

$1\alpha(\text{OH})\text{D}_3$ One-alpha-hydroxy- cholecalciferol – an active vitamin D analog

Clinical studies on prophylaxis and treatment of secondary hyperparathyroidism in uremic patients on chronic dialysis

Lisbet Brandt

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Nephrological Department P, Rigshospitalet, Copenhagen, Denmark.

Correspondence: Erichsensvej 17, 2820 Gentofte, Denmark.

E-mail: lisbet.brandt@dadlnet.dk

Official opponents: Peter Schwarz and Børge Nordestgaard.

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1. INTRODUCTION

The topic of this thesis is the use of the vitamin D analog, $1\alpha(\text{OH})\text{D}_3$, in prophylaxis and treatment of secondary hyperparathyroidism in uremic patients treated with chronic dialysis.

1.1 THE VITAMIN D STORY

In 1920, Adolf Windaus, a German physician and chemist, and his colleagues isolated a material from plant sterols that following irradiation with ultraviolet light was active in healing rickets – a disorder characterized by defect mineralization of the skeleton. The substance was termed “vitamin D”. Dr. Windaus and his colleagues further isolated and identified other nutritional forms of vitamin D enabling them to cure diseases caused by lack of vitamin D. Thereby rickets was eliminated as a major medical problem. For this contribution Alfred Windaus received the Nobel Prize in chemistry in 1928 [8].

Besides the effects of vitamin D on bone mineralization, it was discovered in the 1920s and 1930s that an “endogenous factor” played an unequivocal role in calcium homeostasis by changing the intestinal absorption of calcium according to the skeletal need [9]. This “endogenous factor” was later shown to be the vitamin D analog, $1,25(\text{OH})_2\text{D}_3$. Inadequate plasma levels of calcium and phosphate were shown in the 1950s to be of importance for the defective mineralization of the skeleton in vitamin D deficiency. At that time it was discovered that another major function of vitamin D was to mobilize calcium from bone [10]. How vitamin D exerts this mobilization of calcium is still not known in details, but it became clear in the 1970s that both parathyroid hormone (PTH) and vitamin D, independently, were necessary for this process [11]. In the 1980s, the influence of vitamin D and PTH on renal calcium absorption was discovered [12] leading to vitamin D being accepted as a hormone and not just considered as a vitamin.

Already in 1960, Kodieck et al. observed that the inactive vitamin D compound was converted to an active form after digestion [13, 14]. In 1968, $25(\text{OH})\text{D}_3$ was purified and chemically identified as the first active metabolite of vitamin D [15]. Radio labelled $25(\text{OH})\text{D}_3$ was shown to be metabolized to more polar metabolites [16, 17] and in 1971 the structure of the active vitamin D metabolite

was unequivocally demonstrated to be $1,25(\text{OH})_2\text{D}_3$ [18]. The ultimate proof of $1,25(\text{OH})_2\text{D}_3$ being the active metabolite was obtained in 1973 when Vitamin D-dependent rickets type 1 (an autosomal recessive disorder with a defect in the 1α -hydroxylase enzyme) was successfully treated by what later was found to be physiological doses of synthetic $1,25(\text{OH})_2\text{D}_3$, whereas much larger amounts of vitamin D_3 or $25(\text{OH})\text{D}_3$ were needed to heal the disorder [19]. A few years later, two chemical different structures $1\alpha,25(\text{OH})_2\text{D}_3$ [20] and $1\beta,25(\text{OH})_2\text{D}_3$ [21] were synthesized, with $1\alpha,25(\text{OH})_2\text{D}_3$ being the active metabolite.

The stringent, physiological control of plasma $1,25(\text{OH})_2\text{D}_3$ levels is exerted through the coordinated action of classic mineral regulating organs: the kidney, intestine, bone and parathyroid glands. In chronic renal failure, formation of $1,25(\text{OH})_2\text{D}_3$ is impaired [22, 23].

1.2 INTRACELLULAR ACTIONS OF $1,25(\text{OH})_2\text{D}_3$

In the beginning of the 1960s, Zull et al. showed that cellular nuclear activity was required for vitamin D to carry out its function [24]. The first clear demonstration of the existence of a nuclear vitamin D receptor took place in 1973 [25] and in 1988 the Vitamin D receptor was purified and the full coding sequence for the Vitamin D receptor (VDR) described [26]. VDRs are not only present in the classical target tissues (intestine, kidney, bone) regulating calcium homeostasis, but also in a wide variety of non-classical tissues including keratinocytes, malignant cells and cells belonging to the immune system [27]. Numerous genes induced or suppressed by the $1,25(\text{OH})_2\text{D}_3$ /VDR complex are relevant for the efficacy of $1,25(\text{OH})_2\text{D}_3$ therapy. The biological actions of $1,25(\text{OH})_2\text{D}_3$ on genes include the classical calcium homeostasis in bone, intestine and kidney; the regulation of the rates of synthesis and catabolism of $1,25(\text{OH})_2\text{D}_3$, the suppression of PTH synthesis, modulation of immune responses, and suppression of cell proliferation [27]. The simultaneous presence of $25(\text{OH})\text{D}_3$ - 1α -hydroxylase and VDRs in several tissues suggests a paracrine role for $1,25(\text{OH})_2\text{D}_3$ locally modulating cell proliferation and differentiation [28]. The current model of $1,25(\text{OH})_2\text{D}_3$ -VDR action is that $1,25(\text{OH})_2\text{D}_3$ after entering the cell can either be inactivated by mitochondrial 24 -hydroxylase to $24,25(\text{OH})_2\text{D}_3$ or bind to VDR. Ligand binding activates the VDR to translocate from the cytosol to the nucleus where it heterodimerizes with its partner – the retinoid X receptor RXR. The VDR/RXR complex binds to specific sequences in the promoter region, the vitamin D response element (VDRE), of the target genes – and recruits basal transcription factors and co-regulator molecules to either increase or suppress the rate of gene transcription by RNA-polymerase II [27].

Results of several animal studies have suggested that $1,25(\text{OH})_2\text{D}_3$ also can exert direct action on its target cells via rapid effects on the cell membrane [29, 30]. In 1998, the first paper was published identifying a membrane receptor for $1,25(\text{OH})_2\text{D}_3$ mediating a rapid activation of protein kinase C in rat chondrocytes. Until now, a similar membrane receptor has not been demonstrated in human tissues.

In this thesis first a short introduction to the calcium and phosphate homeostasis in the normal man will be given. Then an overview of the causes, development and consequences of secondary hyperparathyroidism in chronic uremic patients is presented. Finally, follows a short overview of conventional treatment modalities of secondary hyperparathyroidism in dialysis patients at the time when the first of the presented studies was initiated. The clinical data presented are from 3 different experimental scenarios:

1. Short and long-term experiences with intermittent intravenous $1\alpha(\text{OH})\text{D}_3$ treatment to patients on hemodialysis, and a short-term cross-over study between intermittent oral and intermittent intravenous administration.
2. Experience with intermittent oral and intravenous $1\alpha(\text{OH})\text{D}_3$ treatment, combined with reduced calcium concentration in the dialysis fluid to patients on CAPD (Continuous Ambulatory Peritoneal Dialysis) and on hemodialysis and at the same time a

simultaneous change from aluminum- to calcium-containing phosphate binders.

3. Studies on the pharmacokinetic properties of $1\alpha(\text{OH})\text{D}_3$ as compared to active vitamin D, $1,25(\text{OH})_2\text{D}_3$, in normal subjects and uremic patients.

The results obtained will be discussed from diagnostic and treatment perspectives with respect to new knowledge obtained from studies during the recent years. At the end, some aspects of treatment in the future will be discussed.

2. NORMAL CALCIUM AND PHOSPHATE HOMEOSTASIS

2.1 CALCIUM HOMEOSTASIS IN NORMAL MAN

Regulation of the calcium homeostasis involves several organs: Intestine, kidneys, skeleton, and the parathyroids, and different hormones: Parathyroid hormone, vitamin D and calcitonin [31]. The concentration of ionized calcium (Ca^{2+}) in the extracellular fluid (ECF) is 104 times higher than the concentration in the intracellular fluid (ICF) [32, 33]. Most of the calcium in the body (99%) is stored in the skeleton, which serves as a relatively inexhaustible reservoir. Approximately 47% of the extracellular calcium is ionized, 46% is proteinbound and the remainder is complexed to small ions. Approximately 75% of the proteinbound fraction is bound to albumin [32, 33]. Thus less than 1% of the calcium present in ECF is present as Ca^{2+} [32, 33].

Non-ionized calcium, predominantly found in skeletal bone, provides an important structural function to the body, whereas Ca^{2+} is responsible for a variety of physiological and cellular effects that are characteristic of that particular cell type (e.g. secretion, neuromuscular impulse formation, contractile functions etc.).

Normal adult man ingests about 20 mmol of calcium per day of which approximately 40% (i.e. 8 mmol) is absorbed in the duodenum and upper jejunum, although absorption varies from 10-90% (2-18 mmol). About 10% of intestinal calcium absorption occurs passively via independent paracellular, non-saturable pathways while the major part is absorbed via specialized vitamin D dependent saturable, transcellular pathways [31, 32]. Skeletal bone liberates and reabsorbs approximately 500 mmol of calcium per day from an exchangeable pool of 100 mmol [32]. About 250 mmol of ionized calcium is filtered per day in the kidneys by glomerular filtration. About 65% (i.e. 165 mmol) is reabsorbed passively together with sodium in the proximal tubule, 20-25% (i.e. 60 mmol) is reabsorbed in the thick ascending loop of Henle, and finally 10% (i.e. 25 mmol) is absorbed in the distal convoluted tubuli [32]. Parathyroid hormone (PTH) increases calcium absorption in the thick ascending loop of Henle as well as in the distal convoluted tubule, but has no effect on calcium reabsorption in the proximal tubule. The urinary excretion of calcium is 2.5-7.5 mmol per day and represents about 3-5% of the filtered calcium. Approximately 60% (i.e. 12 mmol) of the oral daily intake is excreted with the feces [32].

In healthy man, plasma Ca^{2+} does not vary by more than 5% and is maintained constant mainly by the actions of PTH and vitamin D [31, 32, 34]. Calcitonin is largely secreted in hypercalcemic conditions [32, 33].

2.2 PHOSPHATE HOMEOSTASIS IN NORMAL MAN

An intimate relationship exists between the homeostasis of phosphate and calcium.

Phosphate is needed for mineralisation of bone, for cellular structural components (e.g. phospholipids, nucleotides, phosphoproteins), for energy storage in ATP, for oxygen transport in red blood cell in 2,3-DPG, and in the acid base balance of the organism as cellular and urinary buffers [32]. Phosphates in blood exist as organic (ester and lipid phosphates) and inorganic compounds. Plasma phosphate denotes the inorganic component. Total plasma phosphate in adults ranges from 0.80 to 1.35 mmol/L and is distributed between 15% protein-bound, 45% ionized and 40% in complexed

forms with calcium and magnesium. The intracellular concentration of phosphate is approximately 100 mmol/l with 5 mmol/l as inorganic phosphate and 95 mmol/l in an organic form (i.e. bound in ATP, ADP, creatine phosphate, nicotinamide, adenine dinucleotide etc.). These intracellular forms are readily exchangeable [32].

The normal daily oral phosphate intake is approximately 40 mmol of which approximately 60-70% (i.e. 25-30 mmol) is absorbed in the duodenum and upper jejunum. The minimum oral requirement of phosphate is about 20 mmol per day. Normal urinary phosphate excretion ranges between 10 and 40 mmol per day and about 15 mmol per day is excreted with feces. Approximately 180 mmol phosphate is filtered in the kidneys per day of which 70-85% is reabsorbed in the proximal tubule and 15-30% in the distal nephron. PTH induces phosphaturia by an inhibition of the sodium-phosphate cotransport in the proximal tubule. There is no tubular secretion of phosphate. Ca^{2+} controls urinary phosphate excretion indirectly via PTH secretion. Tubular reabsorption of phosphate increases up to a maximum (TmPO_4); whereafter phosphate is excreted in the urine. PTH, decreases TmPO_4 [32].

Plasma Pi level is maintained within a narrow range through a complex interplay between intestinal absorption, exchange with intracellular and bone storage pools, and renal tubular reabsorption [32, 35]. Most of the factors identified until now, controlling Pi homeostasis, decrease renal reabsorption and intestinal uptake [36]. The key factors are the type 2 Na^+ -Pi cotransporters NPT2a, b and c [35, 36]. NPT2a is a major molecule expressed in the renal proximal tubular cells and in osteoclasts [37, 38], NPT2c is expressed in the same tissues as NPT2a, but must be different from NPT2a since it cannot compensate for functional defects in the NPT2a transporter [39]. The cotransporter NPT2b is expressed in the small intestine, lung, testis and mammary gland [40, 41].

Recent studies of inherited and acquired hypophosphatemia (X-linked hypophosphatemic rickets/osteomalacia, autosomal dominant hypophosphatemic rickets/osteomalacia and tumor-induced rickets/osteomalacia), which exhibit similar biochemical and clinical features, have led to the identification of novel genes, PHEX and FGF23, that also take part in the regulation of Pi homeostasis [42].

In the last decade, a new hormone, klotho, involved in the ageing process [43] has been shown to play a role in the phosphate/calcium metabolism as well [44]. The klotho protein binds to fibroblast growth factor receptors and regulates FGF23 signalling [45]. Klotho also exhibits enzymatic activity modifying the sugar chains of the transient receptor potential vanilloid-5 channel (TRPV5) thereby regulating its activity which involves a durable calcium channel activity and membrane calcium permeability in the kidney [46]. The klotho protein seems to be negatively controlled by dietary Pi [35, 47]. The possible importance in uremic patients remains to be established.

3. METABOLIC CALCIUM DISORDERS IN PATIENTS ON CHRONIC DIALYSIS TREATMENT

In the 1920s and 1930s, an association between bone disease and chronic renal failure was described in different terms such as "renal dwarfism" [48] and "renal rickets" [49]. Based on pathological studies, the term "renal osteitis fibrosa cystica" was introduced in 1937 [8]. In 1943, the term "renal osteodystrophy" was suggested by Liu et al. [50] to unify the concept of bone disorders found in chronic renal failure. The clinical characteristics of renal osteodystrophy were summarized in 1948 by Albright et al. as the combination of renal insufficiency, phosphate retention, a tendency to low plasma calcium, and hyperplasia of the parathyroid glands [51]. They suggested, however, that the cause of the bone disease was acidosis and not hyperparathyroidism. Therefore, their therapeutic strategy was supplementation by a large amount of alkali in combination with oral calcium and native vitamin D-50.000 IU/day. The skeletal symptoms of the patients' diminished and radiological findings improved in approximately 2 months. Other authors reported

improvement of the bone lesions with even higher vitamin D doses, 100.000 IU/day and disagreed on the suggested role of the acidosis [52]. The dispute could not be settled at that time. In 1968, however, the phenomenon "lack of osteoid" in azotemic osteitis fibrosa was described, and the significance of the parathyroid glands in chronic renal failure was proposed [53].

The annual mortality rate in uremic patients corrected for age, sex and race is significantly higher than in the general population. This is primarily due to vascular calcifications and cardiovascular events [54]. In the late 1990s an association between the "plasma calcium \times plasma phosphate product" referred to as the "Ca \times P product", mortality and severe coronary artery calcification [55, 56] was observed in young uremic adults. Over the last few years it has further been shown that disturbances in calcium and phosphate metabolism per se may contribute to the pathogenesis of vascular calcifications [55, 57]. Especially the Ca \times P product has been identified as an independent risk factor of mortality [55]. Therefore, the new K-DOQI guidelines recommend that plasma calcium should be maintained within the normal range – preferably toward the lower end – in patients on chronic dialysis [58].

4. PARATHYROID HORMONE, SECONDARY HYPERPARATHYROIDISM AND PARATHYROID HYPERPLASIA IN UREMIC PATIENTS

Parathyroid hormone is an 84 amino acid polypeptide hormone synthesised in the four parathyroid glands [59]. The human PTH gene is placed on the short arm of chromosome 11, band 11p15. It consists of 3 exons and 2 introns, which are spliced out before transcription to PTH mRNA. On the ribosome, the PTH mRNA is translated to pre-proPTH, which consists of 115 amino acids. During the passage through the rough endoplasmic reticulum, 25 amino acids are spliced off and the product becomes the pro-PTH. In the Golgi apparatus, further 6 amino acids are spliced off before the final PTH 1-84 is stored in vesicles in the cytoplasm of the chief cells in the parathyroid glands [59]. This final PTH molecule is not very stable and may, if plasma Ca^{2+} is high, be degraded to inactive fragments by mechanisms not yet clarified [60-62].

