The role of vitamin D in the pathogenesis and management of secondary hyperparathyroidism in chronic renal failure

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Summary

Chronic renal failure (CRF) causes alterations in calcium-phosphate metabolism promoting the development of secondary hyperparathyroidism (HPT) and renal osteodystrophy. Recent data suggest that these alterations may play a crucial role in determining cardiovascular calcifications and, thus, cardiovascular mortality among CRF patients. An impaired 1α-hydroxylation of 25-hydroxycholecalciferol (25(OH)D) to 1,25-dihydroxycholecalciferol ($1,25(OH)_2D$) with decreased circulating $1,25(OH)_2D$ levels is commonly observed in patients with creatinine clearance below 70 ml/min. The reduction in $1,25(OH)_2D$ production triggers the upregulation of PTH synthesis, through a decreased suppression on PTH gene transcription and a decreased intestinal calcium absorption. Low vitamin D stores often contribute to the decrease in production and tissue resistance to vitamin D worsens $1,25(OH)_2D$ deficiency. A reduced expression of vitamin D receptor (VDR) and a less efficient binding of the complex $1,25(OH)_2D$-VDR to specific DNA segments account for the resistance to $1,25(OH)_2D$ in target cells. Thus, absolute and relative $1,25(OH)_2D$ deficiency is one of the causes of secondary HPT in patients with CRF, together with phosphate retention and skeletal resistance to PTH.
Consistently with these pathophysiological mechanisms, the therapeutic use of $1,25(OH)_2D$ still represents a milestone for the treatment of uremic secondary HPT and renal osteodystrophy, even though hypercalcemia and hyperphosphatemia are common adverse events and may increase the risk of cardiovascular calcifications. Furthermore, adynamic bone disease may develop after vitamin D therapy. Low levels of serum 25(OH)D are associated with more severe osteodystrophy and HPT, even among dialysis patients. To separate these adverse effects from anti-PTH activity, $1,25(OH)_2D$ analogues with lower hypercalcemic effect have been synthesized and are now available for clinical use.

KEY WORDS: vitamin D, calcium, parathyroid hormone, phosphate, chronic renal failure.

Vitamin D metabolism

Cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2) derive from dietary sources (animal and fish liver, eggs, fish oils). Cholecalciferol is also produced in the skin from 7-dehydrocholesterol (pre-vitamin D_3), through the nonenzymatic effect of sunlight ultraviolet B rays (UVB, wavelengths 295-305 nm). 7-dehydrocholesterol is mostly stored in the cytoplasm of cells at the dermal-epidermal border and the effectiveness of its conversion to cholecalciferol is related to the amount of photochromatic energy entering the skin. Photochromatic energy penetrating the skin greatly depends from the incident angle of UVB rays: in winter, the shallow incident angle of sunlight results in lower energy reaching the epidermis and dermis and, therefore, in lower production of vitamin D_3.
Cholecalciferol, ergocalciferol are hydroxilated at carbon 25 in the liver and at carbon 1 in kidney tubules thanks to enzymatic systems including cytocrome P-450 and located at the inner mitochondrial membrane. PTH and hypophosphatemia enhance the activity of renal 1α-hydroxylase, but not that of liver 25-hydroxylase.
The final product, 1,25-dihydroxycholecalciferol ($1,25(OH)_2D$), is the active metabolite of vitamin D, although its serum concentrations do not correlate with vitamin D stores. $1,25(OH)_2D$ promotes active and passive intestinal absorption of calcium and phosphate, and bone mineralization. Conversely, $1,25(OH)_2D$ suppresses PTH synthesis and parathyroid cell proliferation through a genomic activity. $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$ have the same potency in activating intestinal calcium absorption and bone mineralization, whereas $1,25(OH)_2D_2$ is less potent in suppressing parathyroid gland activity (1). Genomic effect of $1,25(OH)_2D$ 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 $1,25(OH)_2D$-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 $1,25(OH)_2D$ was found in intestinal cells.
The monohydroxylated metabolite, 25-hydroxycholecalciferol (25(OH)D), is 500 times less active than $1,25(OH)_2D$, but its serum concentration is the best indicator of vitamin D body stores. In spite of its low affinity for VDR, 25(OH)D maintains some biological effects, because its serum concentrations are 1000 times higher than those of $1,25(OH)_2D$ and compensate for the low affinity for VDR (2). The physiopathological relevance of 25(OH)D has been recently revaluated in population studies showing that low serum concentrations of 25(OH)D were associated with higher serum PTH in healthy elderly individuals (3, 4). In these studies, the serum 25(OH)D concentra-

tion above which all values of serum PTH were normal, was 30 ng/ml (75 nmol/L). This threshold for secondary hyperparathyroidism (HPT) was also confirmed in elderly individuals with elevated creatinine clearance (5) and in hemodialysis patients (6, 7). Based on these findings, the normal range of 25(OH)D serum concentrations was recently redefined and concentration above 30 ng/ml (75 nmol/L) are now generally recommended to prevent secondary HPT (4, 8). Lower 25(OH)D serum concentrations were associated with increased risk of fracture and low bone mineral density (BMD) at different bone sites in young and elderly healthy individuals of both sexes (9, 10). Accordingly, osteopenia and osteoporosis were more frequent in patients with 25(OH)D serum concentrations below 30 ng/ml (75 nmol/L) and osteomalacia was found in patients with 25(OH)D concentrations below 10 ng/ml (25 nmol/L) (4, 8, 11). Serum 25(OH)D concentrations of 30 ng/ml (75 nmol/L) or higher were proposed as the target for the treatment of osteodystrophy, even in hemodialysis patients (6, 7). Hemodialysis patients with lower levels of 25(OH)D had more marked Looser's zone on X-rays and decreased bone formation at bone histology regardless of $1,25(OH)_2D$ levels (12, 6). However, excessively high 25(OH)D levels were associated with low turnover osteodystrophy. Thus, concentrations between 20 and 40 µg/ml have been proposed as the most appropriate 25(OH)D target range for hemodialysis patients by other authors (12). The need to maintain normal vitamin D stores suggests that unknown vitamin D metabolites, besides $1,25(OH)_2D$, may have a beneficial effect on bone and parathyroid metabolism in end stage renal disease.

