRs re-expression.

In uremic rats, $1,25(OH)_2D_3$ suppresses uremia-induced PT hyperplasia both *in vitro* (26) and *in vivo* (27). Naveh-Many et al (28) showed that PTH mRNA was much higher in PT glands from vitamin D-deficient normocalcemic rats than in controls, and that in vitamin D-deficient hypocalcemic rats the upregulation of PTH mRNA was even more pronounced. Moreover, many studies have been conducted to assess whether supplementation with vitamin D sterols can prevent or ameliorate SHPT in CKD. In the early-uremia rat model (7 days of renal failure), $1,25(OH)_2D_3$ and the less hypercalcemic vitamin D analog 1,25-dihydroxy-19-norvitamin D_2 (19-norD_2) controlled both serum PTH levels and PT hyperplasia similarly to what is described with phosphate restriction (21). The suppression of uremic rat PT cell growth by vitamin D treatment can be partially accounted for by the increased expression of p21. Furthermore, studies in patients with secondary HPT suggest an important role for increased p21 expression in PT growth arrest (27).

The efficacy of either $1,25(OH)_2D_3$ or 19-norD_2 in arresting PT hyperplasia and PT gland enlargement was associated with prevention of TGFα and EGFR expression in the PT glands (29). Since the TGFα activation of its receptor induces both TGFα and EGFR gene expression, it is also possible that $1,25(OH)_2D_3$ inhibition of EGFR activation mediates the suppressive effects of the sterol on TGFα and EGFR expression (30).

In CKD patients the inability to synthesize $1,25(OH)_2D_3$ is sustained by decreased 1α-hydroxylation of 25(OH)D in tubular cells and occurs prior to the increase in PTH secretion (31). Serum concentrations of $1,25(OH)_2D_3$ start to decrease at values of creatinine clearance near 70 ml/min. Therefore, patients are pre-

disposed to SHPT even in the early phases of CKD (32). Because vitamin D sensitizes the PT gland to Ca, it is possible that vitamin D deficiency in early CKD may contribute to the development of SHPT, even in the absence of overt hypocalcemia.

The role of the fibroblast growth factor 23 (FGF-23)

Different hormonal mechanisms have been proposed to understand the P homeostasis regulation, even if the knowledge of this complex system remains incomplete. PTH effectively reduces renal P resorption, which counteracts hyperphosphatemia in CKD, and increases renal calcitriol synthesis, leading to enhanced gastrointestinal P absorption and also increasing P renal resorption. In the last 5 years, new factors have been investigated in the physiology of P homeostasis and in the pathogenesis of hyperphosphatemia in CKD. In this sense, the discovery of the Fibroblast Growth Factor 23 (FGF23) certainly represents a major milestone (4).

Parathyroid glands are a target tissue for FGF23 (33). In fact, FGF23 acts on PT glands both directly, reducing PTH synthesis, and indirectly, suppressing 1-alpha-hydroxylase. Over-production of FGF23 in subjects with normal renal function induces hypophosphatemia, low plasma $1,25(OH)_2D_3$ levels, high serum PTH concentration, and severe bone demineralization (34, 35). In contrast, the decline of renal function in CKD patients is associated with a progressive augmentation of circulating FGF23 concentration, which can be due to serum P accumulation and/or the decrease of the renal clearance of FGF23 (36, 37). Once on dialysis therapy, serum FGF23 levels are markedly increased and positively correlated with serum P levels (38). In addition, in dialysis patients, high serum FGF23 concentration seems to predict the occurrence of refractory SHPT as reported by several groups (39, 40). Furthermore, a very recent study by Gutierrez et al (3) showed that increased FGF23 levels seem to be independently associated with mortality among patients who are beginning HD treatment.

CONCLUSIONS

In summary, the hyperphosphatemia associated with CKD most likely triggers FGF23 production to promote renal P excretion, reflected by the severely elevated FGF23 levels in CKD patients. However, given the history of protein hormone assays in patients with renal failure, these findings should be interpreted with caution, as even “intact” hormone levels may not accurately reflect true physiology in renal disease.

In the clinic, increased knowledge of the alterations from mineral metabolism disorders in chronic kidney disease (CKD) may be used to improve diagnostics and target selection for future metabolism treatments. Therefore, the discovery of FGF23 is a major novelty in our understanding of the pathogenesis of P handling and SHPT in renal disease.

Conflict of interest statement
Authors declare there is no potential conflict of interest in relation to this scientific work.

Address for correspondence:
Mario Cozzolino, MD, PhD
Renal Division
S. Paolo Hospital, University of Milan
Via A. di Rudinì, 8
20142 Milan, Italy
e-mail: mariocozzolino@hotmail.com

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