In early renal failure, decreased levels of plasma $1,25(\text{OH})_2\text{D}_3$ induce a change in PTH gene expression and increased synthesis and secretion of PTH [63-65]. The decreased levels of plasma $1,25(\text{OH})_2\text{D}_3$ is due to a decreased renal 1α -hydroxylase activity and a decreased phosphate excretion. The changes are followed by a decrease in plasma Ca^{2+} due to reduced intestinal absorption of calcium and diminished direct feed-back inhibition of $1,25(\text{OH})_2\text{D}_3$ on the parathyroid glands. Ca^{2+} exerts its direct effect on the parathyroid glands through an activation of a seven-transmembrane G-protein-coupled receptor – the Ca^{2+} -sensing receptor (CaR) [66]. The activated receptor triggers a cascade of intracellular responses that eventually decreases synthesis and secretion of PTH and increases degradation of preformed PTH [60]. The relationship between plasma Ca^{2+} and PTH secretion is in normal subjects a sigmoidal S-shaped curve [66]. The CaR is downregulated in moderate to severe secondary hyperparathyroidism resulting in a diminished inhibition of the PTH secretion at a given plasma Ca^{2+} concentration [67, 68]. Furthermore, the down regulation of the CaR is involved in the regulation of the parathyroid cell proliferation resulting in the hyperplasia of the parathyroids [69, 70], that is typically seen in patients with chronic renal failure [71]. A direct suppressive effect of $1,25(\text{OH})_2\text{D}_3$ on the PTH gene transcription which results in decreased synthesis and secretion of PTH, has been demonstrated in rats [72, 73] and humans [74].

$1,25(\text{OH})_2\text{D}_3$ levels are decreased and the vitamin D receptor in the parathyroid cells is down regulated in uremic patients [68, 75, 76]. The consequence is a reduced suppression of PTH transcription and consequently an increased synthesis of PTH. Furthermore, $1,25(\text{OH})_2\text{D}_3$ may also have a role in regulating parathyroid cell proliferation in chronic renal failure [69].

Increased plasma phosphate is a major stimulus to secondary hyperparathyroidism [77-80], maybe by an inhibited release of calcium from intracellular stores [81]. Much of the effect of both decreased Ca^{2+} and increased phosphate has, however, been demonstrated to be due to an increased stability of PTH mRNA and thereby an increased translation into PTH [61, 62].

In advanced stages of chronic renal failure, the mode of growth is changed in the parathyroid cells. Nodular formations within the diffuse hyperplastic tissue has been observed in glands removed from patients with advanced secondary hyperparathyroidism [71, 82]. This type of growth may be both monoclonal resulting in diffuse hyperplasia and polyclonal resulting in nodules [83]. The reason for the high frequency of monoclonal proliferation is unclear. Monoclonal, recurrent changes are present in more than 50% of glands removed from uremic patients with secondary hyperparathyroidism [82]. Chromosomal changes such as mutations or deletions of tumour suppressor genes or activation of tumour enhancer genes have been suggested [84], but mutations or losses of heterozygosity of the CaR or the VDR have not been identified thus far [84].

5. CLINICAL CHALLENGES IN THE TREATMENT OF RENAL OSTEODYSTROPHY AND CARDIOVASCULAR DISEASE

Renal osteodystrophy is the term used to describe many different histological patterns of the skeletal abnormalities in chronic renal failure [85]. The three main conditions are: 1. osteitis fibrosa, characterized by high bone turnover, increased osteoclastic and osteoblastic activity, and high levels of circulating PTH, and 2. an adynamic bone disease, osteomalacia and aluminum induced osteomalacia characterized by low bone turnover and low levels of circulating PTH [85, 86] and 3. a mixed disease. The gold standard for the diagnosis of renal osteodystrophy is a bone biopsy [87]. Long time before uremic patients present with clinical symptoms from the skeleton, biochemical parameters and bone histology become abnormal [87-89]. The histological pattern reported in patients on chronic dialysis treatment is very varied. Osteitis fibrosa is reported in 30-50% of patients in CAPD and HD and adynamic bone disease in 20-66% of the patients [90-94]. The highest frequency of adynamic bone diseases has been found in centers that simultaneously reported the highest frequency of positive aluminum staining in the bone biopsies [91].

In Denmark, as in many other countries, bone biopsies have been replaced by combinations of less invasive methods, for practical reasons, involving blood samples and a variety of imaging methods as a guide to management. Measurement of plasma PTH remains the single most useful biochemical test predicting bone histology in an individual patient [95-99]. Newer biochemical markers of bone turnover may provide useful supplementary information in the future [100, 101].

For years, focus has mainly been on the relationship between plasma PTH, plasma Ca^{2+} , and plasma phosphate on one side and cardiovascular calcifications and mortality on the other side [55, 56, 102-107], e.g. the relation found between the total calcium load in uremic patients due to treatment with vitamin D analogs and calcium containing phosphate binders and mortality [108, 109]. Excessive vascular calcification is seen as a non cell-mediated process of metastatic calcification [55, 102, 110] and also as a condition which resembles developmental osteogenesis [111]. It is well known that hyperphosphatemia is directly involved in vascular calcification [57, 112, 113], but whether $1,25(\text{OH})_2\text{D}_3$ itself also is a contributing factor is still not clarified [114-116]. However, clinical studies suggest that treatment with vitamin D_3 analogs per se may improve the prognosis in uremic patients suffering from cardiovascular disease [117]. Studies have demonstrated better cardiac performance and reduced left ventricular hypertrophy in uremic patients treated by intravenous $1,25(\text{OH})_2\text{D}_3$ [118] and in chronic uremic patients following parathyroidectomy [119, 120]. Recently, increased survival in patients treated with 19-nor- $1,25(\text{OH})_2\text{D}_3$ [121] and a decreased

cardiovascular mortality in patients treated by $1\alpha(\text{OH})\text{D}_3$ [122] has been reported.

6. PHOSPHATE RESTRICTION

In the early 1970s, it became clear that phosphate retention and hypocalcemia in endstage renal disease were of great importance for the pathogenesis of secondary hyperparathyroidism, and that phosphate restriction could prevent the development [77, 123, 124]. As the glomerular filtration rate (GFR) declines in uremia, phosphate retention will ensue, and dietary phosphate restriction alone will not be sufficient to maintain phosphate balance even during hemodialysis on the usual schedule – 4-5 hours 3 times a week [125]. Phosphate retention is associated with a number of complications including besides hyperparathyroidism, renal osteodystrophy [123, 124, 126], and increased mortality [55, 104, 106, 107, 127]. Additional treatment with aluminum containing oral phosphate binders to overcome phosphate retention was initiated already in the 1960s and used extensively until aluminum toxicity was disclosed in the mid-1980s [128].

Consequently, the use of aluminum salts has now been limited to short periods of time in patients with hyperphosphatemia which is difficult to control [58]. Instead calcium carbonate was introduced as a phosphate binder [129] reducing plasma phosphate, but at the expense of an elevation of plasma Ca^{2+} [129]. Calcium acetate was demonstrated to have higher phosphate binding capacity [130] and a lower frequency of hypercalcemia [130] than calcium carbonate, although not in patients on concomitant $1,25(\text{OH})_2\text{D}_3$ treatment [131]. Calcium carbonate and calcium acetate are still the most commonly used phosphate binders worldwide. Although calcium containing phosphate binders are efficacious and cost-effective, the long-term safety of these agents has become the subject of an intense debate because of their possible, but not yet confirmed, role in the progression of soft tissue and cardiovascular calcification in dialysis patients [56, 103, 109, 131-133]. Therefore, the development of an aluminum- and calcium-free phosphate binder became an important goal. Sevelamer HCl was the first developed aluminum- and calcium-free cationic polymer binding phosphate anion acting by ionexchange and hydrogenbonding. It is not absorbed in the gastrointestinal tract and is excreted in feces [134]. Besides phosphate it also binds and sequesters bile salts [135], a property that may account for its ability to lower serum cholesterol concentrations [135]. This may be of importance in uremic patients with cardiovascular disease. Several studies have demonstrated that sevelamer HCl is able to reduce plasma phosphate, reduce the $\text{Ca} \times \text{P}$ product [109, 136, 137] and in two studies, to slow the progression of vascular calcifications [109, 138]. Other intestinal phosphate binders such as lanthanum carbonate [139, 140] and iron-based compounds [141] are now in use. In animal experiments, several groups are investigating intestinal inhibitors of $\text{Na}^+\text{-Pi}$ cotransport [142, 143], but no human in vivo studies are available at the moment.

7. VITAMIN D ANALOGS

Besides reducing the phosphate stimulus of PTH secretion, vitamin D analogs have been used for suppression of PTH synthesis. Shortly after synthetic 1α -hydroxylated metabolites of vitamin D such as $1,25(\text{OH})_2\text{D}_3$ and $1\alpha(\text{OH})\text{D}_3$ were introduced [20, 144, 145] they were provided to uremic patients in order to increase the intestinal calcium absorption and improve the skeletal abnormalities. When the direct suppressive effects of $1,25(\text{OH})_2\text{D}_3$ on PTH synthesis and secretion were discovered [146-148], the use in uremic patients of active vitamin D analogs was expanded. The combined treatment with calcium containing oral phosphate binders and active vitamin D, however, unveiled induction of hypercalcemia as a clinical problem [129, 149, 150]. Intermittent intravenous administration of vitamin D analogs was proposed to solve this problem [74], but prove not to be successful, and subsequently dialysis fluids with reduced calcium content (low-calcium) were introduced [151, 152].

Besides the classical effects of active vitamin D on mineral metabolism, vitamin D is known to affect immunity, muscle and vasculature, reproduction and growth and differentiation of many cell types [27]. Therefore, new vitamin D analogs have been developed in an attempt to increase potency on PTH suppression or to identify analogs with specific effects [153, 154]. In chronic renal failure, focus has been on preventing hypersecretion of PTH without inducing hypercalcemia and hyperphosphatemia [153, 154]. Four analogs with potentially less calcium toxicity, as compared to $1,25(\text{OH})_2\text{D}_3$, have been approved for treatment of secondary hyperparathyroidism in uremia: 19-nor- $1,25(\text{OH})_2\text{D}_2$ [155-160], $1\alpha(\text{OH})\text{D}_2$ [161-165], 22-oxa- $1,25(\text{OH})_2\text{D}_3$ [166, 167] and 26,27- F_6 - $1,25(\text{OH})_2\text{D}_3$ [168, 169]. Very few comparative studies have been performed between the different new analogs and the genuine hormone, $1,25(\text{OH})_2\text{D}_3$. 26,27- F_6 - $1,25(\text{OH})_2\text{D}_3$ has been compared to $1\alpha(\text{OH})\text{D}_3$ [168] and was found to control PTH more effectively; but the results were hampered by a low number of patients enrolled in the study. 19-nor- $1,25(\text{OH})_2\text{D}_2$ has been compared to $1,25(\text{OH})_2\text{D}_3$ and was found to suppress PTH a little faster than $1,25(\text{OH})_2\text{D}_3$ [157]. In a long-term non-controlled study on 19-nor- $1,25(\text{OH})_2\text{D}_2$ PTH was not adequately suppressed because of necessary reductions of dose due to the development of hypercalcemia and an elevated $\text{Ca} \times \text{P}$ product [158]. Coyne et al. found less elevation of serum calcium and phosphate levels in patients on hemodialysis after administration of one large dose of 19-nor- $1,25(\text{OH})_2\text{D}_2$ when compared to $1,25(\text{OH})_2\text{D}_3$ provided in 6 and 8 times smaller doses based on weight, respectively [170].

8. $1\alpha(\text{OH})\text{D}_3$ – AN ACTIVE VITAMIN D ANALOG

In this thesis focus is on the active vitamin D analog $1\alpha(\text{OH})\text{D}_3$. $1\alpha(\text{OH})\text{D}_3$ is an analog of vitamin D_3 which is hydroxylated at position number 1, and administration of $1\alpha(\text{OH})\text{D}_3$ therefore bypasses the impaired 1α -hydroxylation in the diseased kidneys of patients with chronic renal failure. $1\alpha(\text{OH})\text{D}_3$ has to be hydroxylated at the 25-position by the liver to be converted to the active metabolite $1,25(\text{OH})_2\text{D}_3$ [171, 172].

$1\alpha(\text{OH})\text{D}_3$ was produced by LEO Pharma in 1973 as an oral formulation which was convenient, stable and inexpensive [144]. Preliminary reports showed a therapeutic effect of $1\alpha(\text{OH})\text{D}_3$ already in 1973 [173] and it became available in 1974 in Denmark. Since then and until now, it has been the main active vitamin D analog used in Denmark. Already in 1980, Dr. Søren Madsen published his doctoral thesis on the effects of oral administration of $1\alpha(\text{OH})\text{D}_3$ on calcium and phosphate metabolism in chronic renal failure [174]. His conclusion was that “under careful control of plasma calcium and plasma phosphate, all normo- and hypocalcemic dialysis patients who were dialyzed in dialysis units should receive $1\alpha(\text{OH})\text{D}_3$ (or $1,25(\text{OH})_2\text{D}_3$) treatment with the intention to abolish secondary hyperparathyroidism and restore defective vitamin D metabolism”. Shortly after the introduction of $1\alpha(\text{OH})\text{D}_3$ on the market, $1,25(\text{OH})_2\text{D}_3$ also became available for medical treatment [175]. The two vitamin D analogs were used in different geographical areas: In Europe, $1\alpha(\text{OH})\text{D}_3$ was mainly used, while $1,25(\text{OH})_2\text{D}_3$ was mainly used in USA. Surprisingly few studies have been performed comparing these two analogs.

Very little was known about possible differences in actions of $1\alpha(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ when one of the presented studies of this thesis was initiated [7]. A few papers had raised the question whether $1\alpha(\text{OH})\text{D}_3$ needed to be hydroxylated to $1,25(\text{OH})_2\text{D}_3$ before exerting its effects [144, 176, 177]. The possibility that 25-hydroxylation wasn't necessary was supported by in vitro studies from our laboratory which showed that $1\alpha(\text{OH})\text{D}_3$ induced a suppression of PTH secretion from bovine parathyroid cells similar to that of $1,25(\text{OH})_2\text{D}_3$ [178]. Furthermore, studies have demonstrated that the OH-group in the 1α -position and the 6-s-trans conformation of the molecule [179] are of importance for the nuclear VDR activation while the 25-OH-group is of less importance.

The relative therapeutic potency of 1,25(OH)₂D₃ and 1α(OH)D₃ is not clear. Similar µg doses of 1,25(OH)₂D₃ and 1α(OH)D₃ do stimulate intestinal calcium absorption and reverse renal bone disease to the same degree [175, 180, 181]. A delayed effect on intestinal calcium absorption of 1α(OH)D₃ when compared to 1,25(OH)₂D₃ has been published [182], but this delay vanished after long-term treatment.

Both analogs have a significant suppressive effect on plasma PTH in secondary hyperparathyroidism [183]. The potency of 1α(OH)D₃ has however been reported both to be equal to [184] and half that of 1,25(OH)₂D₃ [185, 186] when used in chronic uremic patients.

Only sporadic information was available on the possible pharmacokinetic differences between 1α(OH)D₃ and 1,25(OH)₂D₃ at the time when the present pharmacokinetic study was initiated [6]. After oral administration of 1α(OH)D₃ both a significantly smaller peak concentration (C_{max}) and a smaller area under the curve (AUC) of 1,25(OH)₂D₃ have been reported, than after oral administration of similar doses of 1,25(OH)₂D₃ [186-188]. Increasing peak concentrations of 1,25(OH)₂D₃ were found following increasing doses of intravenous 1α(OH)D₃ [189], but the levels of 1,25(OH)₂D₃ achieved were much lower than those described in studies on intravenous administration of even smaller doses of 1,25(OH)₂D₃ [74, 190]. The possible therapeutic consequences of these findings are not clear. It has been shown that mouse osteoblasts can convert 1α(OH)D₃ to 1,25(OH)₂D₃ [191]. This is maybe also the case in human osteoblasts [192]. A direct suppressive effect of intravenous 1,25(OH)₂D₃ on the secretion of PTH in acutely uremic patients was reported for the first time in 1981 by S. Madsen et al. [148]. Followed in 1984 by a study by Slatopolsky et al. who demonstrated that intermittent intravenous administration of 1,25(OH)₂D₃ induced a marked suppression of PTH without inducing hypercalcemia in patients on chronic hemodialysis [74]. No experience with 1α(OH)D₃ intravenously neither in short- nor long-term studies or intermittent intravenous 1α(OH)D₃ in combination with calcium carbonate binders and low-calcium dialysis fluid was available at the time when the presented studies were initiated [1-5].

In patients treated by CAPD, frequent intravenous administration of 1α(OH)D₃ or 1,25(OH)₂D₃ is impracticable. In the 1990s, studies showed that the effects of intermittent oral and intermittent intravenous 1,25(OH)₂D₃ were similar regarding the suppression of PTH [193-195]. No data on intermittent oral administration of 1α(OH)D₃ in combination with low-calcium dialysis fluid in CAPD patients were available at the time when this particular study was initiated [5].

9. AIM OF THE PRESENT STUDIES

The main purpose of the present studies was to increase the knowledge of the action and effects of different treatment regimes of 1α(OH)D₃, and thereby to improve the prophylaxis and treatment of secondary hyperparathyroidism in uremic patients on chronic dialysis.

The detailed aim of the present series of studies therefore was:

1. To evaluate in patients on chronic hemodialysis whether:

- intermittent intravenous administration of 1α(OH)D₃ would suppress the plasma PTH levels of patients with secondary hyperparathyroidism without inducing the same degree of hypercalcemia that would have been expected from oral administration of similar doses of 1α(OH)D₃ [1]
- it was possible to maintain the marked suppression of plasma PTH seen in short term studies by a long term use of intermittent intravenous administration of 1α(OH)D₃ [2]
- intermittent oral 1α(OH)D₃ treatment could maintain the marked suppression of PTH found after long-term intermittent intravenous administration of 1α(OH)D₃, and further to evaluate whether the route of administration of 1α(OH)D₃ affected

the circulating levels of N- and C-terminal PTH fragments in plasma [3].