Vitamin D may have important functions besides mineral ion homeostasis (13). In patients with congestive heart failure, $1,25(OH)_2D$ concentrations were found to be significantly reduced (14). Recently, it has been demonstrated that vitamin D therapy decreased myocardial hypertrophy in dialysis patients (15). Moreover, vitamin D replacement downregulates the renin-angiotensin system and controls blood pressure in VDR knock-out mice (16). Paradoxically, low $1,25(OH)_2D$ levels have been correlated with increased coronary calcification in patients at high risk for coronary heart disease (17). Indeed, many epidemiological studies have documented an association between vitamin D deficiency and autoimmune diseases, several types of cancers, and cardiovascular disease (18, 19).

Pathogenesis of secondary hyperparathyroidism in chronic renal failure (CFR)

Parathyroid cells are characterized by a low turnover and rarely undergo mitoses (20, 21). However, in the presence of low calcium, high phosphorus, vitamin D deficiency, and uremia, parathyroid cells leave quiescence and divide by increasing the activity of regulatory cell cycle enzymes and/or their inhibitors (22, 23). In secondary HPT, parathyroid gland growth is initially diffuse and polyclonal. Cell proliferation in the nodules then becomes monoclonal and aggressive (24). The rapid de-differentiation of hyperplastic parathyroid cells in culture (25) precludes further assessment of the relative contribution of changes in calcium, phosphate, and vitamin D to the expression of components of the cell cycle critical for growth control.

Role of phosphate

Hyperphosphatemia due to decreased glomerular filtration rate is an important factor in the pathogenesis of secondary HPT (26). Elevated serum phosphate levels induce secondary HPT through indirect and direct mechanisms (27). In addition, hyperphosphatemia inhibits $1,25(OH)_2D$ production (28), with subsequent hypocalcemia.

The direct effects of phosphorus have been demonstrated both *in vitro* and *in vivo* studies. High phosphorus concentrations stimulate PTH secretion in intact rat parathyroid glands (29). Unfortunately, the in vitro effects of phosphorus on PTH secretion could be observed in intact parathyroid tissue preparations, but not isolated, dispersed parathyroid cells (12, 30). Recently, several studies have shown that phosphate may regulate parathyroid function at post-transcriptional level, as it improves PTH mRNA stability through binding of parathyroid cytosolic proteins to the 3'-UTR and especially to the terminal 60 nucleotides of PTH mRNA (31).

Two weeks after 5/6 nephrectomy in rats, uremia-induced mitotic activity is further enhanced by high dietary phosphate, but prevented by phosphorus restriction (22). In contrast to the mitogenic effects of hyperphosphatemia, dietary phosphorus restriction appears to counteract the proliferative signals induced by uremia, thus preventing parathyroid cell replication and the increase in parathyroid gland size (32).

Table I - Values of the parameters of calcium-phosphate metabolism in patients with different creatinine clearance. A significant decrease of serum $1,25(OH)_2D$ is observed since creatinine clearance values of 70 mL/min and is associated with secondary HPT. Serum phosphate increases later, but occurs in the early stages of CRF. Intestinal calcium absorption decreases during the progression of CRF. It was measured using strontium (Sr) as a marker: strontium absorption was determined 4 hours after an oral load and expressed as area under the time-plasma strontium concentration curve (90).

N.	9	10	11	10	18
Creatinine clearance (mL/min)	<20	21-40	41-60	61-80	>80
Age (years)	56±4.7	56±3.4	59±2.6	50±5.5	52±2.1
Serum phosphate (mmol/L)	1.8±0.09	1.26±0.07	1.17±0.05	1.12±0.05	1.1±0.04
Plasma calcium (mmol/L)	2.08±0.1	2.38±0.03	2.42±0.03	2.35±0.03	2.35±0,02
Serum $1,25(OH)_2D$	15±2.9	21±3.2	23±3.6	28±2.9	39±2.8
iPTH (pg/ml)	494±120	129±37	47±7	63±12	29±3
Enteral Ca absorption (mmol of absorbed Sr/l per min)	3.4±0.4	6.2±0.9	6.1±0.4	7.6±0.7	8.1±0.6

