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MAIN ENDOCRINE MODULATORS OF VITAMIN D HYDROXYLASES IN HUMAN PATHOPHYSIOLOGY

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Summary—Vitamin D is considered to be devoid of direct biological activity. It must be first hydroxylated in the liver by a 25-hydroxylase (25OHase), then in the kidney by a 1α-hydroxylase (1αOHase) which is responsible for the synthesis of the active metabolite, 1,25-dihydroxyvitamin D (1,25(OH)2D). The activity of 1αOHase is known to be under the control of a series of endocrine modulators, particularly parathyroid hormone (PTH) and estrogens. We report here our studies in humans concerning the behaviour of vitamin D hydroxylases in some pathological conditions.

In chronic liver disease no severe impairment of vitamin D-25-hydroxylation has been observed, except in the latest stages: this is probably due to the great functional reserve of the liver, so that normal levels of serum 25OHD can be maintained on condition that the vitamin D supply is adequate.

1αOHase is impaired in chronic renal failure due to the decrease in the number of functioning nephrons. It has been demonstrated that kidney transplantation restores normal 1,25(OH)2D levels. A decrease in 1,25(OH)2D production due to reduced PTH stimulation has been observed in hypoparathyroidism: in these patients a subcutaneous substitution therapy with synthetic human parathyroid hormone resulted in restoration of normal 1,25(OH)2D levels. A reduced activity of 1αOHase due to reduced estrogen stimulation plays a key role in postmenopausal osteoporosis. In these patients estrogens increase 1,25(OH)2D levels, as it has been demonstrated directly and indirectly. In the aforementioned pathological conditions an impairment of calcium absorption has been observed; it was directly related to the reduced production of 1,25(OH)2D. Treatment with 1,25(OH)2D3 was effective in restoring normal calcium absorption.

In postmenopausal osteoporosis the reduced levels of 1,25(OH)2D were accompanied by serum levels of 25-hydroxyvitamin D (25OHD) higher than in age-matched control women. In these cases long-term treatment with physiological doses of 1,25(OH)2D3 resulted in a progressive decrease in 25OHD serum levels which approached to the normal range. These findings are likely to be related one to another: the low 1,25(OH)2D levels are responsible for reduced product-inhibition of 25OHase, so that the synthesis of 25OHD increases. A similar mechanism occurs in renal failure and in hypoparathyroidism.

INTRODUCTION

Vitamin D is considered to be devoid of direct biological activity. It must be first hydroxylated in the liver by a 25-hydroxylase (25OHase), then in the kidney by a 1α-hydroxylase (1αOHase), which is responsible for the synthesis of the active metabolite, 1,25-dihydroxyvitamin D (1,25(OH)2D) [1].

25-*Hydroxylation*

25-hydroxyvitamin D (25OHD) is an obligatory intermediate through which all known vitamin D metabolites are synthesized.

Vitamin D-25OHase activity is located predominantly in the liver [2], but not exclusively so, since some 25-hydroxylation can take place in intestine and kidney [3]. The quantitative contributions of these additional sites, however, seem to be quite small, since little 25OHD can be produced by hepatectomized experimental animals [4].

Vitamin D-25OHase activity is associated with the liver microsomal fraction, where a specific NADPH-cytochrome P-450 reductase enzyme system has been identified [5]; this suggests that vitamin D metabolism by the liver occurs via a mixed-function oxidase system, in analogy to what has been demonstrated for the hydroxylation of many steroids and drugs.

A cholesterol-25OHase is also known to exist in liver mitochondria, in addition to the microsomal 25OHase [6]. This hydroxylase is probably involved in vitamin D metabolism only under conditions of high vitamin D intake, so that it can be considered the main factor responsible for the high concentrations of serum 25OHD found in plasma after administration of pharmacological doses of the vitamin.