2. Growing awareness of the toxicity of aluminum containing phosphate binders resulted in changes in the dialysis regimes with introduction of calcium containing phosphate binders and "low-calcium" dialysis fluid (1.25 mmol/l). These new regimes were evaluated in another series of studies regarding:

- the effect of long-term intermittent intravenous use of 1α(OH)D₃ on the secondary hyperparathyroidism and biochemical bone markers in patients on chronic hemodialysis with normal or elevated plasma PTH levels and, further, to evaluate changes of plasma Ca²⁺ during dialysis using two different dialysis solutions [4]
- the effect of long-term intermittent oral use of 1α(OH)D₃ on the secondary hyperparathyroidism in patients on CAPD and to evaluate the changes in the peritoneal mass transfer of calcium, phosphate, magnesium, lactate, creatinine, urea, glucose and albumin after changing to "low-calcium" dialysis fluid [5].

3. Studies were performed to explore pharmacokinetic differences and similarities between 1α(OH)D₃ and 1,25(OH)₂D₃ and to examine whether 1α(OH)D₃ "only" is a pro-drug to 1,25(OH)₂D₃ or has an independent action per se:

- the pharmacokinetics of a single dose of 4 µg of 1,25(OH)₂D₃ and 1α(OH)D₃ in response to intravenous and oral administration in both healthy humans and uremic patients. At the same time, to measure the effects of a single dose of 4 µg of the respective vitamin D analogs on the plasma PTH and plasma Ca²⁺ levels in order to examine whether possible pharmacokinetic differences would result in different biological responses [6]
- the acute effects of a single high dose of 10 µg of 1,25(OH)₂D₃ and 1α(OH)D₃ on the plasma levels of PTH, Ca²⁺ and phosphate in uremic patients on chronic hemodialysis, evaluated by the "Whole" and the "Intact" PTH assays [7].

10. TREATMENT SCHEDULES, PATIENTS AND OUTCOME MEASURES

A total of 168 different patients and 6 healthy volunteers were involved in these three series of studies, which all were performed as single-centre studies. An overview of the number of the treatment schedules, patients, drop-outs, and outcome measures for the three scenarios is presented in the following.

THE FIRST PART (1A, B AND C)

These studies focused on short - (12 weeks) and long-term (103 weeks) effects of intravenous 1α(OH)D₃ on plasma PTH and plasma Ca²⁺ in relation to the doses of 1α(OH)D₃ given. Further, it was examined whether the marked suppression of plasma PTH induced by 300 days of intermittent intravenous treatment with 1α(OH)D₃ could be maintained, when the administration was changed from intravenous to the oral route for 16 further weeks and eventually back to intravenous administration for another 16 weeks.

Patients

In part 1a, 24 patients were included. One patient died from a cerebral thrombosis and 2 patients underwent kidney transplantation during the study. Thus, 21 patients completed the study. One patient was treated outside the protocol with exactly the same schedule. He had not finished 12 weeks of treatment when the results were evaluated and was therefore not included in the publication [1].

In part 1b, a total number of 22, the 21 patients from study 1a plus the extra patient, continued the treatment. A total of 9 patients were withdrawn from the study after 12 weeks of treatment due to severe hypercalcemia (n=1), death from AMI (n=1), transferral to CAPD

(n=1), RAT (n=2) and poor compliance especially to the phosphate binder therapy (n=4). Thirteen patients were followed for 300 days (10 months). Thereafter, 4 patients were excluded due to RAT (n=2), death from aortic stenosis (n=1) and death from severe infection (n=1). Nine patients were followed for 523 days (14 months), whereafter 3 patients were withdrawn due to severe hyperphosphatemia (n=1), RAT (n=1) and severe infection and illness (n=1). Thus, 6 patients were followed for 720 days (2 years). Twenty patients were treated outside the protocol at exactly the same schedule, but not included in the publication [2].

In part 1c, 6 patients from the previous study and the 20 patients treated after the same schedule were included. Seventeen patients completed 300 days. 6 patients completed the following oral phase and 5 patients all three parts. 21 patients were withdrawn for the following reasons: Hyperphosphatemia (n=8), RAT (n=6), death from AMI (n=2), severe infection (n=3), hypercalcemia (n=1) and transferral to CAPD (n=1).

Methods

Blood samples were drawn immediately before the dialysis at day 0 and then at regular intervals for 1-4 weeks. $1\alpha(\text{OH})\text{D}_3$ was given at the end of each dialysis session in increasing doses (maximally 4 μg per dialysis) under careful control of plasma Ca^{2+} . If hypercalcemia developed, $1\alpha(\text{OH})\text{D}_3$ was temporarily discontinued or a lower dose given. When necessary, the dosage of oral phosphate binder was adjusted.

Outcome measures

Outcome measures were plasma PTH, plasma Ca^{2+} , and the doses of $1\alpha(\text{OH})\text{D}_3$.

THE SECOND PART (2A AND B)

These studies focused on long-term effects (88 weeks in hemodialysis patients and 52 weeks in CAPD patients) of a treatment combining $1\alpha(\text{OH})\text{D}_3$, CaCO_3 phosphate binders (instead of aluminum containing binders) and a decreased calcium concentration in the dialysis fluid (1.25 mmol/l) in an attempt to avoid hypercalcemia.

Patients

In part 2a, 60 patients on hemodialysis were included of who 54 completed the first 12 weeks. One patient had tertiary hyperparathyroidism and was scheduled for parathyroidectomy, 2 patients were transferred to CAPD, 2 had RAT, and 1 died in septicemia.

Eighteen patients were withdrawn after 12 weeks due to AMI (n=5), RAT (n=4), parathyroidectomy (n=1), severe hyperphosphatemia due to poor compliance (n=4), transfer to other dialysis units (n=3), and infection (n=1). Thus, 36 patients completed 52 weeks of the study. Seven patients were withdrawn after 52 weeks of treatment due to AMI (n=2), RAT (n=3) and death of severe infection (n=2). Thus, 29 patients were followed for 88 weeks.

In part 2b, 41 patients treated by CAPD were included. Thirty-nine of these completed the first 12 weeks of treatment. One patient had a RAT and 1 was excluded due to peritonitis. Further 9 patients were withdrawn after 12 weeks due to AMI (n=1), RAT (n=3), exacerbation of chronic obstructive pulmonary disease and inability to perform CAPD (n=1), transferral to hemodialysis after own wish (n=1), severe peritonitis (n=2), and operation for ventral hernia (n=1). Thus, 30 patients were followed for 52 weeks.

Methods

Two separate sets of blood samples were obtained as basal values. CaCO_3 was then initiated as oral phosphate binder therapy in patients who received aluminum containing phosphate binder at inclusion. Aluminum containing phosphate binders were, however, still allowed, if judged necessary by the investigator.

The calcium concentration in the dialysis fluid was decreased from 1.75 or 1.50 mmol/l, depending on the previous treatment concen-

tration, to 1.25 mmol/l in the patients treated by hemodialysis and from 1.75 mmol/l to 1.25 mmol/l to patients treated by CAPD.

In patients on chronic hemodialysis who already were being treated with oral $1\alpha(\text{OH})\text{D}_3$ the administration route was changed to intravenous administration. Patients already treated with intravenous $1\alpha(\text{OH})\text{D}_3$ remained initially on an unchanged dose, and patients on CAPD who were already treated by intermittent oral $1\alpha(\text{OH})\text{D}_3$ remained initially at unchanged doses.

Blood samples were obtained immediately before the dialysis from patients treated by hemodialysis and in the morning in the outpatient clinic from patients treated by CAPD. Blood samples were drawn at day 0 and then at regular intervals of 1-4 weeks. The maximum dose of $1\alpha(\text{OH})\text{D}_3$ was 12 $\mu\text{g}/\text{week}$ given under careful control of plasma Ca^{2+} . If hypercalcemia developed, $1\alpha(\text{OH})\text{D}_3$ was temporarily discontinued or a lower dose given. If necessary the dosage of oral phosphate binder was adjusted.

Outcome measures

Outcome measures in the two groups of patients (treated by hemodialysis and CAPD) were plasma levels of PTH, Ca^{2+} and phosphate, the doses of oral phosphate binder used, and the doses given of $1\alpha(\text{OH})\text{D}_3$.

In patients treated with hemodialysis the biochemical bone markers, osteocalcin, alkaline phosphatases, and procollagen type 1 c-terminal extension peptid (P1cP) were measured. BMC was measured in the lumbar spine, femoral neck and also the femoral shaft, as suggested by Ruedin et al. in uremic patients treated with $1,25(\text{OH})_2\text{D}_3$ [196]. Plain X-rays of hands, lumbar spine and hip were performed, too.

Actual plasma Ca^{2+} , pH and plasma Ca^{2+} adjusted to pH=7.4 were analysed at the beginning and at the end of two hemodialysis sessions with the 2 different dialysis solutions.

The dialysis fluid used for CAPD was a commercial solution, and simultaneously with the decrease in the calcium concentration, the magnesium concentration was decreased from 0.75 mmol/l to 0.25 mmol/l, and the lactate concentration increased from 35 mmol/l to 40 mmol/l. Therefore, a peritoneal mass transfer (PET) evaluating calcium, phosphate, magnesium, lactate, creatinine, urea, glucose and albumin transport was performed.

THE THIRD PART (3A AND B)

These studies focused upon the pharmacokinetic differences between intravenous and oral administration of $1,25(\text{OH})_2\text{D}_3$ and $1\alpha(\text{OH})\text{D}_3$ and upon the acute effects of different doses of the two compounds on the plasma levels of PTH, Ca^{2+} and phosphate.

Patients

In part 3a, 6 healthy volunteers and 12 terminal uremic patients were included, 7 on CAPD and 5 on hemodialysis. All patients completed the planned schedules.

In part 3b, 11 uremic patients on chronic hemodialysis were included. Eleven patients completed the first 3 schedules. Between the third and fourth part of the study, one patient died due to a severe pulmonary infection, two patients started on regular treatment with $1\alpha(\text{OH})\text{D}_3$ and one patient did refused to participate. Thus, 7 patients completed this part of the study.

Method

In part 3a, 6 patients received 4 μg of $1\alpha(\text{OH})\text{D}_3$ intravenously and 4 μg of $1\alpha(\text{OH})\text{D}_3$ orally, while 6 other patients received 4 μg of $1,25(\text{OH})_2\text{D}_3$ intravenously and 4 μg of $1,25(\text{OH})_2\text{D}_3$ orally. For comparison, 6 healthy volunteers passed through all 4 schedules.

Two separate sets of blood samples were obtained as basal values and subsequently at regular intervals for the following 72 hours.

In part 3b, 11 uremic patients on chronic hemodialysis were included. The study was divided into 4 parts. In part one, 10 ml of isotonic NaCl was injected as a bolus and blood samples obtained at

specific times for the following 72 hours. With an interval of at least 1 week, the same procedure was performed, but with intravenously administration of 10 µg of 1,25(OH)₂D₃ or 10 µg of 1α(OH)D₃ in a randomised fashion. With a further interval of at least 3 weeks, the schedule was repeated, but now with administration of 10 µg of the other vitamin D analog.

An additional fourth part was added to the study, including only 1,25(OH)₂D₃ in a smaller dose (6 µg), because no calcemic response was observed during the first 24 hours after injection of 10 µg of 1α(OH)D₃ while a significant increase in plasma Ca²⁺ was seen after injection of 10 µg of 1,25(OH)₂D₃. The treatment schedule was the same, except that the patients were only followed for 24 and not 72 hours.

Outcome measures

Outcome measures in part 3a were pharmacokinetic parameters as bioavailability, volume of distribution (Vd) and the metabolic clearance rate (MCR), plasma PTH and plasma Ca²⁺ in relation to time.

Outcome measures for part 3b were plasma Ca²⁺ and plasma levels of PTH measured by one "Whole" and two "Intact" PTH assays in relation to time.

11. METHODS

11.1 PLASMA CA²⁺

Ionized calcium (Ca²⁺) is the biological active form of calcium [197-199]. Since the mid-1970s, reliable automatic methods based on ion-selective electrodes have been available for analysis of plasma Ca²⁺ [200]. In the studies included in the present review, plasma Ca²⁺, pH and plasma Ca²⁺ adjusted to pH=7.4 were all analyzed by a calcium ion electrode analyzer (ICA1) [1], ICA 2 [2-5], and ABL555 [6, 7], produced by Radiometer, Copenhagen, Denmark.

Based on the knowledge that calcium is the major regulator of PTH synthesis and secretion [60, 66], the intention in these studies was to stabilise plasma Ca²⁺ at the upper end of the normal range [1-5] thus minimizing PTH secretion.

Blood samples for determination of plasma Ca²⁺ and pH were placed on ice and measured immediately.

11.2 PARATHYROID HORMONE

PTH 1-84 is rapidly cleared from the circulation with a disappearance half-time of about 2 minutes [59]. Removal of PTH from the blood occurs by extraction in the liver for 60-70% and in the kidneys for 20-30% [59, 201]. The Kupffer cells are responsible for both the rapid clearance and the extensive proteolysis of the PTH molecule that occur in the liver. Less than 10-20% of secreted PTH 1-84 is metabolized peripherally to circulating C-terminal fragments. However, 50-90% of the total circulating PTH immunoreactivity is C-terminal fragments because the clearance of C-terminal PTH fragments, occurring mainly via glomerular filtration, is significantly slower than that of PTH 1-84. Furthermore, C-terminal fragments are secreted together with PTH 1-84 by the parathyroid glands [59, 202]. Generated N-terminal PTH fragments are rapidly cleared by the liver and normally difficult to demonstrate in plasma [201].

Radioimmunoassays for analysis of PTH have been available since 1963, but results obtained by different assays varied considerably [203, 204]. The reason was that different assays used polyclonal antisera that were generated against different regions of the PTH molecule. Most antisera were directed toward the middle or C-terminal end of the PTH molecule. Thus, both the PTH 1-84 and the C-terminal fragments of PTH present in the circulation were measured by these assays [203]. Efforts were therefore directed towards developing radioimmunoassays measuring the N-terminal region of the PTH molecule – which is responsible for the known biological effects of PTH. Although such assays had clear theoretical benefits, their poor sensitivity limited clinical use [205]. Nussbaum et al. developed a two-site immunometric method that was offering the possibility of extracting what was thought to be the pure PTH 1-84

from the complex mixture of PTH 1-84 and fragments in the circulation [206]. Most available data that are comparing plasma levels of PTH with bone biopsies are obtained by such first generation immunoradiometric assays. These assays have proved to be adequate screening tools separating high turnover bone disorders with high PTH from low turnover bone disorders with low PTH [89]. The "Intact" PTH assay (Allegro, Nichols Institute Diagnostics, CA, USA) is a commonly used assay, which also was employed in the present studies [1-7]. This immunoradiometric assay makes use of a goat antibody immobilised onto plastic beads binding only to PTH 39-84, i.e. the C-terminal part, and another radiolabelled goat antibody that binds to the N-terminal PTH region – the specific binding-site is, however, not exactly known [207]. Such an assay is supposed to measure all PTH 1-84. Similar assays are available, and in one of our studies [7] the samples were measured simultaneously by another Intact assay, too (Total PTH, a part of the DUO PTH Kit from Scantibodies Laboratories, Santee, CA, USA). This assay uses goat polyclonal anti-PTH 39-84 coated beads as a universal solid phase and a second polyclonal antibody directed against the PTH 7-34 region. To evaluate a possible effect of 1α(OH)D₃ on the peripheral metabolism of PTH, plasma PTH was further analysed by a C-terminal iPTH 53-84 (MILAB, Malmö Immunlaboratorium AB, Sweden) assay, in three of the studies [1-3], and in one study [3] also by a N-terminal iPTH 1-34 radioimmunoassay (Allegro, Nichols Institute, USA).

The optimal concentration of plasma PTH to prevent extraskel-etal complications in chronic renal failure, when measured by the intact PTH assay (Allegro, Nichols Institute Diagnostics, CA, USA) is not precisely known [96, 208, 209]. The K/DOQI- guidelines recommend 150 – 300 pg/ml when analysed by this intact PTH assay [58]. It has previously been shown that a level of PTH 2-4 times the upper normal limit was necessary [96, 208] in order to maintain the coupled processes of bone resorption, formation and normal features of skeletal remodelling in uremic patients. This remarkable finding has been explained as a consequence of the uremic state in it self [210], by "skeletal resistance to PTH" due to down- regulation of the PTH1-receptor (PTH1R) [211], by activation of the RANK-RANKL system and elevated OPG-level [212], and, finally, by decreased levels of growth factors and/or increased levels of inhibitory substances blocking the action of growth hormone such as bone morphogenetic proteins (BMP) [213].