Recent studies on the effects of dietary phosphate on parathyroid gland growth demonstrated that the low phosphorus-induction of p21 (an inhibitor of kinases of the cell-cycle) mRNA and protein content in parathyroid glands contributes to the antiproliferative effects of phosphate restriction on uremia-induced parathyroid cell growth (33). In this uremic rat model, the temporal increases in p21 protein expression correlate inversely with the parathyroid levels of a marker of mitotic activity, the proliferating nuclear cell antigen (PCNA) (33).
In the search for the mitogenic stimuli triggered by high dietary phosphate, we next focused on transforming growth factor-α (TGFα). TGFα, known to promote growth not only in malignant transformation but also in normal tissues (34), is increased in hyperplastic and adenomatous human parathyroid glands (35). High phosphate diet worsens uremia-induced parathyroid hyperplasia by increasing parathyroid expression of TGFα. The rapid return of parathyroid TGFα content to normal after phosphate restriction suggests that low phosphorus may counteract uremia-induced parathyroid cell growth not only through induction of p21 expression, but also by preventing the increase in parathyroid TGFα (33, 36).
Increase in parathyroid TGFα induces cell growth through autocrine and paracrine mechanisms through activation of its receptor, the epidermal growth factor receptor (EGFR) (37). In human parathyroid glands, Gogusev et al. (35) demonstrated the presence of EGFR protein in 4 out of 5 adenomas, in 13 out of 15 tissue samples of hyperplasia secondary to renal failure, and in most samples of normal parathyroid tissue. No differences in the expression patterns were observed between groups. However, studies in 104 human hyperplastic parathyroid glands, which failed to detect EGFR protein, showed higher EGFR mRNA expression in carcinoma and primary hyperplasia compared to adenomas and hyperplasia secondary to renal failure (38).
The concept that co-expression of TGFα and EGFR could contribute to non-neoplastic parathyroid hyperplasia led us to examine the dietary-phosphate regulation of parathyroid EGFR expression in rat parathyroid glands. Similarly to the changes in TGFα expression, high dietary phosphate increases parathyroid EGFR content to above normal levels, while phosphorus restriction prevented the increases in EGFR levels (39, 40).
These findings indicate that the uremia-induced parathyroid co-expression of TGFα and its receptor, EGFR, acts as a mitogenic signal, which can be blunted by phosphate restriction and counteracted through the induction of p21. These new insights into the molecular mechanisms of parathyroid hyperplasia suggest that, in addition to phosphate restriction or use of phosphate binders, therapeutic approaches focusing on induction of p21 and inactivation of TGFα/EGFR growth-promoting signals may slow down the progression of secondary HPT.

Role of calcium

Calcium is a key regulator factor in secondary HPT progression. Low serum calcium levels decrease the activation of the Calcium-Sensing Receptor (CaSR), a plasma membrane G-protein coupled molecule that allows parathyroid cells to sense calcium in the extracellular fluid, thus greatly promoting PTH synthesis and secretion (41). In contrast, hypercalcemia activates the CaSR, rapidly suppressing secondary HPT. Recent evidence suggests that signaling through the CaSR plays an important role on parathyroid hyperplasia (42). Moreover, calcium-depending signaling through the CaSR may prevent parathyroid hyperplasia even in tissues that are not-responders to vitamin D (43).
The extracellular calcium concentration may also regulate the level of PTH mRNA (44, 45) and parathyroid cell proliferation (46). Moreover, calcium regulates VDR mRNA and protein expression in parathyroid cells independently of $1,25(OH)_2D$ (47). Clearly, serum calcium levels could also indirectly regulate PTH levels through a feedback of $1,25(OH)_2D$ on the parathyroid glands.
Reduced expression of CaSR in hyperplastic parathyroid glands has been demonstrated in an animal model (48). Parathyroid hyperplasia appears 48 hours after 5/6 nephrectomy in rats fed a high phosphate diet, while CaSR expression starts to decrease 48 hours later. Furthermore, in humans, CaSR content declines by about 60% in hyperplastic parathyroid glands compared to normal controls (49).
Further support for the pathophysiological relevance of changes in the expression of parathyroid p21, TGFα and EGFR in controlling proliferative activity came from studies, evaluating the expression of these three proteins after suppression of parathyroid cell growth by high-calcium intake or its further enhancement by low dietary calcium. High dietary calcium controlled uremia-induced parathyroid hyperplasia, reducing both parathyroid gland size and the expression of two markers of mitotic activity, Ki67 and PCNA (36). Furthermore, high calcium diet increased parathyroid p21 levels and prevented the rise in parathyroid content of TGFα and EGFR induced by uremia (36, 40). The mechanisms for high-calcium induction of p21 and prevention of the increase in TGFα and EGFR are unknown. Studies in vitamin D receptor-ablated mice showed the ability of a calcium-enriched diet to prevent the development of parathyroid hyperplasia in both hypocalcemic and normacalcemic states (50).
In relation to high-calcium control of TGFα/EGFR growth promoting signal, hypercalcemia and low plasma levels of TGFα were recently associated in cancer patients, suggesting the possibility of the systemic control of TGFα expression by calcium (51). The existence of such an association in CRF patients could partially explain the suppression of parathyroid growth by hypercalcemia.
The changes in p21, TGFα and EGFR in the parathyroid glands of uremic rats fed a high Ca diet suggest that increases in serum calcium or in intracellular calcium, induced by vitamin D therapy, could enhance the effects of vitamin D itself in increasing p21 expression and reducing TGFα and EGFR content.

Role of vitamin D

In the 5/6 nephrectomized rats, $1,25(OH_2)D_3$ suppresses uremia-induced parathyroid cell proliferation both *in vitro* (52), and *in vivo* (53).
Naveh-Many showed that PTH mRNA was much higher in parathyroid glands from vitamin D-deficient normocalcemic rats than controls, and that in vitamin D-deficient hypocalcemic rats the upregulation of PTH mRNA was even more pronounced (54). Moreover, many studies have been conducted to assess whether supplementation with vitamin D sterols can prevent or ameliorate secondary HPT in CRF.
In the early-uremia rat model (7 days of renal failure), $1,25(OH_2)D$ and the less hypercalcemic vitamin D analog 1,25-dihydroxy-19-norvitamin D2 (19-norD_2) controlled both serum PTH levels and parathyroid hyperplasia similarly to what is described with phosphate restriction (36). The suppression of uremic rat parathyroid 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 parathyroid growth arrest (55).
The efficacy of either $1,25(OH_2)D$ or 19-norD_2 to arrest parathyroid hyperplasia and parathyroid gland enlargement, was associated with prevention of TGFα and EGFR expression in the