1α-*Hydroxylation*

Further metabolism of 25OHD to 1,25(OH)2D appears to take place only in the kidney [7] (and, in pregnancy, in placental tissue), despite the *in vitro* demonstration of some. 1αOHase activity in extrarenal sites, namely in bone cells [8]. In fact, no measurable quantities of 1,25(OH)2D3 are detectable *in vivo* in anephric animals given radioactive 25OHD [9].

1αOHase is exclusively associated with the mitochondrial fraction of the renal cortex; the enzyme system consists of a three-component mixed-function oxidase, which involves NADPH reduction of a flavoprotein termed "renal ferredoxin reductase";

this enzyme in turn reduces a protein called "renal ferredoxin", which supplies reducing equivalents to the cytochrome P-450 [10].

Regulation of vitamin D hydroxylases

As to the regulation of the aforementioned enzyme activities, it should be stressed that our level of understanding of the 25-hydroxylation reaction is minimal in comparison to the bulk of data available concerning the biochemistry of renal hydroxylation processes.

Some authors have even denied the possibility that the step of conversion of vitamin D to 25OHD might be subject to physiological regulation [3]. However, available observations indicate that even if this metabolic step is not particularly stringently regulated, there is certainly some kind of control. In fact, it has been reported that a greater fraction of vitamin D is converted to 25OHD in vitamin D deficient subjects than in patients who have received substantial quantities of vitamin D [11]. However, 25-hydroxylation cannot be considered a major site of regulation of vitamin D metabolism, since high serum levels of 25OHD can be accumulated under conditions of large doses of vitamin D.

The activity of renal 1αOHase is known to be under the control of several factors, some of which are hormonal in nature, the main endocrine modulators of 1,25(OH)2D synthesis being parathyroid hormone [12, 13] and estrogens [14, 15].

There are strict analogies between the control of the synthesis of adrenal steroids and that of 1,25(OH)2D (which, in consideration of its chemical structure, regulated synthesis and mechanism of action can be considered a true steroid hormone).

We report here our experience in human pathological conditions which have provided useful data concerning the main endocrine modulators of vitamin D hydroxylases.

VITAMIN D METABOLISM IN CHRONIC LIVER DISEASE

The liver is not involved in vitamin D function only as the site of 25-hydroxylation. It plays other major roles: it is the source of bile salts, which are essential for a normal intestinal absorption of dietary vitamin D; it is the organ where the specific plasma carrier proteins for vitamin D and its metabolites are synthesized and is also believed to be the site of binding of both vitamin D and 25OHD to these binding proteins [16]; finally, the liver is involved in the enterohepatic circulation of vitamin D and its metabolites [17]. Thus, any kind of hepatic malfunction may potentially interfere not only with the metabolism of vitamin D, but also with its absorption and subsequent plasma transport.

Chronic liver disease is sometimes complicated by various degrees of metabolic bone involvement, which is responsible for a reduced mechanical resistance of the skeleton and manifests itself with pain and increased incidence of fractures [18].

In patients with prolonged cholestatic liver disease the prevailing histologic pattern is that of osteomalacia [19, 20]. This can be accounted for by a reduced intestinal absorption of dietary vitamin D, as a part of the characteristic fat malabsorption, and by the interruption of the enterohepatic circulation of 25OHD.

In patients with hepatocellular dysfunction the most common histologic pattern is osteoporosis [18]. The pathogenesis of this is not fully understood yet, but an impairment of vitamin D metabolism has been suggested as an important factor in this condition as well. Low serum levels of 25OHD have been reported in these patients [21], but whether these are due to impaired liver hydroxylation or to reduced substrate availability remains an unanswered question.

Vitamin D metabolites in chronic liver disease

We studied a large number of patients, with either alcoholic cirrhosis or post-infectious chronic hepatitis without cholestatic complication, who were considered to represent a good model of severe hepatic dysfunction.

There were 34 men and 26 women, aged 28–90 yr; 37 of the patients had alcoholic cirrhosis and 23 post-infectious chronic hepatitis. In these patients the serum levels of the main vitamin D metabolites were determined, in addition to a series of liver function parameters.