PTH 1-84 acts through a specific PTH1R receptor. The receptor requires the N-terminal amino acids of PTH to be activated. In plasma from uremic patients, non 1-84 PTH circulating fragments interfering significantly with the commercial "Intact PTH assays" have been demonstrated [214, 215]. This iPTH was co-migrating together with the PTH 7-84 fragment when fractionated by HPLC, and the concentration of the fragments in plasma increased progressively with decreasing GFR [216]. These observations together with reports that there might exist another PTH receptor with binding specificity for the C-terminal region of PTH [217, 218] initiated examinations of the possible relevance of such a potential C-PTH receptor as a modulator of bone cell activity. By infusion studies in rats, it has been demonstrated that the C-terminal fragments of PTH 7-84, 39-84 and 53-84 inhibit the increase in plasma Ca²⁺ induced by PTH 1-84 and PTH 1-34 in thyro-parathyroidectomized rats [219, 220]. In vitro studies have further demonstrated that bone resorption, as measured as ⁴⁵Ca released from labelled neonatal mouse calvariae, induced by 1,25(OH)₂D₃ and PTH 1-84 was diminished after incubation with PTH 7-84 [221]. Also impaired formation of osteoclast-like cells in response to 1,25(OH)₂D₃ in murine bone marrow cultures after incubation with PTH 7-84 and PTH 39-84 was seen [221]. Thus these large C-terminal fragments may play a role in the regulation of osteoclastogenesis and may be part of the mechanisms behind the observed skeletal resistance to PTH in uremia [219]. To overcome the problem of co-measured large C-terminal fragments, newer PTH assays have now been developed – the

second generation immunoradiometric assay ("Whole PTH", Scantibodies Laboratories, Santee, CA, USA). This assay uses a specific goat polyclonal PTH antibody directed against the N-terminal PTH 1-4 region and polyclonal anti-PTH 39-84 coated beads as the universal solid phase. The results obtained with this assay were in one of our studies [7] compared to results obtained by two first generation immunoradiometric assays – "Total" PTH, (Scantibodies Laboratories, Santee, CA, USA) and the "Whole" PTH assay (part of DUO PTH Kit). Sufficient knowledge has not been accumulated to establish the predictive power of these newer assays with regard to secondary hyperparathyroidism and renal bone disease.

Blood samples for determination of plasma PTH was immediately placed on ice, centrifuged at 4°C and stored at -20°C until analysed.

11.3 PLASMA-1,25(OH)₂D₃ AND 25(OH)D₃

Plasma 1,25(OH)₂D₃ levels in patients suffering from chronic uraemia were previously assumed to be independent of the plasma levels of 25(OH)D₃ [222] although the existence of an extra-renal 1 α -hydroxylation and synthesis of 1,25(OH)₂D₃ in chronic renal failure is well-known [223]. Studies on plasma 25(OH)D₃ in patients with varying degrees of renal failure described a correlation between the concentrations of p-1,25(OH)₂D₃ and p-25(OH)D₃ [23]. Some authors have suggested that low levels of p-25(OH)D₃ in chronic uremic patients may be a risk factor for the development of secondary hyperparathyroidism and reduced BMD in the hip [224-226]. But there is no evidence from patients on dialysis that supplementation with D₂ or D₃ to raise p-25(OH)D₃ increases p-1,25(OH)₂D₃ or lowers the elevated levels of PTH. Nevertheless, it is now generally recommended that p-25(OH)D₃ should be kept higher than 30 ng/ml in patients on chronic dialysis [58].

In the present studies, p-1,25(OH)₂D₃ was measured in different conditions after administration of 1 α (OH)D₃ or 1,25(OH)₂D₃. P-25(OH)D₃ was also determined in one study to establish the level in our population of uremic patients [1].

1,25(OH)₂D₃ and 25(OH)D₃ were extracted from plasma with diethylether and the extracts chromatographed [227]. 25(OH)D₃ was measured by a competitive protein-binding assay. The binding protein was obtained from the cytosolic fraction of rachitic rat kidneys [227]. 1,25(OH)₂D₃ was measured by a competitive protein binding assay using calf thymus cytosol as the source of binding protein [227]. From 1996 and onwards, the analyses for 1,25(OH)₂D₃ and 25(OH)D₃ were available in our own laboratory.

Blood samples for the determination of 1,25(OH)₂D₃ were immediately placed on ice, centrifuged at 4°C and stored at -20°C until analysis.

11.4 BIOCHEMICAL BONE MARKERS

– INDIRECT MARKERS OF BONE DISEASE

While bone biopsies are the gold standard in directly following ongoing bone disease of uremic patients, a few simple biochemical parameters, besides PTH may constitute the first-line in diagnostic algorithms in daily clinical practice.

Indirect markers reflecting bone formation are osteocalcin, procollagen type 1 c-terminal extension peptide (P1cP) and alkaline phosphatases. No useful markers reflecting bone resorption in renal osteodystrophy have so far been identified [88, 100, 228].

Plasma alkaline phosphatase were measured in 6 studies [1-6] by a standard laboratory method which was not discriminating between the bone fraction and other fractions.

Plasma osteocalcin was measured in 3 of the studies [2-4] by an ELIZA method.

Plasma P1cP was measured in one study [4] by a radioimmunoassay (Farnos Diagnostica, Finland) [229].

Aluminum adversely affects the differentiated function of osteoblasts and was previously a major factor in the development of low turnover bone disease [230]. Aluminum toxicity was suspected when p-aluminum was >100 μ g/l [231]. Serum-aluminum was

measured in 5 of the studies [1-5] by electrothermal atomic absorption photometry.

11.5 OTHER ANALYSES

Total plasma calcium and plasma inorganic phosphate (Pi) were measured by photometry. Hemoglobin, thrombocytes, leucocytes, plasma lactate dehydrogenase, plasma ALAT and plasma ASAT, p-L og D-lactate, plasma PP (factor 2,7 and 10), plasma protein, plasma creatinine, plasma urea, plasma magnesium, plasma glucose and serum albumin were all measured by standard laboratory tests.

Blood Pressure was measured by a manual mercury sphygmomanometer in a sitting position.

Weight was measured on the same electronic weight each time.

The standardized Peritoneal Equilibrium Test [232] was used to calculate mass transfer of calcium, phosphate, magnesium, lactate, creatinine, urea, glucose and albumin.

11.6 IMAGING TECHNIQUES – MARKERS OF RENAL BONE DISEASE AND EXTRASKELETAL CALCIFICATIONS

X-ray examinations and evaluation of bone mineral content (BMC) have been used over time to demonstrate bone disease secondary to prolonged secondary hyperparathyroidism [233]. These investigations may provide further information when combined with measurements of indirect biochemical bone markers, but no combination has so far been able to replace bone-biopsies [233, 234]. The radiological presentation of the bone in advanced secondary hyperparathyroidism is very characteristic. The increased osteoclastic bone resorption secondary to excess PTH results in radiographically evident subperiosteal erosions most often present in the hands along the radial margins of the middle phalanges of the second and third fingers [233]. Osteosclerosis is caused either by excessive accumulation of poorly mineralized osteoid, which radiographically will appear denser than normal bone, or by an exaggerated osteoblastic response following osteoclastic bone resorption. The increased bone density may be generalized or more often found in the axial skeleton where the midplane of the vertebral bodies shows a normal density while the endplate exhibits sclerosis (a so called rugger-jersey spine) [233]. Periosteal reactions may also be seen, most often in the metatarsal, femoral and pelvic bones [235]. These last mentioned radiological features are now rarely seen due to improved therapeutic management. The correlation between radiographic changes and clinical signs and symptoms of bone disease is poor [236].

The diagnosis of adynamic bone disease rests on histomorphometric and histodynamic findings of a low bone turnover combined with a lack of increased thickness of osteoid seams and osteoid. This condition cannot be diagnosed based on plain X-ray pictures [86].

Other complications are now pictured on X-rays such as metastatic and vascular calcifications [237] that in the extreme clinical form may present as calciphylaxis [238]. A score based on calcifications observed on plain x-rays of the abdominal aorta has been developed [239] and showed a good correlation to the more sophisticated Electronic Beam Computer Tomography (EBCT) in determining cardiovascular calcification. Such methodologies may prove useful to guide further therapeutic choices in dialysis patients in the future. In one of the present studies [4], radiographic evaluations including anterior-posterior and lateral views of the columna lumbaris, anterior-posterior view of both hands and both hips including the femoral shaft were performed. At the end of the study, all radiographs of each patient were collected for comparison and evaluated by the same radiologist. The pictures were examined systematically for bone changes only and not for possible vascular calcifications.

The increasing availability of Dual photon- and now Dual-X-ray absorptiometry (DEXA) facilitates measurement of bone mineral density (BMD g/cm²)/bone mineral content (BMC) in both healthy people and patients with chronic renal failure. BMD/BMC is useful in diagnosing osteoporosis and predicting the fracture risk in patients with normal renal function, but the method has not been

validated in patients with chronic renal failure [240]. In renal osteodystrophy, BMD/BMC can both be decreased and increased when compared to healthy man. This is due to regional differences in the skeleton, and the presence of severe secondary hyperparathyroidism and adynamic bone disease [88, 241, 242]. False elevated high BMD, not reflecting changes in bone, may be due to vascular calcifications [233]. The risk of fractures is increased in patients with chronic renal failure, but the correlation to BMD is poor [243-246]. BMD is, however, only one of several assessment tools in evaluating renal osteodystrophy and should be judged in context with biochemical variables and bone histology if available [246]. In one study [4], we evaluated BMC by dual photon absorptiometry in the lumbar spine, femoral neck and the femoral shaft. All calculations for one particular patient were performed with reference to the previous measurement in that person.

12. RESULTS AND DISCUSSION

12.1 BENEFITS OF TREATMENT BY INTRAVENOUS $1\alpha(\text{OH})\text{D}_3$ ON THE SECONDARY HYPERPARATHYROIDISM IN PATIENTS ON CHRONIC HEMODIALYSIS

Oral treatment with $1,25(\text{OH})_2\text{D}_3$ and $1\alpha(\text{OH})\text{D}_3$ has been used with great benefit as treatment of secondary hyperparathyroidism and renal osteodystrophy in uremic patients for many years [149, 175, 180, 181, 247]. Only sparse experience with intravenous administration of $1,25(\text{OH})_2\text{D}_3$ and $1\alpha(\text{OH})\text{D}_3$ was available on these conditions until the end of the 1980s. In 1989, results of our first study on intravenous administration of $1\alpha(\text{OH})\text{D}_3$ to patients on chronic hemodialysis were published [1]. In a short-term study (84 days) [1], we found a marked suppression of plasma PTH by $67\pm 6\%$, **Figure 1**.

Our results were in agreement with those obtained in a small Swedish study including 7 patients [248]. In the Swedish study, intravenous administration of $1\alpha(\text{OH})\text{D}_3$ at the end of each hemodialysis session induced a significant suppression of plasma PTH by $40\pm 20\%$. Similar results on intravenous administration of other vitamin D analogs have been reported [74, 157, 165, 167]. Although the studies were not quite similar in design and patient material, they were on the whole comparable. The patients included were all treated by chronic hemodialysis, all had elevated plasma PTH levels, and all were treated with a vitamin D analog intravenously with the doses being adjusted to the plasma Ca^{2+} level. Slatopolsky et al. administered $1,25(\text{OH})_2\text{D}_3$ intravenously for 8 weeks and reported a suppression of plasma PTH by $70.1\pm 3.2\%$ [74]. Akizawa et al. administered 22-oxa- $1,25(\text{OH})_2\text{D}_3$ intravenously for 12 weeks and reported a suppression of plasma PTH from 905.1 ± 66.7 pg/ml to 590.3 ± 73.8 pg/ml ($\approx 35\%$) [167]. Sprague et al. administered 19-

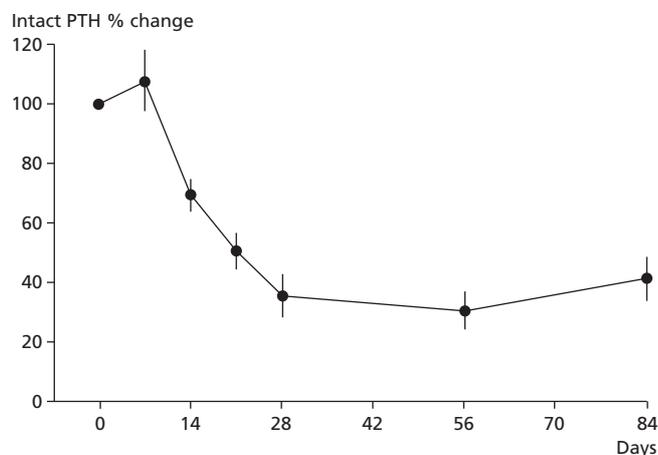


Figure 1. Intact PTH in relation to time of treatment. Twenty-one patients on chronic hemodialysis participated in the study, which lasted for 84 days. $1\alpha(\text{OH})\text{D}_3$ was given intravenously at the end of the hemodialysis 3 times a week. PTH values are presented as percent changes from the initial value due to great variation in individual PTH levels [1]. Mean \pm SEM.

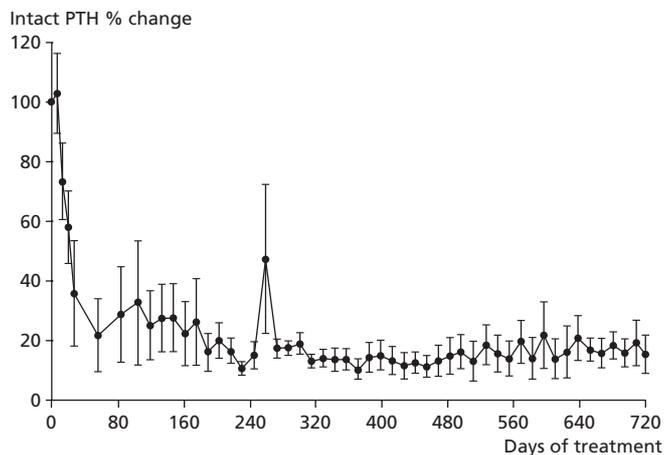


Figure 2. Plasma intact PTH during treatment with $1\alpha(\text{OH})\text{D}_3$ of 6 patients on chronic hemodialysis. $1\alpha(\text{OH})\text{D}_3$ was administered intravenously at the end of each hemodialysis 3 times a week for 720 days. PTH values are presented as percent changes due to great variation in the individual PTH levels [2]. Mean \pm SEM.

nor- $1,25(\text{OH})_2\text{D}_2$ intravenously for 12 weeks and reported a suppression of plasma PTH from about 700 to 300 pg/ml ($\approx 57\%$) [157]. Maung et al. administered $1\alpha(\text{OH})\text{D}_2$ intravenously for 8 weeks and reported a suppression of plasma PTH by $65\pm 3.9\%$ [165].

The long-term effect of intravenous $1\alpha(\text{OH})\text{D}_3$ was addressed in our following studies [2-4]. When plasma phosphate and plasma Ca^{2+} were carefully controlled, it was possible, in our group of patients with elevated PTH, to keep plasma PTH stable for more than 2 years [2-4], **Figure 2**.

In advanced chronic renal failure, where the proliferation of the parathyroid glands appears to be more polyclonal [76], the number of receptors for $1,25(\text{OH})_2\text{D}_3$ and calcium are reduced in the glands [68, 75, 76]. The enhanced growth of parathyroid cells in secondary hyperparathyroidism has been attributed to hyperphosphatemia per se [64] and a relative or absolute deficiency of $1,25(\text{OH})_2\text{D}_3$ with or without concomitant hypocalcemia [69]. In vitro studies in primary cultures of bovine parathyroid cells have shown that $1,25(\text{OH})_2\text{D}_3$ completely inhibit cell proliferation [249]. The need for parathyroidectomy in chronic uremic patients increases with the time on dialysis [250] maybe as a consequence of the nodular growth [251] and resistance to treatment with $1,25(\text{OH})_2\text{D}_3$. Regression of parathyroid hyperplasia, as shown by ultrasound, in patients on hemodialysis following oral $1,25(\text{OH})_2\text{D}_3$ treatment has been reported by some authors [252], but not by others [253]. The reason for this discrepancy could be differences in the nodular hyperplastic stage of the parathyroid glands at initiation of treatment in the two studies.

The long-term effect of intravenous treatment by $1\alpha(\text{OH})\text{D}_3$ observed in our study may indicate a delay in the proliferation of the parathyroid cells although no imaging methods to monitor parathyroid gland size were used. The direct effect of intravenous $1\alpha(\text{OH})\text{D}_3$ was, in our studies, a marked decrease in plasma PTH before any increase in plasma Ca^{2+} occurred [1-4]. The reduction in plasma PTH was dependent on changes in plasma Ca^{2+} and the doses of the vitamin D-analog used when evaluated by multivariate analysis [1, 2], **Figure 3**.

The long-term sustained effect achieved by intravenous administration of $1\alpha(\text{OH})\text{D}_3$ may be due to an upregulation of the numbers of VDR-receptors in the parathyroid glands in response to $1,25(\text{OH})_2\text{D}_3$ [254]. An influence of $1,25(\text{OH})_2\text{D}_3$ on the calcium sensing receptor gene transcription in the parathyroid cells cannot be excluded either [255]. A normalisation of the set point of the parathyroid hormone- Ca^{2+} relation curve [256] and a shift of the sigmoidal curve to the left when compared to that observed in untreated chronic renal failure [257] have also been reported.