parathyroids (39). Since the TGFα activation of its receptor induces both TGFα and EGFR gene expression, it is also possible that 1,25(OH)D inhibition of EGFR activation mediates the suppressive effects of the sterol on TGFα and EGFR expression (56).
In addition, vitamin D therapy suppresses parathyroid growth even in an established rat model of secondary HPT. 5/6 nephrectomized rats fed a high phosphorus diet for 8 weeks and received either vehicle or the vitamin D analog 19-norD_2. No increase in parathyroid gland size was observed in the vitamin D treated uremic animals compared to the untreated rats (36).
In patients with CRF (Table I), the inability to synthesize $1,25(OH)_2D$ is sustained by decreased 1α-hydroxylation of 25(OH)D in tubular cells and occurs prior to the increase in PTH secretion (57). Serum concentrations of $1,25(OH)_2D$ start to decrease at values of creatinine clearance near 70 mL/min. Therefore, patients are predisposed to secondary HPT even in the early phases of CRF (58). Because vitamin D sensitises the parathyroid gland to calcium, it is possible that vitamin D deficiency in early CRF may contribute to the development of secondary HPT, even in the absence of overt hypocalcemia.
Alteration of renal 1α-hydroxylase is frequently associated with other defects that may further diminish $1,25(OH)_2D$ synthesis. A reduction in vitamin D stores may be found in a significant proportion of CRF patients, but also of healthy individuals in industrial countries (3, 59). Among healthy elderly Europeans 36% of men and 47% of women had serum 25(OH)D levels below 12 ng/mL (30 nmol/L), with the highest prevalence in Southern countries (60, 61). Similar frequencies were also observed in non-European populations (62, 11). Ninty-three percent of Spanish elderly individuals with plasma creatinine higher than 1 mg/dL, and 79% of Italian patients with creatinine clearance between 30 and 70 mL/min, had serum 25(OH)D below 30 ng/ml (75 nmol/L) (5, 63). In these populations, 25(OH)D deficiency was not necessarily associated with a decrease in serum concentrations of $1,25(OH)_2D$ (5). Inactivity with insufficient exposure to sunlight or low dietary intake may be responsible for vitamin D insufficiency in CRF patients. Patients with CRF may also develop a reduced capability to produce 25(OH)D and resistance to UVB, due to skin hyperpigmentation caused by retention of melanocyte-stimulating hormone. An additional factor may be patient's age, since aging is usually associated with lower skin concentration of 7-dehydrocholesterol and decreased responsiveness of renal 1α-hydroxylase to PTH (64). Underlying renal disease may also affect vitamin D status. Urinary loss of vitamin D bound to vitamin D-binding protein occurs in nephrotic syndrome. Likewise, patients undergoing peritoneal dialysis lose vitamin D-binding protein with peritoneal effluent.
$1,25(OH)_2D$ concentrations were directly related with those of 25(OH)D in CRF patients (62), but not in healthy individuals. Thus, the activity of renal 1α-hydroxylase in CRF appears to be substrate-dependent and is depressed by low serum concentrations of 25(OH)D (63, 62, 65). Interestingly, a positive correlation between 25(OH)D and $1,25(OH)_2D$ was also observed in hypercalciuric stone formers with normal creatinine clearance (66). Therefore, careful assessment of 25(OH)D status is today proposed as an integral part of the therapeutic strategy to prevent secondary HPT and bone disease in CRF patients.

Clinical consequences of secondary hyperparathyroidism

Secondary HPT causes both skeletal and extra-skeletal complications. Bone-associated consequences include with osteitis fibrosa and renal osteodystrophy, while non-skeletal consequences include cardiovascular calcification, soft-tissue calcification, endocrine disturbances, compromised immune system, neurobehavioral changes, and altered erythropoiesis (67, 68).

Renal osteodystrophy

Renal osteodystrophy refers to a bone disorder that occurs in patients with CRF, characterized by abnormal bone turnover (69). PTH directly stimulates calcium mobilization from the bone by acting on the osteoclasts and indirectly by stimulating bone resorption and formation through the increase of vitamin D synthesis in the kidney. It is well known that vitamin D stimulates osteoblast activity and increases both osteoclast amount and function (69). In CRF patients vitamin D deficit leads to a loss in normal osteoblast activity and a reduction in bone mineralization. Furthermore, high serum PTH levels stimulate osteoclasts to mobilize more calcium from bone tissue, with bone mass reduction (70, 71).
In CRF patients renal osteodystrophy is characterized by five types of bone lesions. Osteitis fibrosa and mixed osteodystrophy are high-turnover bone diseases. Osteomalacia, aluminium-induced bone disease, and adynamic bone disease are low-turnover bone diseases (69). High-turnover bone diseases are characterized by rapid bone turnover induced by abnormally high serum PTH levels and abnormal mineralization. Conversely, low-turnover bone diseases are caused by over-suppression of PTH or aluminium at the site of bone mineralization (69). Figure 1 displays the complex interaction among the multiple factors that may cause the different bone lesions in renal osteodystrophy.