Vitamin D metabolites were measured, after lipid extraction of samples, column chromatography and HPLC, by competitive protein binding assay, using rat serum as the source of binding protein for 25OHD [22] and a calf thymic cytosol for 1,25(OH)2D [23].

Lower serum levels of 25OHD and 1,25(OH)2D than in age-matched control subjects were observed in our patients.

The mean value of serum 25OHD was 6.16 ng/ml (normal values: 5.0–40.0 ng/ml). The data obtained, however, showed a non-Gaussian distribution (as it is usually found in normal subjects), with a mode of 4.2 ng/ml. The data were transformed logarithmically and the significance of differences between patients with chronic liver disease and a group of 149 age-matched controls was evaluated by unpaired Student's t-tests; the difference was found to be highly significant ($t = 3.03$; $P < 0.01$).

The mean value of serum 1,25(OH)2D was 24.9 ± 12.2 SD pg/ml; it was significantly lower than the mean observed in a group of age-matched healthy subjects (29.9 ± 17.0 pg/ml) [24].

Vitamin D cutaneous synthesis and metabolism in chronic liver disease

We have studied the endogenous synthesis and metabolism of vitamin D in a group of seven patients with liver cirrhosis, by evaluating the changes in-

duced in the serum concentrations of the main vitamin D metabolites in response to whole-body u.v. irradiation.

A mercury arc lamp of known spectrum and energy was used, with progressively increasing irradiation times, a total of 13.5 min being delivered over 5 days. Blood samples were obtained before and at various intervals up to 21 days after the initiation of the study.

The results showed a lower response of 25OHD in cirrhotic patients as compared with 18 normal subjects (the maximum increases were 16.6 ± 7.6 and 22.8 ± 11.2 ng/ml, respectively); the difference, however, was not statistically significant. 1,25(OH)2D showed a significantly higher response than in 11 controls (maximum increases 51.5 ± 24.5 and 24.2 ± 22.8 pg/ml, respectively) [25] (Fig. 1).

The conclusion can be drawn that in liver cirrhosis the vitamin D cutaneous synthesis and liver hydroxylation in response to u.v. irradiation are not significantly impaired. The higher response of 1,25(OH)2D is likely to result from increased 1αOHase activity and is consistent with the view that these patients are vitamin D deficient.

Vitamin D 25-hydroxylation in chronic liver disease

The step of liver vitamin D-25-hydroxylation was then studied in detail by administering vitamin D by subcutaneous injection to cirrhotic patients and normal volunteers as a control.

This kind of study has always been hampered by the unavailability of a suitable form of vitamin D for parenteral use. In fact, vitamin D, that is a lipid-soluble substance, is generally administered as slow-release oil preparations that fail to cause significant changes in the serum levels of 25OHD within a reasonably short time. On the other hand, the evaluation of the 25OHD response after oral administration of the parent vitamin is heavily influenced by the intestinal absorption of the sterol, which has been reported to be impaired in such patients [19, 26].

We have obtained a rapid-release parenteral preparation of vitamin D3, using a surface-active compound as a solubilizing agent and a single dose of this vitamin D3 (1.25 mg) was administered. The data obtained were transformed logarithmically and the areas below the response curves were calculated. Unpaired Student's *t*-tests were used to evaluate the statistical significance of differences between cirrhotic patients and controls.

The serum concentrations of 25OHD increased remarkably after D injection, reaching a maximum in most cases within 60 h and decreasing slowly thereafter. Wide variations among subjects were observed. The difference between the response of cirrhotic patients and that of control subjects was not statistically significant (Fig. 1).

These results confirm that, despite the subnormal serum concentrations of 25OHD and 1,25(OH)2D, the liver vitamin D-25 hydroxylation is not severely impaired in patients with chronic liver disease.