Long-term studies of intravenous administration of a vitamin D analog in patients on chronic hemodialysis have only been sparsely

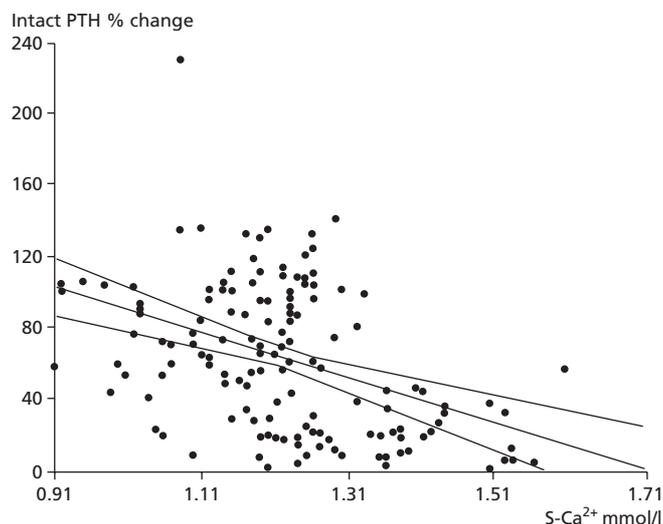


Figure 3. Linear regression and correlation between changes in intact PTH levels and plasma Ca^{2+} during treatment with $1\alpha(\text{OH})\text{D}_3$ administered intravenously to 21 patients on chronic hemodialysis ($R^2 = 0.169$, $p < 0.001$) [1].

reported. Rodriguez et al. reported that intravenous administration of $1,25(\text{OH})_2\text{D}_3$ for 42 weeks at the end of each dialysis resulted in a suppression of plasma PTH from 890 ± 107 to 346 ± 119 pg/ml [258]. Akizawa et al. reported that intravenous administration of 22-oxa- $1,25(\text{OH})_2\text{D}_3$, at the end of each dialysis for 12 months kept plasma PTH at the level achieved after 12 weeks [167]. Finally, Lindberg et al. reported that intravenous administration of 19-nor- $1,25(\text{OH})_2\text{D}_2$ at the end of each dialysis for 13 months resulted in a suppression of plasma PTH from 628.3 ± 27.65 to 409 ± 35 pg/ml [159].

12.2 LIMITATIONS IN THE CLINICAL USE OF INTRAVENOUS $1\alpha(\text{OH})\text{D}_3$

As for $1,25(\text{OH})_2\text{D}_3$ the use of $1\alpha(\text{OH})\text{D}_3$ in doses that effectively suppress plasma PTH may be limited by the development of hypercalcemia, hyperphosphatemia and an increased $\text{Ca} \times \text{P}$ product due to increased intestinal absorption and mobilization of calcium and phosphate from the bone [27]. The toxicity may be further aggravated by the concomitant use of large doses of oral phosphate binders containing calcium. This problem has large implications and is very serious as evidence is increasing that cardiovascular mortality is correlated to abnormalities in the $\text{Ca} \times \text{P}$ product in uremia [8, 56]. In our studies [1-4], all patients had low to normal p-Ca^{2+} at the entrance. During the treatment period it was necessary to reduce the doses of $1\alpha(\text{OH})\text{D}_3$ due to an increase in plasma Ca^{2+} . In our study, the accepted levels of plasma Ca^{2+} were in the upper normal range or slightly elevated. With reference to the recent K/DOQI guidelines [58], plasma Ca^{2+} should be in the mid-normal to low-normal end of the range.

As expected, all patients in our studies had plasma phosphate levels above the normal range despite treatment with oral phosphate binders [1-4]. During treatment, many patients experienced increases in plasma phosphate to >2 mmol/l [1-4]. This problem was handled by an increase in the doses of phosphate binder. Severe hyperphosphatemia per se has been reported to cause failure of active vitamin D treatment to suppress PTH [258, 259]. Data showing an increased mortality due to hyperphosphatemia and an increased $\text{Ca} \times \text{P}$ product in uremic patients were not available until 1998 [55]. Although the $\text{Ca} \times \text{P}$ product was not calculated, the obtained mean values of plasma total calcium and plasma phosphate in our studies [1-4] indicate that at least part of the patients must have had elevated $\text{Ca} \times \text{P}$ product values above the now recommended 4.4 mmol²/l² [58].

No direct comparison has been made between intravenous use of $1\alpha(\text{OH})\text{D}_3$ and other vitamin D analogs. However, intravenous administered 19-nor- $1,25(\text{OH})_2\text{D}_2$ has been directly compared to in-

travenous administered $1,25(\text{OH})_2\text{D}_3$ in patients on chronic hemodialysis [157]. Although animal experiments have demonstrated a lower potency of 19-nor- $1,25(\text{OH})_2\text{D}_2$ than $1,25(\text{OH})_2\text{D}_3$ in stimulating intestinal calcium and phosphate absorption [260], a direct comparison between 19-nor- $1,25(\text{OH})_2\text{D}_2$ and $1,25(\text{OH})_2\text{D}_2$ in hemodialysis patients was not able to demonstrate any differences in the frequency or severity of hypercalcemia and hyperphosphatemia. Fewer episodes of sustained hypercalcemia and increased $\text{Ca} \times \text{P}$ product were, however, observed in the 19-nor- $1,25(\text{OH})_2\text{D}_2$ group [157]. In a dose equivalence study between 19-nor- $1,25(\text{OH})_2\text{D}_2$ and $1\alpha(\text{OH})\text{D}_2$ the incidences of hypercalcemia, hyperphosphatemia and elevated $\text{Ca} \times \text{P}$ product were similar for doses with a comparable PTH suppression [261]. In a crossover study between 19-nor- $1,25(\text{OH})_2\text{D}_2$ and $1,25(\text{OH})_2\text{D}_3$, lower serum calcium, lower $\text{Ca} \times \text{P}$ product and lower PTH levels were obtained with 19-nor- $1,25(\text{OH})_2\text{D}_2$ [160] than with $1,25(\text{OH})_2\text{D}_3$. 22-oxa- $1,25(\text{OH})_2\text{D}_3$ has been reported to have less hypercalcemic effect than $1,25(\text{OH})_2\text{D}_3$ in uremic rats by some [262], but not by others [263]. In a clinical study, intravenous administration of 22-oxa- $1,25(\text{OH})_2\text{D}_3$ induced an increase in plasma Ca^{2+} , and a reduction in dose was necessary [166]. $1\alpha(\text{OH})\text{D}_2$ was found less calcemic than $1\alpha(\text{OH})\text{D}_3$ in rat studies [264], but still a dose reduction was needed in clinical studies [164, 165]

12.3 THE OPTIMAL MODE OF ADMINISTRATION

The first report on the use of high doses of intravenous $1,25(\text{OH})_2\text{D}_3$ in 20 long-term chronic hemodialysis patients (8 weeks) was published by Slatopolsky et al. [74]. Subsequently, it was shown that intravenous administration of $1,25(\text{OH})_2\text{D}_3$ in uremic patients effectively promoted healing of osteitis fibrosa resistant to oral $1,25(\text{OH})_2\text{D}_3$ treatment [265]. A higher peak concentration of $1,25(\text{OH})_2\text{D}_3$ and a greater "area under the curve" were found using intravenous instead of oral administration [74]. Following these findings, intravenous treatment with active vitamin D was preferred in patients treated by chronic hemodialysis instead of the oral route. We have shown [6], that the peak concentration of plasma $1,25(\text{OH})_2\text{D}_3$ and the "area under the curve" is higher when $1\alpha(\text{OH})\text{D}_3$ is administered intravenously instead of orally. In general, however, significantly lower concentrations were found after administration of $1\alpha(\text{OH})\text{D}_3$ than after administration of $1,25(\text{OH})_2\text{D}_3$ both when given intravenously and orally [6]. The administration of active vitamin D was changed from daily to intermittent – mainly 3 times a week given at the end of each dialysis [193]. Several short-term (<24 weeks) comparative studies have been performed both as cross-over and parallel studies including $1,25(\text{OH})_2\text{D}_3$, $1\alpha(\text{OH})\text{D}_3$, and $1\alpha(\text{OH})\text{D}_2$ [194, 253, 266, 267]. No convincing evidence for the intravenous route has been found in these short-term studies. Initially a faster suppression of PTH was seen in two studies, when the active vitamin D was given intravenously, but after 4 months of treatment no difference existed between the two groups [194, 253]. Further long-term studies are needed to clarify whether the intravenous and oral routes are equal regarding efficacy and side effects. The available studies indicate that the intermittent administration rather than the intravenous route is of importance. When comparing daily oral and intermittent intravenous administration in patients on chronic hemodialysis, no significant differences were found regarding hypercalcemia and hyperphosphatemia [195]. A study in rats suggested, however, that the dose of calcitriol delivered directly to the target organs such as the parathyroid glands might be of importance for the suppression of PTH [69]. An interesting question was whether it is possible to maintain the initial suppression of PTH found after intravenous administration of $1\alpha(\text{OH})\text{D}_3$ by subsequent intermittent oral administration. We addressed this question in one study [3] and although the number of patients completing the study was small, we showed that the marked suppression of plasma PTH obtained by treatment with intravenous $1\alpha(\text{OH})\text{D}_3$ could indeed be maintained after shift-

ing to intermittent oral treatment. After 16 weeks of oral treatment the route of administration was shifted back to intravenous and no further suppression of PTH was induced, **Figure 4**.

In patients treated by CAPD, repeated intravenous administration of $1,25(\text{OH})_2\text{D}_3$ is not practical. Therefore high pulse doses of oral and intraperitoneal administration of $1,25(\text{OH})_2\text{D}_3$ one to three times a week became the standard treatment in these patients [268-273]. Intraperitoneal $1,25(\text{OH})_2\text{D}_3$ is practicable, effective and safe in the treatment of secondary hyperparathyroidism in CAPD patients, but has no advantages when compared to oral treatment [274]. We studied the effect on secondary hyperparathyroidism of intermittent oral $1\alpha(\text{OH})\text{D}_3$ in combination with "low calcium dialysis fluid" in CAPD patients [5]. The doses of $1\alpha(\text{OH})\text{D}_3$ were lower in our study than in other CAPD studies which have observed an improvement of the secondary hyperparathyroidism by the use

of "low calcium dialysis fluid" and oral intermittent $1,25(\text{OH})_2\text{D}_3$ [275, 276]. This was also the case when compared to results obtained in our previous study on hemodialysis patients, who had intravenous administration of $1\alpha(\text{OH})\text{D}_3$ [4]. It is notable that the prescribed weekly doses of $1\alpha(\text{OH})\text{D}_3$ in our present CAPD study were lower after 52 weeks of attempted optimal treatment (1.4 ± 0.31 $\mu\text{g}/\text{week}$), than in our study performed in patients treated by hemodialysis (2.88 ± 0.52 $\mu\text{g}/\text{week}$) including patients with exactly the same degree of secondary hyperparathyroidism (PTH in CAPD patients 151 ± 41 pg/ml [5], and PTH in hemodialysis patients 151 ± 44 pg/ml [4]). A transient increase in plasma PTH took place when calcium was changed to low calcium dialysis fluid in the CAPD patients, but no further aggravation of the secondary hyperparathyroidism was observed thereafter [5].

Moe et al. demonstrated in 1998 in a randomized trial that pulse

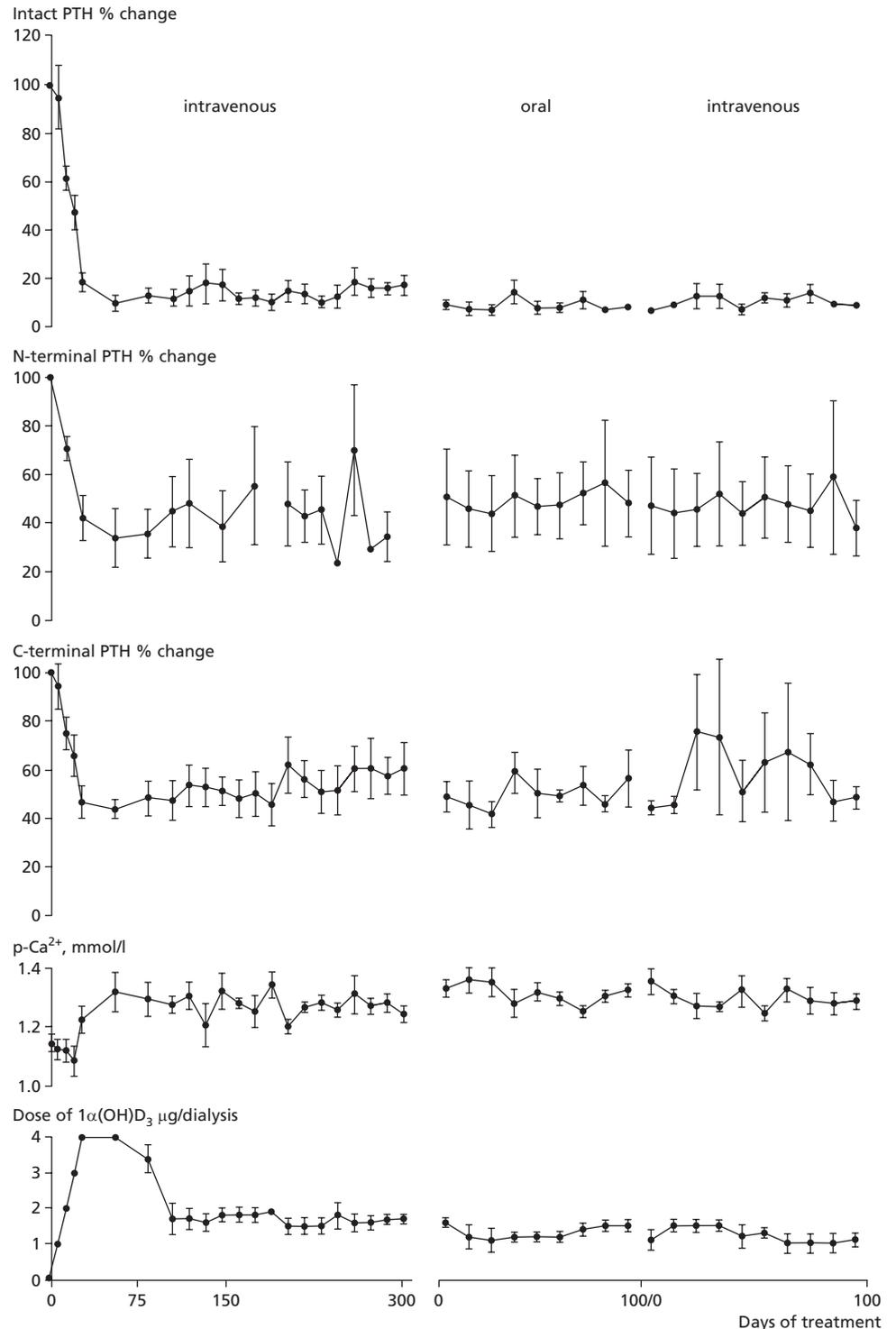


Figure 4. Percentage changes in plasma intact PTH, N-terminal PTH, C-terminal PTH and plasma Ca^{2+} in relation to intermittent intravenous and oral doses of $1\alpha(\text{OH})\text{D}_3$ and time of treatment. Five patients on chronic hemodialysis completed the study. The treatment protocol was divided into 3 consecutive parts: 1) $1\alpha(\text{OH})\text{D}_3$ administered intravenously for > 300 days, 2) $1\alpha(\text{OH})\text{D}_3$ administered orally for 100 days followed by 3) $1\alpha(\text{OH})\text{D}_3$ administered intravenously again for 100 days. $1\alpha(\text{OH})\text{D}_3$ was given intravenously or orally at the end of each hemodialysis 3 times per week. PTH values are presented as percentage changes due to great variation in individual PTH levels [3]. Mean \pm SEM.

therapy and daily administration of calcitriol are similarly effective and safe for the treatment of mild to moderate secondary hyperparathyroidism in CAPD patients, despite higher peak levels of $1,25(\text{OH})_2\text{D}_3$ found with pulse therapy [277].

12.4 INTRODUCTION OF A NON-CALCIUM CONTAINING PHOSPHATE BINDERS AND OF LOW CALCIUM DIALYSIS FLUID (1.25 MMOL/L) – CONSEQUENCES

Changes in plasma Ca^{2+} during dialysis and possible effects on renal bone disease

In order not to increase the total calcium load to the patients when the phosphate binder therapy was changed from aluminum to calcium containing binders calcium containing, an introduction of "low calcium dialysis fluid" was necessary [152, 278-281].

Sperschneider et al. showed that pre-existing microcalcifications progressed and new appeared in vessels and soft-tissue areas of the hand during a 3-year study on uremic dialysis patients when the calcium concentration in the dialysis fluid was 1.75 mmol/l ("high calcium dialysis fluid"), no active vitamin D was administered, and CaCO_3 was given as an oral phosphatebinder [281]. When "low calcium dialysis fluid" was introduced it was feared that a decrease in plasma Ca^{2+} , would aggravate secondary hyperparathyroidism and thereby accelerate bone turnover [282]. We addressed this problem in our unit in patients treated by both hemodialysis [4] and CAPD [5]. In patients treated by hemodialysis, we found a decrease in plasma Ca^{2+} during the dialysis [4], Figure 5, while others have reported no change [283].