Cardiovascular calcification

CRF patients have a dramatically higher incidence of cardiovascular morbidity and mortality compared to the general population. In the last ten years, several studies pointed out that vascular calcification is a major cause of cardiovascular disease in the dialysis population. In CRF patients, high levels of plasma calcium, serum phosphate and PTH play a critical role

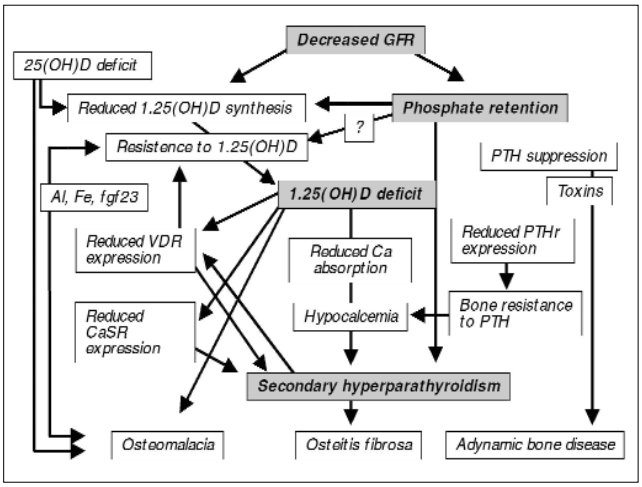

Figure 1 - This figure displays the complex interaction between the different components of the system controlling calcium and phosphate in blood. The decrease of glomerular filtration rate (GFR) leads to phosphate retention and $1,25(OH)_2D$ deficiency that induce parathyroid hyperplasia and stimulate PTH secretion. Various endogenous or exogenous toxins interfere with bone mineralization processes.

in the pathogenesis of cardiovascular events.
Patients develop extensive medial calcification, which causes increased arterial stiffness and high morbidity and mortality due to cardiovascular events (72, 73). A variety of risk factors are associated with vascular calcification in dialysis patients (time on dialysis, uremic toxins, history of diabetes, inflammation), but abnormalities in bone mineral metabolism may play a critical role (74). In fact, elevated serum phosphate, calcium-phosphate product, and PTH levels contribute to vascular calcification, although their roles are incompletely understood (75, 76).
Elevations of serum phosphate and calcium-phosphate product levels may worsen cardiovascular events in the uremic population, through a progressive increase in calcium deposition in the coronary arteries and heart valves (77). Furthermore, calcium-containing phosphate binders can increase calcium load (78). Recently, an elegant study by Goodman et al. (79) in hemodialysis children and young adults showed a correlation between coronary artery calcification detected by electron beam computed tomography (EBCT) and age of dialysis, serum phosphorus, calcium-phosphate product levels, and daily intake of calcium. Moreover, experimental observations in uremic rats (80, 81) and in dialysis patients (82) showed that a new calcium and aluminium-free phosphate binder (Sevelamer HCl) reduced the progression of cardiovascular calcification more than calcium-based phosphate binders.

Treatment of secondary hyperparathyroidism: role of vitamin D and its analogues

Prevention and treatment of secondary HPT commonly requires control of both phosphate and $1,25(OH)_2D$ levels in serum. Phosphate levels are usually controlled by reducing dietary phosphate intake, by dialysis, and by using phosphate-binders. $1,25(OH)_2D$ effectively suppresses PTH production and improves bone histology in some patients (77). Vitamin D and its analogues act after binding to VDR in parathyroid cell cytosol. The vitamin D-VDR complex controls the rate of PTH gene transcription by interacting with VDRE, located 100-120 bases upstream from the PTH gene (83). Furthermore, vitamin D upregulates CaSR expression on cell membrane, thus leading to a greater inhibition on PTH secretion (41). Through CaSR the increase in circulating calcium ions can inhibit tonic PTH secretion by stimulation of mitogen-activated protein kinase pathways (84). VDRE regions are present in the two promotors of the CaSR gene (85). Calcium ions inhibit PTH secretion not only through CaSR, but also by interacting with cytosolic proteins affecting the stability of PTH mRNA. Hypocalcemia favors the activity of cytosolic proteins protecting PTH mRNA from the degrading effect of ribonucleases. On the other hand, hypercalcemia favors the activity of proteins degrading PTH mRNA (86). Furthermore, a trascriptional activity of calcium has been hypothesized (87). Postranscriptional and transcriptional effects of calcium ions are indirectly influenced by the calcium-repleting activity of vitamin D.
The therapeutic use of $1,25(OH)_2D$ in CRF mainly aims to raise intestinal absorption of calcium, to protect bone against osteomalacia and to control parathyroid function. However, also the function of other organs expressing VDR, as kidney, pancreas, myocardium and testis, might be improved by vitamin D administration. $1,25(OH)_2D$ inhibitory effect on PTH synthesis and parathyroid hyperplasia is well established in CRF. Vitamin D analogues with a lower hypercalcemic and hyperphosphatemic activity, are now available as an efficient alternative to $1,25(OH)_2D$ treatment. Furthermore, normal serum levels of 25(OH)D are relevant for the prevention or treatment of secondary HPT, due to the capacity of 25(OH)D to support renal 1α-hydroxylase activity or the synthesis of active vitamin D metabolites different from $1,25(OH)_2D$ (6, 62, 63, 65).

Table II - Objectives for renal osteodystrophy treatment in patients with different levels of CRF recommended in K/DOQI guidelines. Stage 1 and 2 of renal function are characterized by creatinine clearance above 90 mL/min and between 60 and 90 mL/min.