The low basal values of vitamin D metabolites reported are likely to result from reduced availability

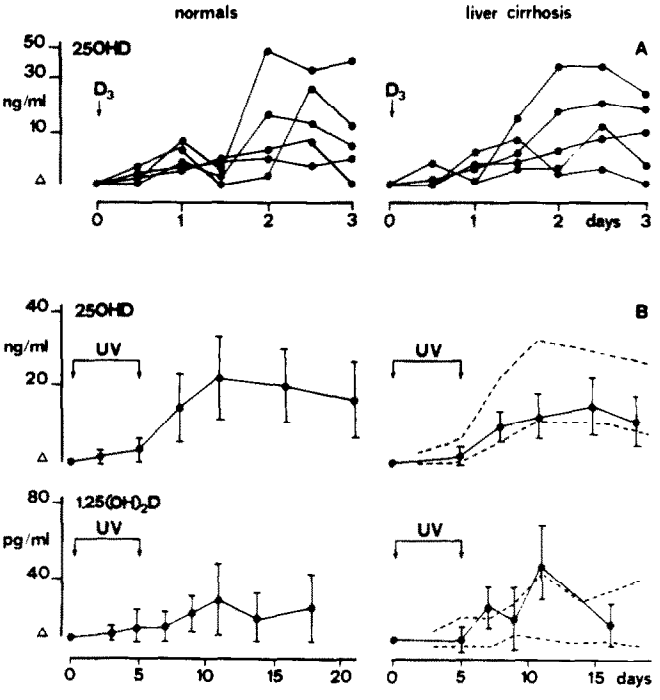

Fig. 1. (A) Response of serum 25OHD to the s.c. injection of vitamin D3 (1.25 mg) in normal subjects and cirrhotic patients. (B) Response of serum 25OHD and 1,25(OH)2D to whole-body artificial u.v. irradiation in normal subjects and cirrhotic patients.

of the parent vitamin D, as a consequence of low dietary intake, impaired intestinal absorption and reduced sunlight exposure.

When the vitamin D supply is adequate the levels of its metabolites increase rapidly up to the range of the normal controls.

This is probably due to a great functional reserve, which enables a chronically diseased liver to maintain its role in vitamin D metabolism; this function is likely to decline only in the latest stages of the disease.

VITAMIN D METABOLISM IN CHRONIC RENAL DISEASE

The production of 1,25(OH)2D is impaired in chronic renal failure when the functioning renal mass is lower than 20%.

Indeed, very low serum levels of 1,25(OH)2D have been found in patients with chronic renal failure and it has been demonstrated that anephric or uremic individuals are not capable of metabolizing labelled vitamin D properly [27, 28].

This is considered the main pathogenetic factor of the abnormalities in calcium metabolism of patients with chronic renal disease (including a severe impairment of intestinal calcium absorption) which can lead to the development of renal osteodistrophy.

This condition, characterized by a mixed histologic pattern of osteomalacia and secondary hyperparathyroidism, can be effectively treated by a substitution therapy with calcitriol (synthetic 1,25(OH)2D3), i.e. by the exogenous administration of the active metabolite that cannot be synthesized in adequate amounts by the diseased kidney.

The subject has been extensively studied, so that an enormous volume of data is available at present concerning the pathogenesis of renal osteodistrophy [29], its histologic aspect [30] and treatment [31].

The etiologic treatment of renal osteodistrophy is represented by renal transplantation, which can be followed by a satisfactory restoration of renal function. A rise in serum 1,25(OH)2D concentrations has been observed in uremic patients who had undergone a successful renal transplant, whereas this metabolite was virtually absent prior to the surgical procedure [32]. According to Lund *et al.* serum 1,25(OH)2D was undetectable in a group of nephrectomized patients routinely treated with a daily oral supplement of 1200 U of vitamin D3, very low in patients with end-stage renal failure on chronic haemodialysis receiving the same oral dose of vitamin D3 (mean serum level: 6.4 ± 5.9 pg/ml) and much higher in a group of patients who had received kidney transplants (18.3 ± 10.2 pg/ml) [33].