Contrary to patients treated by hemodialysis, patients treated by CAPD are exposed to "low calcium dialysis fluid" 24 hours a day and not only for 4-5 hours 3 times a week. We found in this group of patients [5] both a decrease in plasma Ca^{2+} and a change from positive to negative calcium mass transfer (MT) when the calcium concentration in the dialysis fluid was reduced from 1.75 mmol/l to 1.25 mmol/l (as shown in Figure 5 and Figure 6). These findings are in accordance with others [284-286]. These changes increase the risk of aggravation of an existing secondary hyperparathyroidism and often require an adjustment of the doses of both phosphate binding CaCO_3 and $1\alpha(\text{OH})\text{D}_3$ [5, 287, 288]. The increased plasma Ca^{2+} ,

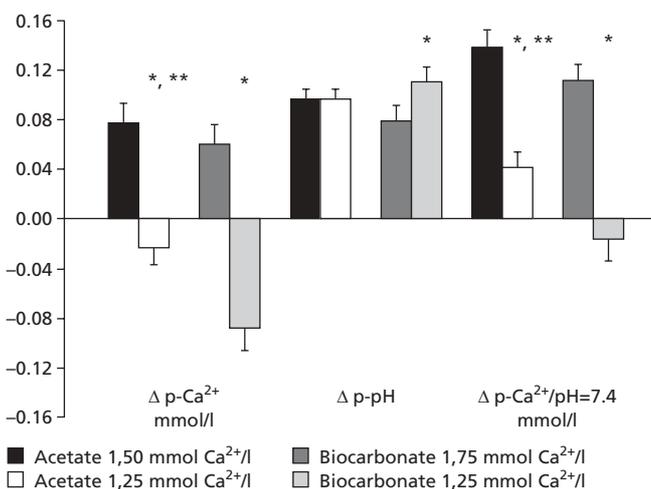


Figure 5. Changes in plasma Ca^{2+} , plasma pH, and plasma Ca^{2+} measured at $\text{pH}=7.4$ (mean \pm SEM) during dialysis by "standard" and "low-calcium" dialysis solutions. The samples were drawn from the bloodline of the dialyser immediately before and after dialysis. The investigations were performed with 1 week's intervals at the beginning of the study (week 0). Twenty-seven patients were dialysed by acetate dialysis (calcium concentration 1.50 mmol/l and 1.25 mmol/l) and 27 patients were dialysed by bicarbonate dialysis (calcium concentration 1.75 mmol/l and 1.25 mmol/l). Only for historical reasons, there were differences in the calcium concentrations in the two "standard" dialysis solutions.
*) differences between "standard" and "low-calcium" solution ($p < 0.0001$).
**) differences between "low-calcium" acetate and "low-calcium" bicarbonate solution ($p < 0.012$) [4].

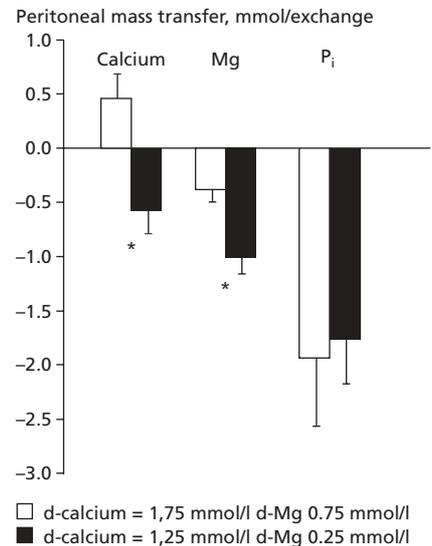


Figure 6. Peritoneal mass transfer (mmol per exchange) of calcium, magnesium and phosphate (P_i) during a 4 hour peritoneal equilibrium test (PET) (2 l glucose 22,7 mg/ml) in 7 patients on CAPD when using 2 different dialysis solutions – Dianeal® (d-calcium 1.75 mmol/l, magnesium 0.75 mmol/l) and PD4 (d-calcium 1.25 mmol/l, magnesium 0.25 mmol/l).
*) $p < 0.0003$ [5].

observed secondary to the extra oral calcium intake when CaCO_3 was used as the principal oral phosphate binder, was found in some studies to reduce the secondary hyperparathyroidism [278, 279, 289]. In other studies, however, an increase in plasma PTH was observed and treatment with active vitamin D necessary, especially in long-term studies [280, 281]. Saisu et al. combined increased doses of calcium containing phosphate binder with unchanged doses of active vitamin D in a group of uremic patients treated by hemodialysis using "low-calcium dialysis fluid" [290]. After 1 year of treatment they observed a trend towards increased plasma alkaline phosphatases and plasma osteocalcin [290]. While high turnover bone disease may be less frequent than previously found, low turnover bone disease (adynamic bone disease) unrelated to aluminum has been described increasingly often in dialysis patients [291, 292]. Adynamic bone disease is presumably more often observed in patients treated by CAPD than in patients treated by hemodialysis [293]. Patients with adynamic bone disease are often asymptomatic and whether the condition is deleterious on a long-term basis is still a matter of debate [86, 245, 294]. The exact pathogenesis of the disorder is unknown, but it is found in patients with prevailing low PTH which may therefore be a pathogenetic factor [86, 294].

Treatment with active vitamin D, which may depress PTH in dialysis patients where PTH is normal or only slightly elevated, is therefore generally avoided. Oversuppression of PTH is reversible, and elevated plasma PTH induced by low calcium dialysis may be an advantage if oversuppression of PTH is suspected [295]. No bone biopsies were performed in our studies and whether the patients with normal or decreased plasma PTH included [4, 5], suffered from low turnover bone disease is an open question. The prevalence of adynamic bone disease in a Danish dialysis population is unknown. Looking at usual risk factors for the development of adynamic bone disease – age, sex, dialysis duration, prevalence of diabetes mellitus, parathyroidectomy or failed transplanted [291], the existence of the condition in our patients cannot be excluded.

Changes in plasma Ca^{2+} , plasma PTH and doses of $1\alpha(\text{OH})\text{D}_3$

After introduction of "low calcium dialysis fluid" in CAPD, a significant initial decrease in plasma Ca^{2+} was observed together with a significant increase in PTH [5]. The doses of $1\alpha(\text{OH})\text{D}_3$ were initially increased and subsequently adjusted under careful control of plasma Ca^{2+} . After 12 weeks of treatment, plasma PTH decreased to the initial levels [5]. In patients treated by hemodialysis no changes in p-Ca^{2+} were observed initially when measured just before the beginning of the next dialysis. Patients with an initial normal PTH demonstrated a temporary increase in PTH after 64 weeks while no changes were observed in patients with initial elevated PTH. In both

groups, it was possible to increase the dose of $1\alpha(\text{OH})\text{D}_3$ initially. The dose was subsequently adjusted, mostly reduced, with consideration to plasma Ca^{2+} . After 88 weeks, PTH did not differ from initial levels in the patients with an initial normal PTH, and was close to normal in the patients with initially elevated PTH [4].

Changes in type of phosphate binder

It was possible to increase the dosages of the oral phosphate binder CaCO_3 after introduction of “low-calcium dialysis fluid” for patients treated by both CAPD and hemodialysis [4, 5]. The CaCO_3 doses used were however higher, and plasma Ca^{2+} and plasma phosphate lower in patients on hemodialysis than in patients treated by CAPD. As reported on 50 patients treated by CAPD by Hutchison et al. [296], it was possible to reduce, but not totally exclude aluminum containing phosphate binders. A negative mass transfer for phosphate of -1.9 mmol/exchange and contemporary changes in the dialysate/plasma ratio of phosphate were demonstrated in our study. These changes were not affected by the calcium concentration in the dialysis fluid, in agreement with other studies [284, 297]. Despite negative MT for phosphate, inadequate removal of phosphate by CAPD is well-known. A positive phosphate balance will be the inevitable outcome in a well-nourished CAPD patient unless diet prescription and oral phosphate binders are used [297].

Changes in biochemical bone markers

The influence on biochemical parameters, that indirectly may reflect bone turnover, in hemodialysis patients treated with “low calcium dialysis fluid” combined with intravenous $1\alpha(\text{OH})\text{D}_3$ and CaCO_3 as the principal oral phosphate binder was addressed in one study [4]. Plasma P1cP, a bone marker which has been introduced as a noninvasive index of bone formation in different metabolic bone diseases [298-302], was evaluated. We observed an initial increase in plasma P1cP followed by a decline to basal levels after 6 weeks, which is in accordance with others [300, 301]. Coen et al. compared bone biopsies and different biochemical bone markers in predialysis patients with secondary hyperparathyroidism on treatment with active vitamin D and demonstrated a positive correlation between plasma P1cP and bone formation. However, no significant correlations were found between plasma P1cP and plasma osteocalcin, plasma PTH or alkaline phosphatases [300]. In our study, only a weak but significant correlation was seen between P1cP and osteocalcin. This finding is in accordance with results from Hamdy et al. [301]. Plasma osteocalcin levels have been shown to reflect bone formation in patients on chronic dialysis [303]. Different assays for osteocalcin measure different absolute values due to different antibodies used [304]. Despite the marked suppression of intact PTH levels, no suppression of osteocalcin was observed in our investigation. The future use of biochemical bone markers, individually or in combination with other methods, will undoubtedly improve the diagnosis and the treatment of the complex disorder, renal osteodystrophy [100].

Changes in Bone Mineral Content

Bone lesions and reduced BMC are frequently seen in patients with chronic renal failure. The reduced BMC correlates to age, sex, PTH, duration of the uremia, and number of transplantations [89, 305]. Oral administration of $1\alpha(\text{OH})\text{D}_3$ to uremic patients has previously been shown to bring the decrease to a stop and in some patients even to increase BMC of the forearm [306], femoral head and neck [307], and lumbar spine and femoral shaft [196]. In our study, prevention of a further decrease of BMC was observed in the lumbar spine, and femoral neck and shaft in hyperparathyroid patients while a small, but significant decrease was still found in the femoral shaft of patients with normal PTH levels [4].

None of the studies in this thesis have focused directly on the effect of “low calcium dialysis fluid” and intravenous $1\alpha(\text{OH})\text{D}_3$ on cardiovascular events. Although only seen in a few patients, soft-tis-

sue calcification disappeared or was reduced as was a single bone cyst on the “low calcium dialysis regime” [4].

Taken together, dialysis using “low calcium dialysis fluid” combined with a change of oral phosphate binder to mainly calcium containing phosphate binders and the introduction of intermittent intravenous/oral $1\alpha(\text{OH})\text{D}_3$ can be used without aggravating secondary hyperparathyroidism and without decreasing BMC in uremic patients when plasma Ca^{2+} is carefully controlled. “Low calcium dialysis fluid (1,25 mmol/l)” has been recommended as “normal (standard) calcium dialysis fluid” in the USA since 2003 [58] and in Denmark since 2005 [308].

12.5 PLASMA PTH MEASUREMENTS DEPEND ON THE ASSAYS USED – ARE BETTER TOOLS FOR MANAGEMENT OF SECONDARY HYPERPARATHYROIDISM OBTAINABLE?

The actually measured changes in plasma concentrations of PTH induced by intravenous and oral administration of $1\alpha(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ depend upon which PTH assay is used. Plasma PTH was measured in the three studies concerning short-term and long-term intravenous administration of $1\alpha(\text{OH})\text{D}_3$ both by the intact PTH assay and by a C-terminal PTH assay measuring PTH 53-84 [1-3]. The initial levels as measured by the C-terminal PTH assay were about 10 times higher than those obtained with the intact PTH assay. Also the suppression of plasma PTH measured by the intact PTH assay was more pronounced (about 60%) than the PTH suppression measured by the C-terminal PTH assay (about 40%). Although we found great differences in the exact levels, there was a highly significant correlation ($R^2=0.72$) between the concentrations measured in the two assays. In one of the studies, we further evaluated whether the route of administration (oral or intravenous) of $1\alpha(\text{OH})\text{D}_3$ affected the circulating levels of N- and C-terminal fragments in plasma [3]. The data are shown in Figure 4. As expected, we found a reduction in plasma PTH when measured by both N-terminal-, C-terminal- and Intact PTH assays. The suppression of plasma PTH, when analysed by the Intact PTH, was more pronounced than when analysed by the N-terminal assay, which was again more pronounced than when analysed by the C-terminal PTH assay. A very high correlation between changes in plasma Intact PTH and plasma N-terminal PTH was found which may reflect that the N-terminal PTH assay mainly measures intact PTH, as has been shown in vitro [309]. A less marked correlation was found between changes measured by the intact PTH and C-terminal PTH assay (Figure 7), and between the N-terminal and C-terminal assays, presumably reflecting the accumulation of C-terminal fragments secondary to the reduced renal clearance [202, 207].

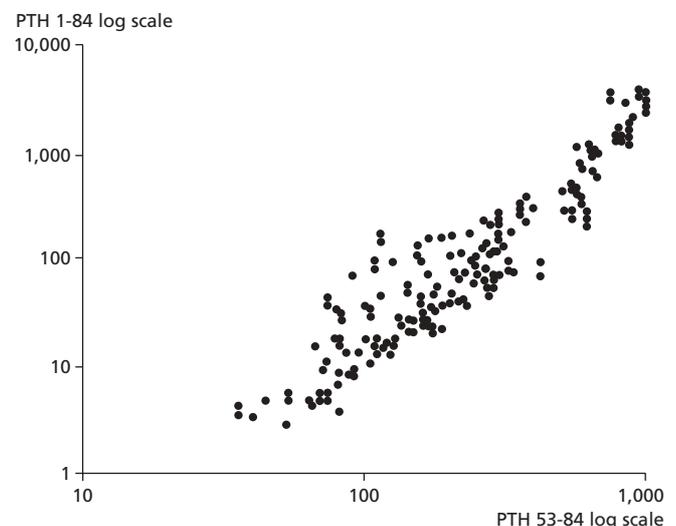


Figure 7. Correlation between intact PTH and C-terminal PTH in 21 patients on chronic hemodialysis during intravenous treatment for 84 days with $1\alpha(\text{OH})\text{D}_3$ ($R^2 = 0.72$, $p < 0.001$) [1].

The route of administration of $1\alpha(\text{OH})\text{D}_3$ did not change the correlations. A new PTH assay (the whole PTH assay) detecting only the bioactive whole PTH 1-84 [219, 310] was hoped to offer a better tool for the management of secondary hyperparathyroidism. This whole PTH 1-84 assay was used in one study where the suppression of plasma PTH was followed after administration of one large intravenous dose of either $1,25(\text{OH})_2\text{D}_3$ and $1\alpha(\text{OH})\text{D}_3$ and the measurements compared to that of two intact PTH assays [7]. In accordance with previously published studies on chronic uremic patients, the specific PTH values were significantly lower when analysed by the whole PTH assay than when analysed by the intact PTH assays [311]. The results obtained by the whole PTH and intact PTH assays were highly correlated within the whole range investigated, **Figure 8**.

The higher levels of plasma PTH found when analysed by an intact PTH assay were ascribed to accumulation of large C-terminal PTH fragments (non PTH 1-84) [7, 219, 310]. A possible modulating effect of large C-terminal PTH fragments on the stimulatory effects of PTH 1-84 on bone turnover was postulated and gave rise to the hypothesis that a ratio between PTH 1-84 and C-terminal PTH had a higher predictive power in distinguishing low- and high-bone turnover than the concentrations measured [209]. Other groups were unable to confirm this hypothesis [311-315]. The whole PTH assay gives more exact measurements of PTH, but whether it will change the understanding of secondary hyperparathyroidism in chronic uremia remains an open question.

12.6 $1\alpha(\text{OH})\text{D}_3$ – A PRECURSOR TO $1,25(\text{OH})_2\text{D}_3$ OR A FREE ACTIVE VITAMIN D ANALOG PER SE?

In expectation of a more effective inhibitory effect on the parathyroid glands [148], intravenous administration of $1,25(\text{OH})_2\text{D}_3$ instead of oral was introduced in 1984 [74]. This expectation was based upon the finding of a higher peak-concentration of $1,25(\text{OH})_2\text{D}_3$ after intravenous injection than after oral administration of the same doses [74, 316]. $1\alpha(\text{OH})\text{D}_3$ is known to be hydroxylated to $1,25(\text{OH})_2\text{D}_3$ by the liver [171, 172]. In many studies regarding intravenous administration of $1\alpha(\text{OH})\text{D}_3$, blood samples for measurements of plasma $1,25(\text{OH})_2\text{D}_3$ were drawn immediately before the next dialysis i.e. 2 or 3 days after administration of the last dose. By using this setup we observed [1, 2] a small increase in plasma $1,25(\text{OH})_2\text{D}_3$ in accordance with others [189] which correlated with the doses of $1\alpha(\text{OH})\text{D}_3$ administered. The peak plasma concentration

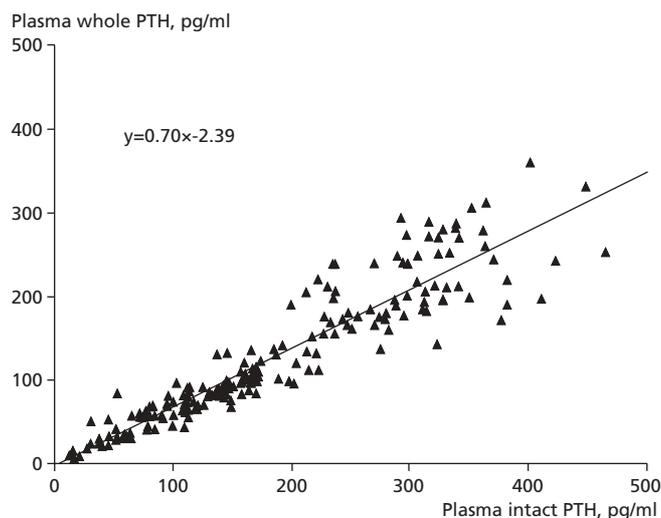


Figure 8. Correlation between plasma PTH as measured by whole PTH assay (Scantibodies) and intact PTH assay (Nichols) in 11 patients on chronic hemodialysis following an intravenous bolus administration of isotonic NaCl, $10\ \mu\text{g}$ of $1\alpha(\text{OH})\text{D}_3$ and $10\ \mu\text{g}$ of $1,25(\text{OH})_2\text{D}_3$ [7]. Administration of each of the two vitamin D analogs (and placebo) resulted in similar correlations between plasma intact PTH and plasma whole PTH. Therefore, the data of all 3 investigations were combined in this Figure. $R^2 = 0.86$, $p < 0.0001$.