	Stage of renal function		
	Stage 5	Stage 4	Stage 3
Creatinine clearance (mL/min)	<15	16-30	30-60
iPTH (pg/mL)	150-300	70-110	35-70
Serum phosphate (mmol/L)	1.13-1.78	0.87-1.49	0.87-1.49
Plasma calcium (mmol/L)	2.1-2.37	2.2-2.5	2.2-2.5
Ca-P product ($mmol^2/L^2$)	< 4.5	< 4.5	< 4.5

Clinical targets for serum concentrations of phosphate, calcium and PTH (Table II) have been proposed in the 2003 guidelines proposed by the Kidney Dialysis Outcomes Quality Initiative (K/DOQI) Work Group (88). Target for plasma concentrations of PTH increases according to creatinine clearance because patients develop a progressive tissue resistance to PTH explained by a diminished expression of PTH receptors and by $1,25(OH)_2D$ deficiency or resistance (89, 90). The low target for calcium concentrations aims to avoid calcium overload and an excessively high calcium-phosphate product.
A particular bone complication of the therapy with vitamin D metabolites occurs in case of excessive parathyroid suppression. As defined in the K/DOQI guidelines, the target for PTH concentration is 150-300 pg/mL in end-stage CRF (Table II). If the values of circulating PTH are suppressed below these limits, bone cells become quiescent and bone remodeling is far slowed (91). The histology pattern of these functional alterations is adynamic bone disease, characterized by decreased number of bone cells, bone metabolic inactivity and reduced bone formation rate (91). The mechanism of adynamic bone disease is unknown. The clinical consequences of adynamic bone disease may be an increased fracture risk, while its relationship with osteoporosis is still debated (92). At the opposite, osteomalacia may develop in case of $1,25(OH)_2D$ deficiency, even though the toxic effect of aluminum overload has been the most frequent cause of osteomalacia in dialysis population till some years ago (93).

Vitamin D supplementation

Vitamin D deficit is frequent in CRF and its correction prevents or cures secondary HPT and achieves the specific beneficial effects of 25(OH)D on muscles and bones (6, 94). Vitamin D supplements are recommended in patients with creatinine clearance above 15 mL/min and with serum concentrations of 25(OH)D below 30 ng/mL (75 nmol/L), because their kidney is stimulated to produce $1,25(OH)_2D$ by the increase of 1α-hydroxylase substrate, 25(OH)D. Ergocalciferol is the most convenient product to replace vitamin D stores, due to its availability, cost and efficacy. K/DOQI guidelines recommend to administer 50000 IU/month per six months orally in patients with serum 25(OH)D concentrations of 16-30 ng/mL (40-75 nmol/L), or six-month courses with more frequent doses in the presence of lower 25(OH)D levels (88). During this treatment, plasma

calcium concentration should not exceed 2.51 mmol/L (10.5 mg/dL) and serum phosphate 1.49 mmol/L (4.6 mg/dL). To prevent vitamin D deficiency in CRF patients, Schomig and Ritz proposed oral supplementation with 1000 IU day of cholecalciferol, or 10000 IU/week (58). Alternatively, 25(OH)D may be administered orally at the dose of 10-40 µg/day, according to the serum levels of this metabolite and calcium, as usually employed in osteoporosis.

The use of vitamin D in end-stage CRF has been source of debate. Hemodialysis patients with low levels of 25(OH)D had more marked Looser's zones at the pelvis radiograms and decreased bone formation at bone histology, regardless of $1,25(OH)_2D$ levels (12, 6). Gazali et al. suggest to prescribe 1600 IU/day of vitamin D or 20-30 µg/day of 25(OH)D at least during the winter months (6). The doses of vitamin D proposed by these Authors were higher than those commonly indicated for general elderly population (800 IU/day), because the need of vitamin D is increased in CRF patients due to less efficient 25(OH)D synthesis (2, 123).

$1,25(OH)_2D$

$1,25(OH)_2D$ remains a milestone for the treatment of secondary HPT in CRF patients. It may be prescribed to patients with secondary HPT at every stage of CRF, although theoretically it should be used in the presence of normal serum levels of 25(OH)D. Exogenous administration of $1,25(OH)_2D$ becomes inevitable in patients with creatinine clearance below 15 mL/min (Table III), provided that the serum values of calcium, phosphate and PTH indicate its use (88). In CRF patients with creatinine clearance above 15 mL/min, $1,25(OH)_2D$ treatment may begun at the oral dose of 0.25 µg/day, according to the K/DOQI guidelines (88). Clinical trials aimed to study the outcome of $1,25(OH)_2D$ treatment showed that CRF patients, who had received $1,25(OH)_2D$ (0.25-0.5 µg/day per os) for one year, had lower circulating PTH and normal bone histology at the end of follow-up. A significantly higher bone turnover and lower bone mineral density were observed in patients who had not received $1,25(OH)_2D$ (95-97). Therefore, although protective against HPT, $1,25(OH)_2D$ treatment is likely to expose patients with early CRF to the risk of adynamic bone disease and may also accelerate renal function decline, probably due to vascular deposition of calcium-phosphate salts (98). However, the dosage of 0.125-0.25 µg/day was well tolerated in patients with mild-moderate CRF and appeared safe for bone and kidney (99, 100).

$1,25(OH)_2D$ is prescribed to hemodialysis patients as oral daily doses or intravenous (i.v.)/oral thrice-weekly boli. The intermittent supplementation was studied for dialysis patients with the rationale to produce peaks of $1,25(OH)_2D$ concentration in blood. Pharmacokinetic considerations and experimental findings suggested that intermittent supplementation has the advantage of a greater PTH suppression and an easier patient compliance. In spite of this promising background, two trials comparing intermittent with daily administration of $1,25(OH)_2D$ in dialysis patients, showed no advantages of intermittent doses in terms of efficacy and safety (101, 102). About i.v. or oral boli, although K/DOQI guidelines conclude that i.v. boli were more efficient for the control of secondary HPT, a study was unable to find differences between the two administration routes (103). The expectation created by the introduction of the intermittent administration gave rise to a discussion about a possible "pharmacological parathyroidectomy", capable to abolish autonomous parathyroid secretion. Conversely, nephrologists learnt that excessive suppression of PTH was the main cause of adynamic bone disease (121, 122). Plasma concentrations of PTH between 150 and 300 pg/mL are now considered as the target for vitamin D treatment which has to be started only when PTH is above this range.