VITAMIN D METABOLISM IN HYPOPARATHYROIDISM

Parathyroid hormone (PTH) is considered the most important regulator of the activity of renal 1αOHase. Nevertheless, our knowledge of the role of PTH in vitamin D metabolism is mainly based on animal studies, only a small number of indirect observations being available in man [34–36].

The intestinal calcium absorption is adaptively regulated by the organism, so that there is an increased efficiency of calcium absorption under conditions in which the calcium demand is high (e.g. low dietary calcium intake) and vice versa [37]. Much evidence has accumulated to suggest that adaptation to the varying calcium requirements involves an endocrine-mediated regulation of 1,25(OH)2D production [34].

PTH has been shown to be the mediator through which the synthesis of 1,25(OH)2D is adapted to the calcium needs of the organism [35].

A decrease in 1,25(OH)2D production due to reduced PTH secretion has been reported in hypoparathyroidism and it has been demonstrated that the intestinal absorption of calcium is impaired in these patients [38, 39] (Fig. 2) who show, as a major clinical feature, hypocalcemia with a variety of related neurological disorders.

The parenteral administration of PTH would represent the etiologic treatment. However, calcitriol is a valuable oral substitution therapy that results in a pronounced rise in serum calcium level and in an effective control of clinical symptoms.

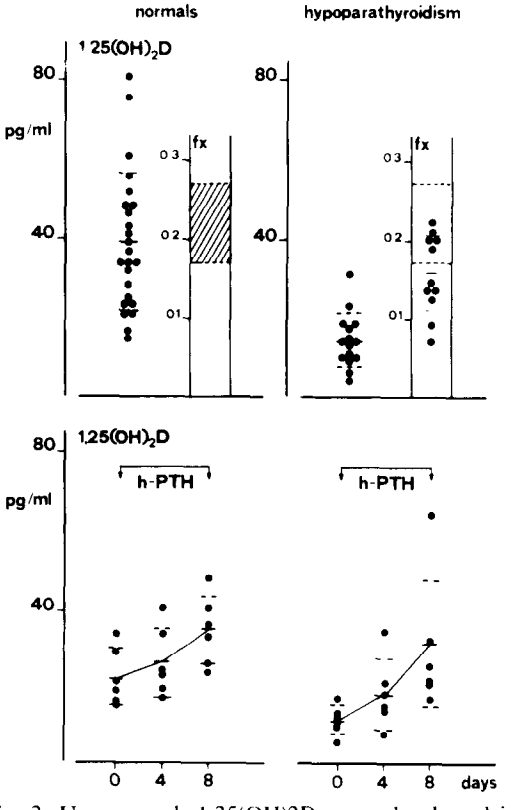

Fig. 2. Upper panel: 1,25(OH)2D serum levels and intestinal calcium absorption (fx) in normal subjects and patients with hypoparathyroidism. Lower panel: effects of a continuous s.c. infusion with 4.1 U/h of synthetic human 1-34 PTH in normal subjects and patients with hypoparathyroidism.

Effects of PTH infusion on 1,25(OH)2D serum levels

We have studied the effects of PTH on the synthesis of 1,25(OH)2D by measuring the changes induced by the infusion of human PTH on the serum levels of 1,25(OH)2D in a group of 6 patients with hypoparathyroidism and in a control group of 6 healthy volunteers.

Synthetic human PTH (1-34 fragment) was infused subcutaneously at the dose of 4.1 U/h (100 U/day) for 8 days. PTH was develivered continuously by a minipump of the type commonly used for insulin infusion in patients with diabetes. Serum 1,25(OH)2D was measured, after lipid extraction of samples and HPLC, by competitive protein binding assay, using a cytosol of intestinal mucosa from rachitic chicks as the source of binding protein [22].

A substantial increase in the serum levels of 1,25(OH)2D was observed during PTH infusion in the normal subjects: the mean serum concentration of 1,25(OH)2D was 22.1 ± 7.4 pg/ml prior to the initiation of hormone infusion and 34.8 ± 8.7 pg/ml at the end of the study.