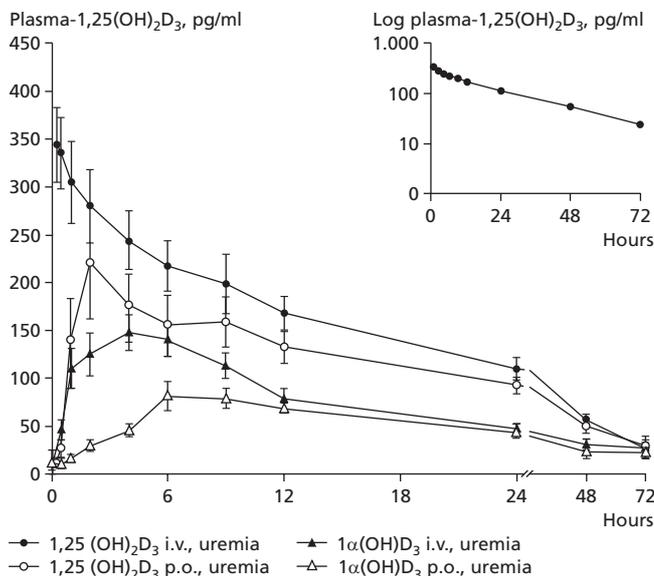


Figure 9. Plasma- $1,25(\text{OH})_2\text{D}_3$ concentrations in relation to time in 6 chronic uremic patients following i.v. and oral administration of $4\ \mu\text{g}$ of $1,25(\text{OH})_2\text{D}_3$ and in another 6 uremic patients following i.v. and oral administration of $4\ \mu\text{g}$ of $1\alpha(\text{OH})\text{D}_3$. A semilogarithmic transformation of the plasma- $1,25(\text{OH})_2\text{D}_3$ course after intravenous administration of $1,25(\text{OH})_2\text{D}_3$ is inserted in upper the right corner [6]. Mean \pm SEM.

of $1,25(\text{OH})_2\text{D}_3$ achieved after oral administration of $1,25(\text{OH})_2\text{D}_3$ is lower than after intravenous administration [6, 74], **Figure 9**.

The peak levels of plasma $1,25(\text{OH})_2\text{D}_3$ achieved in our study after oral and intravenous administration of $1\alpha(\text{OH})\text{D}_3$ were similar [6] to the findings in other studies [74, 185-187, 189] and were only about 50% of the plasma levels achieved after similar doses of $1,25(\text{OH})_2\text{D}_3$. The suppression of PTH was similar after administration of $4\ \mu\text{g}$ of $1\alpha(\text{OH})\text{D}_3$ and $4\ \mu\text{g}$ of $1,25(\text{OH})_2\text{D}_3$. This may be due to a "threshold level" of $1,25(\text{OH})_2\text{D}_3$ in combination with a normal plasma Ca^{2+} as has been suggested by Shigematsu et al. [317]. It might however also be due to a conversion of $1\alpha(\text{OH})\text{D}_3$ to $1,25(\text{OH})_2\text{D}_3$ inside the parathyroid cells [318]. This would explain the benefits found by intermittent oral regimes of both $1,25(\text{OH})_2\text{D}_3$ [194, 195, 253] and $1\alpha(\text{OH})\text{D}_3$ [3, 5]. Since the peak concentration of plasma $1,25(\text{OH})_2\text{D}_3$ after administration of $1\alpha(\text{OH})\text{D}_3$ was markedly lower than that obtained after similar doses of $1,25(\text{OH})_2\text{D}_3$ [6], another explanation could be that the peak concentration of $1,25(\text{OH})_2\text{D}_3$ is of less importance for the direct suppression of PTH secretion than previously assumed. Finally, it's possible that the effect of $1\alpha(\text{OH})\text{D}_3$ on the PTH secretion in uremic patients can not solely be explained by the conversion of $1\alpha(\text{OH})\text{D}_3$ to $1,25(\text{OH})_2\text{D}_3$. Therefore, the theoretical possibility exists that the 25-hydroxyl group on $1,25(\text{OH})_2\text{D}_3$ is not mandatory for the suppression of the PTH gene in the parathyroid glands, but that the 1α -hydroxyl group is the structural feature required. This assumption is supported by results from an in vitro study from our laboratory where the suppression of PTH secretion from bovine parathyroid cells by $1\alpha(\text{OH})\text{D}_3$ was equal to that of $1,25(\text{OH})_2\text{D}_3$ [178], and further supported by equal suppression of plasma PTH in uremic patients after administration of single intravenous doses of $4\ \mu\text{g}$ of $1\alpha(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ [6], **Figure 10**.

No general agreement exists on the acute effects of $1,25(\text{OH})_2\text{D}_3$ and $1\alpha(\text{OH})\text{D}_3$ on plasma PTH neither in healthy nor in uremic man. In normal subjects, both no change after intravenous administration of $1,25(\text{OH})_2\text{D}_3$ [319] and $1\alpha(\text{OH})\text{D}_3$ [320] and a decrease of plasma PTH after intravenous administration of $1\alpha(\text{OH})\text{D}_3$ [6] have been reported. In uremic patients, both no change after intravenous administration of $1,25(\text{OH})_2\text{D}_3$ [321] and $1\alpha(\text{OH})\text{D}_3$ [189, 321] and a decrease of plasma PTH after intravenous administration of $1,25(\text{OH})_2\text{D}_3$ [6, 190, 322] and $1\alpha(\text{OH})\text{D}_3$ [6, 323] have been reported.

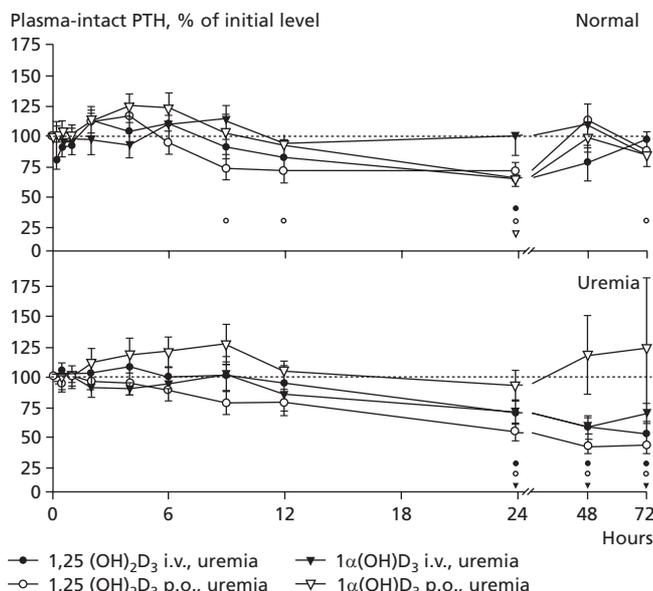


Figure 10. Plasma- Ca^{2+} and plasma intact PTH in relation to time following i.v. and oral administration of $4 \mu\text{g}$ of $1,25(\text{OH})_2\text{D}_3$ and i.v. and oral administration of $4 \mu\text{g}$ of $1\alpha(\text{OH})\text{D}_3$. The same 6 healthy volunteers were included in all 4 parts of the study. The 12 uremic patients were separated into 2 groups, the $1\alpha(\text{OH})\text{D}_3$ group and the $1,25(\text{OH})_2\text{D}_3$ group, due to the great volume of blood needed for plasma- $1,25(\text{OH})_2\text{D}_3$. A paired t-test was performed guided by a two-way analysis of variance. Values significantly different from baseline values ($p < 0.05$) are marked below the curve by the symbol of the relevant vitamin D analog. Plasma PTH values are presented as percentage of baseline value due to great variation in individual basal plasma PTH levels [6]. Mean \pm SEM.

In a direct comparative set-up where $1\alpha(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ were administered intravenously in equally high doses of $10 \mu\text{g}$, we observed that plasma Ca^{2+} increased significantly more after administration of $1,25(\text{OH})_2\text{D}_3$ than after $1\alpha(\text{OH})\text{D}_3$, in accordance with a previous report [182]. A dose of $6 \mu\text{g}$ of $1,25(\text{OH})_2\text{D}_3$ caused no increase in plasma Ca^{2+} and suppressed plasma PTH by $\sim 60\%$ ($p < 0.001$) after 24 hours while the suppressive effect of $10 \mu\text{g}$ of $1\alpha(\text{OH})\text{D}_3$ on PTH was $\sim 20\%$ ($p < 0.05$), indicating that $1,25(\text{OH})_2\text{D}_3$ is a more potent vitamin D analog than $1\alpha(\text{OH})\text{D}_3$. This difference in potency may be due to the kinetics of the conversion of $1\alpha(\text{OH})\text{D}_3$ to $1,25(\text{OH})_2\text{D}_3$ and does indicate that $1\alpha(\text{OH})\text{D}_3$ continuously will be converted to $1,25(\text{OH})_2\text{D}_3$. From our previous studies, it is known that the bioavailability of $1\alpha(\text{OH})\text{D}_3$ when measured as $1,25(\text{OH})_2\text{D}_3$ is less than 50% of the amount achieved after administration of similar doses of $1,25(\text{OH})_2\text{D}_3$ [6]. In a study on the vitamin D receptor in the osteoblast using autoradiography following a single intravenous injection of ^3H - $1\alpha(\text{OH})\text{D}_3$ and ^3H - $1,25(\text{OH})_2\text{D}_3$ to neonatal rats [324] it was demonstrated that the nuclear uptake in the osteoblasts was delayed, but sustained longer after administration of $1\alpha(\text{OH})\text{D}_3$ than after administration of $1,25(\text{OH})_2\text{D}_3$.

This observation can also be explained by the relative long time needed for the conversion of $1\alpha(\text{OH})\text{D}_3$ to $1,25(\text{OH})_2\text{D}_3$. In receptor affinity studies in rat intestine comparing $1\alpha(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ in the presence of $1,25(\text{OH})_2\text{D}_3$ the affinity of the receptor for $1\alpha(\text{OH})\text{D}_3$ was 900 times less than for $1,25(\text{OH})_2\text{D}_3$ [325]. This finding supports the concept that the main effect of $1\alpha(\text{OH})\text{D}_3$ takes place after conversion to $1,25(\text{OH})_2\text{D}_3$. Our own study [6] did not exclude a direct effect of $1\alpha(\text{OH})\text{D}_3$ on the parathyroid glands, but our results support that the main effect in vivo of $1\alpha(\text{OH})\text{D}_3$ on PTH suppression is preceded by a 25-hydroxylation to $1,25(\text{OH})_2\text{D}_3$.

The observed pharmacokinetic differences between $1,25(\text{OH})_2\text{D}_3$ and $1\alpha(\text{OH})\text{D}_3$ might explain the different biological responses between single-dose studies and long-term continuous studies. Consequently, the pharmacokinetic differences between $1,25(\text{OH})_2\text{D}_3$ and

$1\alpha(\text{OH})\text{D}_3$ in uremic patients and healthy volunteers were analysed in one study [6]. A 57% lower metabolic clearance rate of $1,25(\text{OH})_2\text{D}_3$ was found in uremic patients as compared to healthy volunteers in accordance with others [274, 326]. This difference in the clearance of $1,25(\text{OH})_2\text{D}_3$ between healthy volunteers and uremic patients may be due to the lack of kidney function in the uremic patients affecting renal metabolism and the elimination of $1,25(\text{OH})_2\text{D}_3$, or due to the presence of toxins in uremic plasma suppressing the degradation of $1,25(\text{OH})_2\text{D}_3$ [326]. The bioavailability as $1,25(\text{OH})_2\text{D}_3$ after intravenous administration of $1\alpha(\text{OH})\text{D}_3$ was only 40% when compared to $1,25(\text{OH})_2\text{D}_3$ in both normal and uremic subjects in accordance with results obtained after intravenous administration of $1\alpha(\text{OH})\text{D}_2$ [327]. Despite the finding that $4 \mu\text{g}$ of both $1,25(\text{OH})_2\text{D}_3$ and $1\alpha(\text{OH})\text{D}_3$ [6] seemed to have the same suppressive effect on plasma PTH, we observed a concomitant small, yet significant increase in plasma Ca^{2+} in the uremic patients after administration of $1,25(\text{OH})_2\text{D}_3$ that was not seen after administration of $1\alpha(\text{OH})\text{D}_3$. This means that no exact conclusions can be drawn on the therapeutic equivalence between the two drugs. However, the clinical experience is that $1\alpha(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ are both effective drugs in the treatment of secondary hyperparathyroidism in uremic patients [2, 4, 184, 193].

13. IN CONCLUSION

Based on the results of my investigations [1-7], it is concluded that:

- 1a. Intravenous administration of $1\alpha(\text{OH})\text{D}_3$ induced a marked suppression of plasma parathyroid hormone levels without causing serious side-effects in patients on chronic hemodialysis. It was possible to prevent hypercalcemia by close monitoring of plasma Ca^{2+} levels and by adjusting the dose of $1\alpha(\text{OH})\text{D}_3$ accordingly.
- 1b. Long-term intermittent intravenous treatment with $1\alpha(\text{OH})\text{D}_3$ was effective in suppressing plasma levels of Intact PTH.
- 1c. When plasma intact PTH by intravenous $1\alpha(\text{OH})\text{D}_3$ was suppressed to a stable level, then the same degree of suppression could be maintained by intermittent oral $1\alpha(\text{OH})\text{D}_3$ therapy. It was not examined whether a similar degree of suppression of severe secondary hyperparathyroidism could be induced by intermittent oral $1\alpha(\text{OH})\text{D}_3$ treatment alone. The response following chronic administration of $1\alpha(\text{OH})\text{D}_3$ on circulating levels of intact PTH and N- and C-terminal PTH fragments did not reveal any significant differences between the actions of intravenous and oral $1\alpha(\text{OH})\text{D}_3$ on the parathyroids.
- 2a. The combination of "low-calcium" dialysis fluid in hemodialysis (1.25 mmol/l), CaCO_3 and pulse intravenous $1\alpha(\text{OH})\text{D}_3$ prevented development of secondary hyperparathyroidism in patients with normal PTH at the initiation of the study, and induced a long-term suppression of PTH in patients with secondary hyperparathyroidism. No clinical or biochemical indication of the development of adynamic bone disease was observed. Intravenous administration of $1\alpha(\text{OH})\text{D}_3$ prevented a decrease of BMC in lumbar spine and femoral neck of hemodialysis patients with both normal as well as elevated PTH levels. It became possible to use larger doses of CaCO_3 and to reduce, but not exclude, the use of aluminum-containing oral phosphate binder in combination with intravenous administration of $1\alpha(\text{OH})\text{D}_3$. A decrease in plasma Ca^{2+} during dialysis was induced, and special care had to be focused on the compliance to CaCO_3 in order not to aggravate the secondary hyperparathyroidism.
- 2b. In patients on CAPD, low-calcium dialysis fluid (1.25 mmol/l) made it possible to use larger doses of CaCO_3 and to reduce, but not exclude the use of aluminum containing phosphate binder in combination with oral pulse $1\alpha(\text{OH})\text{D}_3$. A negative calcium balance was induced, and a reduction of the calcium concentration in the dialysis fluid was recommended only to be used in patients under strict control.

- 3a. The metabolic clearance rate of 1,25(OH)₂D₃ was 57% lower in uremic patients than in normal subjects ($p < 0.03$). The bioavailability in both normal subjects and uremic patients of 1,25(OH)₂D₃ was markedly lower following administration of 1 α (OH)D₃ intravenously and orally than after administration of oral 1,25(OH)₂D₃. Despite lower plasma 1,25(OH)₂D₃ levels after administration of 1 α (OH)D₃ than after 1,25(OH)₂D₃, no significant differences were observed in the PTH suppressive effect in uremic patients of 4 μ g intravenously of either of the two vitamin D analogs.
- 3b. A single, intravenous high dose of 10 μ g of 1 α (OH)D₃ or 1,25(OH)₂D₃ significantly suppressed plasma PTH. The acute suppressive effect of 1,25(OH)₂D₃ was three times greater than that of 1 α (OH)D₃. The increase in plasma Ca²⁺ after intravenous administration of 10 μ g 1,25(OH)₂D₃ was significantly higher than that of 1 α (OH)D₃. Due to the simultaneous effect on plasma Ca²⁺ it is not possible to decide whether 1 α (OH)D₃ has a direct effect per se on the parathyroid glands. The study further cannot tell anything about the therapeutic equivalence during long-term treatment with 1 α (OH)D₃ or 1,25(OH)₂D₃. The PTH response to acute administration of the 1 α (OH)D₃ and 1,25(OH)₂D₃ analogs was in principle the same, when measured by one "whole" PTH and two "intact" PTH assays, resulting mainly in a parallel shift of the PTH response curve. In this study on chronic uremic patients circulating levels of large C-terminal PTH fragments were not affected by differences in plasma Ca²⁺ concentration or by the intravenous administration of 1 α (OH)D₃ or 1,25(OH)₂D₃.