Vitamin D analogues

Several new vitamin D analogues have been developed and investigated with the rationale to treat secondary HPT decreasing the risk of hypercalemia and hyperphosphatemia in CRF patients (83, 84). Vitamin D analogues have variable affinity for the components of the vitamin D system, including the vitamin D-binding protein and the nuclear VDR. Some of the effects are genomic and mediated through changes in the structural configuration of the vitamin D-VDR complex or in the affinity of the vitamin D-VDR complex for the key response elements in various target genes. There are currently three vitamin D analogues approved for use in CRF patients with secondary HPT in the US, 1,25-dihydroxy-22-oxavitamin D_3 (22-oxacalcitriol, OCT), 1,25-dihydroxy-19-norvitamin D_2 (19-norD_2), 1α-hydroxyvitamin D_2 (1αOHD_2). 19-norD_2 is approved for the treatment of secondary HPT in Italy.

OCT is a vitamin D_3 derivate, which differs from $1,25(OH)_2D_3$ for the substitution of carbon 22 with an oxygen. Oral and i.v. preparations are available. Experience in rats showed that it is

Table III - Algorithm for treatment with $1,25(OH)_2D$, OCT or 19-norD_2 in hemodialysis patients according to K/DOQI guidelines. Vitamin D should not be prescribed in patients with serum concentrations of calcium and phosphate higher than those indicated. The initial doses of vitamin D is related to plasma levels of PTH and then regulated on therapy outcome. K/DOQI guidelines consider the i.v. way as preferential with respect to oral way of administration.

To convert plasma calcium concentrations in mg/dL, multiply by 4; plasma phosphate concentrations in mg/dL, multiply by 3.1; calcium phosphate product in mg^2/dL^2, multiply by 12.4.

Plasma PTH (pg/mL)	Plasma calcium (mmol/L)	Serum phosphate (mmol/L)	Calcium-phosphate product ($mmol^2/L^2$)	$1,25(OH)_2D$ dose (µg after HD)	OCT dose (µg after HD)	19-norD_2 dose (µg after HD)
300-600	<2.37	<1.78	<4.5	i.v.: 0.5-1 oral: 0.5-1	i.v.: 2.5-5	i.v.: 2 oral: 5
600-1000	<2.37	<1.78	<4.5	i.v.: 1-3 oral: 1-4	i.v.: 6-10	i.v.: 2-4 oral: 5-10
>1000	<2.5	<1.78	<4.5	i.v.: 3-5 oral: 3-7	i.v.: 10-15	i.v.: 4-8 oral: 10-20

less potent than $1,25(OH)_2D$ to suppress parathyroid glands and much less active on plasma calcium. OCT is commonly administered to hemodialysis (HD) patients three times a week (Table III). In the first trial, episodes of hypercalcemia occurred in 33% of patients and serum alkaline phosphatase decreased significantly after OCT therapy, suggesting the correction of high-turnover bone disease (104). The reduction in PTH and the increment of calcium and phosphate were dose-dependent. The initial OCT oral dose was 5 µg three times a week when plasma iPTH concentration was 300-500 pg/mL and 10 µg three times a week when iPTH was above 500 pg/mL (105). OCT was also successfully used in CRF patients with creatinine clearance above 15 mL/min at the oral dose of 1-5 µg/day with no deleterious effects on renal function (106).
The different activity of OCT and $1,25(OH)_2D$ has been attributed to the different pharmacokinetics of the two compounds. OCT affinity for VDR and vitamin D-binding protein is lower than that of $1,25(OH)_2D$ and, thus, it is rapidly cleared from the blood. OCT treatment is also associated with the inhibition of renal 1α-hydroxylase and a consequent reduction in serum levels of $1,25(OH)_2D$. The short half-life and the $1,25(OH)_2D$ deficiency explain the scarce effect of OCT on intestine and bone and, ultimately, on calcium concentrations. On the other hand, OCT has a prolonged activity on parathyroid glands, where it is retained in the nuclei (107-108).
19-norD_2 has the side chain of vitamin D_2 and differs from $1,25(OH)_2D$ for the lack of carbon 22. It was the first analog approved for use in CRF patients and is available in Italy for i.v. administration, commonly three times a week after hemodialysis (Table III). It may be dosed according to a 4:1 ratio to $1,25(OH)_2D$, even though a ratio 3:1 was proposed for a safer approach (109). Thus, in patients already taking $1,25(OH)_2D$, the initial doses of 19-norD_2 can be calculated as four times that of $1,25(OH)_2D$. Alternatively, its initial dosage may be calculated from circulating PTH concentrations (PTH concentration / 80) or body weight (0.04 µg/kg of dry body weight) (110).
Efficacy of 19-norD_2 and $1,25(OH)_2D$ was compared in a randomized double-blind trial. Reduction in plasma PTH was more rapid in patient taking 19-norD_2, although the PTH reduction target (decrease of 50% of the baseline plasma concentration) was obtained in a similar proportion of patients after 20 weeks of treatment (79% with 19-norD_2 and 69% $1,25(OH)_2D$). Severe hyperphosphatemia (above 2.58 mmol/L or 8 mg/dL) occurred in 9% of patients treated with 19-norD_2 and 14% of patients treated with $1,25(OH)_2D$, while no differences in plasma calcium levels were observed (111). In another randomized double-blind study comparing 19-norD_2 with $1,25(OH)_2D$, hypercalcemia and/or calcium-phosphate product above 6 $mmol^2/L^2$ (75 mg^2/dL^2) occurred at least once in 68% of patients during treatment with 19-norD_2 and 64% with $1,25(OH)_2D$ (112). Conversely, persistent hypercalcemia was observed in 18% of patients treated with 19-norD_2 and 33% with $1,25(OH)_2D$. These trials showed that 19-norD2 was able to control PTH levels reducing the occurrence of hypercalcemia and hyperphosphatemia in comparison with $1,25(OH)_2D$. Therefore, the control of PTH secretion is obtained with lower calcium-phosphate product with 19-norD_2 (111, 112). This reduces the risk of vascular calcification and may contribute to the increased survival observed in hemodialysis patients undergoing 19-norD_2 therapy compared with those taking $1,25(OH)_2D$ (113). Furthermore, in patients taking 19-norD_2 a reduced hospitalization rate was observed (114). However, the values of these studies about survival in patients treated with 19-norD_2 was limited by the fact they were retrospective and not randomized.
The limited hypercalcemic effect of 19-norD_2 results from a lower affinity for VDR in intestine and bone. In addition, 19-norD_2 causes a selective vitamin D deficiency and a reduction in intestinal calcium absorption (115, 116).
1αOHD_2 is a prodrug activated by 25-hydroxylation in the liver. It may be administered daily or three times a week, orally or i.v. Clinical trials demonstrated a good efficacy of 1αOHD_2 in the treatment of secondary HPT in hemodialysis patients. 1αOHD_2, prescribed at the initial oral doses of 4 µg daily or three times a week, normalized PTH concentrations in 88% of patients, but hypercalcemia and hyperphosphatemia complicated the treatment in 42% and 75% of patients respectively (117). This high rate of hypercalcemia was not observed in other studies, although an increase in serum phosphate and calcium invariably occurred. In a larger trial including 99 hemodialysis patients, 1αOHD_2 was prescribed at the oral dose of 10 µg three times a week. Hypercalcemia was observed in 15% of patients and hyperphosphatemia in 19%. Plasma PTH decreased by 45% from baseline levels and became normal in 83% of patients (118). Intravenous and oral preparations of 1αOHD_2 are available. The increase of calcium and phosphate was lower with i.v. than oral administration, but the induced decline of PTH was similar. 1αOHD_2 was also used at the dose of 1-3 µg/day in non-uremic CRF patients. Its use was safe and effective in decreasing plasma PTH and increased bone mineral density (119).
The lower hypercalcemic effect of 1αOHD_2 with respect to 1αOHD_3 has been attributed to the production of 24-hydroxylated metabolites with less hypercalcemic activity (120).

Conclusions

The modern strategies to prevent secondary HPT in CRF patients give great relevance to vitamin D replacement therapy, which requires to take into account the stage of CRF, the underlying renal disorder, the levels of circulating PTH, the condition of the bone, the vitamin D stores, the parameters of bone turnover and the values of calcium and phosphate in serum.
The administration of $1,25(OH)_2D$ or its analogues is unavoidable in dialysis patients when secondary HPT is often already established. Its association with 25(OH)D or ergocalciferol or cholecalciferol may be beneficial for the bones, if a deficit of vitamin D stores is present (serum levels of 25(OH)D below 20-30 ng/mL). Therapeutic use of 25(OH)D or ergocalciferol is indicated in earlier stages of CRF to support renal 1α-hydroxylase activity. At these stages, low doses of $1,25(OH)_2D$ may also be employed to prevent HPT since they are not harmful for renal function. The dose of vitamin D metabolites should be titrated on serum concentrations of calcium and phosphate, to avoid an excessively high calcium-phosphate product, and on serum PTH concentrations, to avoid excessive PTH suppression and an adynamic condition of the bone.
The aim of vitamin D replacement therapy is to prevent HPT since the early stages of CRF, because parathyroid hyperplasia and osteodystrophy cannot be completely reverted once developed. The attention of nephrologists has largely focused on dialysis patients and, unfortunately, few studies have analyzed the outcome of vitamin D therapy in non-uremic patients. Because of the lack of clinical studies in this population no guidelines are available on when to start vitamin D replacement therapy in CRF patients. Therefore, it is likely that HPT and osteodystrophy are undertreated in a significant proportion of CRF patients. We have tried to summarize the criteria proposed in the current literature: one common criteria is that vitamin D therapy should be considered when serum 25(OH)D concentration is below 30 ng/mL. Instead, we do not know when $1,25(OH)_2D$ can be safely prescribed to non-uremic patients and whether there are additional benefits by its association with 25(OH)D or ergocalciferol.
On the other hand, vitamin D therapy is not without problems, the first of which being vascular calcification. Again, we should

make every effort to prevent vascular calcification since the early stages of CRF, because calcification is a non-reversible lesion. The K/DOQI guidelines provide some criteria to minimize the risk of calcium phosphate precipitation in terms of target serum concentration of calcium and phosphate. However, our methods to fight calcification are limited and even the calcium-phosphate product provides insufficient information.

The therapeutical strategies for secondary HPT are now changing. The availability of $1,25(OH)_2D$ analogues warrants inhibition of parathyroid glands with lower effect on calcium and phosphate levels, and perhaps reduces mortality of dialysis patients. In the near future $1,25(OH)_2D$ analogues will be combined with a new drug, cinacalcet, that directly inhibits PTH secretion and parathyroid proliferation. Potentially, the association of cinacalcet with $1,25(OH)_2D$ or its analogues is very promising because of the opposite effects of the two drugs on plasma calcium levels.

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