14. FUTURE ASPECTS – NEW VITAMIN D ANALOGS AND CALCIMIMETICS

Today it is generally accepted that the clinical approach to chronic uremic patients 10 years ago might not have been optimal, and that abnormalities of the skeleton and mineral metabolism might be responsible for the morbidity and mortality observed. Epidemiological studies have shown that hyperphosphatemia in particular may lead to increased morbidity and mortality [55, 57, 104, 106, 107, 127, 154, 328]. Application of principles from normal human physiology on calcium and phosphate metabolism and vitamin D effects in human disease has led to a shift of paradigm in the management of patients with chronic kidney disease: There is now a general agreement on the importance of controlling plasma phosphate, normalise and avoid increases of plasma Ca²⁺, and not to over suppress PTH. Focus is on the potential role of calcium overload in the development of vascular calcifications. Advances in knowledge are now beginning to translate into improvements in patient care. Non-calcium containing phosphate binders have been developed [329]. Among these, sevelamer HCl has been on the market for years and has contributed to a decrease in plasma phosphate without induction of hypercalcemia [136, 137]. Potentially, sevelamer HCl may also attenuate mortality, coronary and aortic calcification [138, 330]. Lanthanum carbonate, another non-calcium containing phosphate binder, has been on the market in Denmark for the last couple of years [331] and is well tolerated. Whether lanthanum accumulates to biologically significant levels in the organism is under debate [139, 140, 259, 332].

Until now, no specific phosphate-receptor has been demonstrated, but a Pi-2Na exchange mechanism has been demonstrated in rat parathyroid glands and intestine [142, 143]. Blocking such transport mechanism could constitute a potential treatment principle in the future.

Many efforts have been used to develop new vitamin D analogs that retain the suppressive effect on PTH secretion without increasing the intestinal calcium absorption. Chemical modifications of the native 1,25(OH)₂D₃ molecule have resulted in many different vitamin D analogs that have proved useful in treatment of cancer, skin disorders and metabolic calcium disturbances. Only a few have,

however, been used in clinical trials in chronic uremic patients. Some of the newer analogs are: 1 α (OH)D₂ (1 α -tachysterol) [161-165, 261, 327], F₆-1,25(OH)₂D₃ (falecalcitriol) [168, 169], 22-oxa-1,25(OH)₂D₃ (maxicalcetriol) [166, 167] and 19-nor-D₂ (paricalcitol) [121, 155-160, 170, 260, 261, 333]. The mechanism by which these new vitamin D analogs maintain their PTH suppressive effect, but with a less effect on calcium and phosphate metabolism, has not yet been fully elucidated. Pharmacokinetic studies of 22-oxa-1,25(OH)₂D₃ showed a smaller binding to vitamin D binding protein and a higher clearance as compared to 1,25(OH)₂D₂ which resulted in a more protracted effect in the parathyroid glands than in the intestine [334]. No difference has been observed in the pharmacokinetics of 19-nor-1,25(OH)₂D₂ parameters when compared to 1,25(OH)₂D₃. Development of a postreceptor resistance towards the drug in the intestines and bone was, however, found in long-term studies in normal rats [333]. 26,27-F₆-1,25(OH)₂D₃ is converted to an active metabolite which accumulates in bone. Retention of different active metabolites of the drug, perhaps in combination with an upregulating mechanism of different genes, may be an explanation for the effect of this particular drug [335]. The mechanisms responsible for the effect of 1 α (OH)D₂ are unknown. It may be attributed to accumulation of 1 α (OH)D₂ in the parathyroid glands activating the vitamin D receptor or, possibly, to an interaction with a novel receptor [336]. All 4 mentioned vitamin D analogs have been compared to placebo and have been shown to reduce PTH, and all 4 cause a small, but significant increase in plasma Ca²⁺.

Interestingly, 19-nor-1,25(OH)₂D₂ and 26,27-F₆-1,25(OH)₂D₃ [156, 168] did not increase plasma phosphate significantly. This effect – or missing effect – on plasma phosphate may prove important in the view of the worrisome findings of calcification in the heart of uremic patients [55, 56, 106, 330]. This possible benefit should be confirmed in larger future studies. Only direct comparative studies between the new analogs and 1 α (OH)D₃ or 1,25(OH)₂D₃ will demonstrate whether these new drugs possess relevant clinical advantages regarding calcemic and phosphatemic effects or a superior effect on PTH suppression or bone histology in chronic uremic patients.

Cloning of the calcium-sensing receptor has led to the development of a quite new group of pharmacological agents – the calcimimetics. The mechanism behind these agents is that they act as an allosteric calcium modulator and lowers the threshold for activation by calcium of the calcium-sensing receptor in the parathyroid glands [337]. Calcimimetics have demonstrated a unique effect on the suppression of PTH simultaneously reducing plasma calcium, plasma phosphate and the Ca \times P product [337], presumably all due to a decreased bone turnover.

Important questions are now raised: What will be the role of vitamin D and vitamin D analogs in the future? Does a combined use of calcimimetics and vitamin D have additive effects on the suppression of parathyroid hormone? Most nephrologists believe that vitamin D supplementation will prove to have advantageous effects in combination with calcimimetics, and that vitamin D analogs will continue to be commonly used in chronic uremic patients because vitamin D is needed for optimal bone health. Further, the odds of survival seem to be increased when active vitamin D is used in uremic patients [121, 122, 338, 339]. At this moment, no data are available showing whether treatment by calcimimetics reduces the mortality rate, reduces cardiovascular events, or reduces the progression of bone disease in chronic uremic patients. Ongoing studies will hopefully solve this problem. In the future, studies of calcimimetics may alter our understanding of the relationship between parathyroid hormone and bone disease. There is an urgent need for the development of algorithms for the use of phosphate binders and vitamin D supplement in combination with calcimimetics focusing upon longterm morbidity and mortality in uremic patients.

15. SUMMARY IN ENGLISH

Chronic uremia is characterized by decreased levels of plasma

1,25(OH)₂D₃ due to decreased renal 1-hydroxylase activity and by decreased renal phosphate excretion. The consequence is an increased synthesis and secretion of parathyroid hormone – secondary hyperparathyroidism – due to the low levels of plasma calcium, low levels of plasma 1,25(OH)₂D₃ and high levels of phosphate. The association between renal bone disease and chronic renal failure is well described. Epidemiological studies have indicated that an association also exists between secondary hyperparathyroidism and increased mortality and cardiovascular calcifications in chronic uremic patients. Treatment of secondary hyperparathyroidism in chronic uremia focuses on avoiding hyperphosphatemia by the use of oral phosphate binders, which bind phosphate in the intestine and a concomitant substitution by a 1 α -hydroxylated vitamin D analog in order to compensate for the reduced renal hydroxylation.

Additional treatment with aluminum containing phosphate binders to overcome phosphate absorption and retention was initiated already in the 1960s and used extensively until aluminum toxicity was disclosed in the mid-1980s. Instead calcium carbonate and calcium acetate were used as phosphate binders.

Until recently, the most commonly used active vitamin D drug was either the natural 1,25(OH)₂D₃, or the 1 α -hydroxylated analog, 1 α (OH)D₃ which after 25-hydroxylation in the liver is converted to 1,25(OH)₂D₃. 1 α (OH)D₃ was produced by LEO Pharma in 1973. The two vitamin D analogs were used in different geographical areas: In Europe 1 α (OH)D₃ was mainly used, while 1,25(OH)₂D₃ was mainly used in the USA.

1,25(OH)₂D₃ increases the intestinal absorption of calcium and improves skeletal abnormalities. The combined treatment with calcium containing phosphate binders and active vitamin D induces an increase in plasma Ca²⁺ and hypercalcemia became a clinical problem. Subsequently therefore, dialysis fluid with reduced a calcium concentration (“low-calcium”) was introduced.

In 1981 Madsen et al. [148] demonstrated for the first time a direct suppressive effect of intravenous 1,25(OH)₂D₃ on plasma PTH in acutely uremic patients. In 1984, Slatopolsky et al. [74] demonstrated that intravenous 1,25(OH)₂D₃ induces a marked suppression of plasma PTH with no increase in plasma Ca²⁺ in chronic uremic patients. In the middle of 1980s, 1 α (OH)D₃ became available not only as an oral, but also as an intravenous formulation.

The main purpose of the present studies was to increase the knowledge of the action and effects of different treatment regimes with 1 α (OH)D₃, and thereby to improve the prophylaxis and treatment of secondary hyperparathyroidism in uremic patients on chronic dialysis. 168 patients on chronic dialysis treatment and 6 healthy volunteers were included in the 7 studies included in this thesis.

The first part of the studies, focused on short- (12 weeks) and long-term (103 weeks) effects of intravenous 1 α (OH)D₃ on plasma PTH and plasma Ca²⁺ in relation to the doses of 1 α (OH)D₃ given. Further, it was examined whether the marked suppression of plasma PTH induced by 300 days of intermittent intravenous treatment with 1 α (OH)D₃, could be maintained when the administration was changed from intravenous to the oral route for 16 further weeks and then shifted back to intravenous administration for another 16 weeks.

The second part focused on long-term effects (88 weeks in hemodialysis patients and 52 weeks in CAPD patients) of a treatment modality combining 1 α (OH)D₃, and CaCO₃ as phosphate binders instead of aluminum containing compounds and a decreased calcium concentration in the dialysis fluid to 1.25 mmol/l in an attempt to avoid development of hypercalcemia.

The third part focused upon the pharmacokinetic differences between intravenous and oral administration of 1,25(OH)₂D₃ and 1 α (OH)D₃ and upon the acute effects of different doses of the two compounds on the plasma levels of PTH, Ca²⁺ and phosphate.

Plasma PTH is biochemical parameter most often used for the diagnosis and monitoring of bone disease in patients with chronic

uremia. The level of plasma PTH measured depends on the assay used. More specific assays measuring only whole PTH 1-84 without co-measuring large C-terminal fragments have been developed. In this thesis, 5 different assays were used – one “N-terminal”, one “C-terminal”, two “Intact” and one “Whole” PTH assay. Each sample was analyzed by 1-3 different assays.

Based on the results of my studies [1-7], it is concluded that:

- 1a. Intravenous administration of 1 α (OH)D₃ induces a marked suppression of plasma PTH without causing serious side-effects in patients on chronic hemodialysis. It is possible to prevent hypercalcemia by closely monitoring plasma Ca²⁺ levels and by adjusting the dose of 1 α (OH)D₃ accordingly.
- 1b. Long-term intermittent intravenous treatment with 1 α (OH)D₃ was effective in suppressing plasma levels of Intact PTH.
- 1c. When plasma intact PTH was suppressed to a stable level by intravenous 1 α (OH)D₃ the suppression could be maintained by intermittent oral 1 α (OH)D₃ therapy. It was not examined whether a similar degree of suppression of severe secondary hyperparathyroidism could be induced by intermittent oral 1 α (OH)D₃ treatment alone. The responses following chronic intravenous or oral administration of 1 α (OH)D₃ on circulating levels of intact PTH and N- and C-terminal PTH fragments did not reveal any significant differences between the two routes of administration on the actions on the parathyroid glands.
- 2a. The combination of “low-calcium” hemodialysis fluid (1.25 mmol/l), CaCO₃ as a phosphate binder, and intermittent intravenous 1 α (OH)D₃ prevented development of secondary hyperparathyroidism in uremic patients with normal PTH at the initiation of the study and induced a long-term suppression of PTH in patients with secondary hyperparathyroidism. No clinical or biochemical indications of development of adynamic bone disease were observed. Intravenous administration of 1 α (OH)D₃ prevented a decrease of BMC in the lumbar spine and femoral neck of hemodialysis patients both with normal and with elevated PTH levels. It was possible to use larger doses of CaCO₃ and to reduce, but not exclude, the use of aluminum-containing phosphate binders in combination with intravenous administration of 1 α (OH)D₃. A decrease of plasma Ca²⁺ was induced during dialysis, and special care had to be taken on the compliance of the patients as to the use of CaCO₃ binders in order not to aggravate secondary hyperparathyroidism.
- 2b. In patients on CAPD, the use of low-calcium dialysis (1.25 mmol/l) made it possible to use larger doses of CaCO₃ phosphate binders and to reduce, but not exclude the use of aluminum containing phosphate binder in combination with oral pulses of 1 α (OH)D₃. A negative calcium balance was induced, and it is therefore recommended that a reduction of the calcium concentration in the dialysis fluid is only used in patients under strict control.
- 3a. The metabolic clearance rate of 1,25(OH)₂D₃ was 57% lower in uremic patients than in normal subjects (p<0.03). The bioavailability of 1,25(OH)₂D₃ in both normal subjects and uremic patients was markedly lower following administration of 1 α (OH)D₃ both intravenously and orally than after administration of oral 1,25(OH)₂D₃. Despite lower plasma 1,25(OH)₂D₃ levels after administration of 1 α (OH)D₃ than after 1,25(OH)₂D₃, no significant difference was observed in the PTH suppressive effect in uremic patients of 4 μ g intravenously of either of the two vitamin D analogs.
- 3b. A single intravenous high dose of 10 μ g of 1 α (OH)D₃ or 1,25(OH)₂D₃ significantly suppressed plasma PTH. The acute suppressive effect of 1,25(OH)₂D₃ was three times greater than that of 1 α (OH)D₃. The increase in plasma Ca²⁺ after intravenous administration of 10 μ g 1,25(OH)₂D₃ was significantly higher than that of 1 α (OH)D₃. Due to the simultaneous effect on plasma Ca²⁺ observed it was not possible to decide whether

1 α (OH)D₃ has a direct effect per se on the parathyroid glands or not. The study further did not give any further knowledge about the possible therapeutic equivalence of long-term treatment with 1 α (OH)D₃ or 1,25(OH)₂D₃. The PTH responses to acute administration of the 1 α (OH)D₃ and 1,25(OH)₂D₃ analogs were in principle the same when measured by one "whole" PTH and two "intact" PTH assays, namely mainly in a parallel shift of the PTH response curve. In this study on chronic uremic patients circulating levels of large C-terminal PTH fragments were not affected by differences in plasma Ca²⁺ concentration or by the intravenous administration of 1 α (OH)D₃ or 1,25(OH)₂D₃.

There is now a general agreement on the importance of carefully controlling plasma phosphate, normalize and avoid increases of plasma Ca²⁺, and not to oversuppress PTH during treatment. Focus today is on the potential deleterious role of calcium overloading in the development of vascular calcifications in uremic patients.

There is an urgent need for a development of an algorithm for the use of phosphate binders and vitamin D supplementation in combination with calcimimetics focusing upon long term morbidity and mortality in uremic patients.

ABBREVIATIONS

1 α (OH)D ₃	One-alpha-hydroxy-cholecalciferol
1,25(OH) ₂ D ₃	1,25-dihydroxycholecalciferol
2,3 DPG	2,3-diphosphate glycerate
ALAT	Alanin amino transferase
AMI	Acute myocardial infarction
ASAT	Aspartate amino transferase
ATP/ADP	Adenosine-tri-phosphate/adenosine-diphosphate
AUC	Area under the curve
BMC	Bone mineral content
BMD	Bone mineral density
BMP	Bone morphogenic protein
Ca \times P product	Calcium phosphate product
Ca ²⁺	Ionized calcium
C _{max}	Maximal plasma 1,25(OH) ₂ D ₃ measured
CAPD	Continuous ambulatory peritoneal dialysis
CaR	Calcium sensing receptor
CRF	Chronic renal failure
DEXA	Dual energy X-ray absorptiometry
EBCT	Electron beam computed tomography
ECF	Extracellular fluid
FGF23	Fibroblast growth factor 23
GFR	Glomerular filtration rate
HD	Hemodialysis
HPLC	High-performance liquid chromatography
ICF	Intracellular fluid
Intact PTH assay	Radioimmuno PTH assay. Parathyroid hormone or hormone fragments reacting in a specific (radio)immunoassay. Molar concentrations given do not reflect exact concentrations, but immunoreactivity compared with the standard used in the assay
iPTH	Immunoreactive PTH
IRMA	Immunoradiometric assay
IU	International units
K-DOQI	The kidney disease outcomes quality initiative
LDH	Lactate dehydrogenase
MT	Mass transfer
MCR	Metabolic clearance rate
Mg	Magnesium
NPT	Natrium phosphate cotransporter
OPG	Osteoprotegerin
P1cP	Procollagen type 1 c-terminal extension peptid
PET	Peritoneal equilibrium test

PHEX	Phosphate-regulating gene with homologies to endopeptidases on the X-chromosome
Pi	Inorganic phosphate
Plasma Ca ²⁺	lasma ionized calcium
PP	Coagulation time, factor 2, 7 and 10
PTH	Parathyroid hormone
PTH/PTHrP	Parathyroid hormone/parathyroid hormone related peptide
PTHr1	Parathyroid receptor type 1
R ²	Determination coefficient
RANK-RANKL	Receptor activator of nuclear factor kappa B-receptor activator of nuclear factor kappa B ligand
RAT	Renal allo-transplantation
RIA	Radioimmunoassay
RXR	Retinoid X receptor
TmPO ₄	Maximum rate of renal tubular re-absorption of phosphate
TRPV5	Transient receptor potential vanilloid-5 channel
Vd	Volume of distribution
VDR	Vitamin D receptor
VDR/RXR	Vitamin D/retinoid receptor heterodimer
VDRE	Vitamin D response elements
Whole PTH assay	Immunoradiometric assay. Determines the full-sized parathyroid hormone of 84 amino acids, not cleaved into smaller fragments

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