Only small increases in 1,25(OH)2D levels were observed during the first days (mean level on the fourth day: 26.3 pg/ml). The magnitude of responses showed no significant relationship with the initial values.

In the patients with hypoparathyroidism the infusion of human PTH induced a greater response of 1,25(OH)2D (mean basal value 11.5 ± 4.0 pg/ml and 31.3 ± 15.9 pg/ml at the end) but the responses varied widely in different patients [40] (Fig. 2).

Effects of PTH infusion in a patient with primary hyperparathyroidism

In a patient with primary hyperparathyroidism, the infusion of synthetic human PTH failed to cause definite changes in the serum levels of 1,25(OH)2D, which were higher than normal in basal conditions.

A few days after surgical removal of a parathyroid adenoma, serum 1,25(OH)2D was much lower than in control subjects; the infusion of identical doses of PTH resulted in a remarkable increase in serum 1,25(OH)2D up to pre-treatment levels.

VITAMIN D METABOLISM IN POSTMENOPAUSAL OSTEOPOROSIS

Osteoporosis is defined as a condition of bone loss, without significant changes in mineralization. Histologically, a decrease in thickness and number of the trabeculae of calcellous bone is seen, without increases in osteoid volume.

In a large number of postmenopausal women bone loss is so rapid that the skeletal mass soon becomes lower than that required for a normal function of mechanical support: these patients are considered to have postmenopausal osteoporosis.

Considerable evidence has been gathered indicating that a severe impairment of intestinal calcium absorption plays a fundamental role in the pathogenesis of post-menopausal osteoporosis [41, 42].

Estrogens and vitamin D metabolites in postmenopausal osteoporosis

Estrogens represent another endocrine stimulator of 1,25(OH)2D synthesis, as has been demonstrated in experimental animals and humans. It is known that in hens estrogens and progesterone markedly stimulate 1αOHase at the time of eggshell formation, so providing high plasma levels of 1,25(OH)2D that bring about a better utilization of calcium from intestine and medullary bone to meet the needs of eggshell production [43]; moreover, marked increases in the renal 1αOHase activity have been demonstrated in chicks and cockerels as a consequence of estrogen and gestogen injection [14].

As it concerns humans, a double-blind study, carried out in postmenopausal osteoporotic women, gave interesting indications. The patients were treated with an estrogen–gestogen combination or with a placebo: the treatment restored a normal intestinal calcium transport, whereas the placebo was ineffective [44] (Fig. 3). Since calcium absorption is considered to be under the control of 1,25(OH)2D, it is likely that the increase in calcium absorption observed under estrogen–gestogen treatment was a consequence of a rise in 1,25(OH)2D production.

More recently a direct effect of estrogens on the renal synthesis of 1,25(OH)2D has been demonstrated in humans [45, 46].

A decrease in 1,25(OH)2D production due to reduced estrogen stimulation appears to play a key role in postmenopausal osteoporosis.

Estrogens have long been considered an effective etiologic treatment of postmenopausal osteoporosis; indeed, they stimulate 1αOHase activity, increase the serum levels of 1,25(OH)2D and consequently improve the intestinal absorption of calcium [46]. However, due to some potential untoward side-

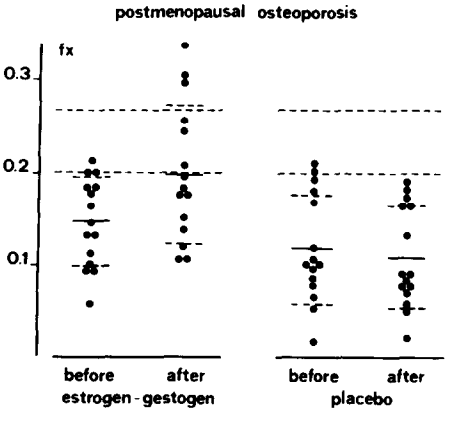

Fig. 3. Effects of an estrogen–gestogen combination and placebo on the intestinal absorption of calcium (fx) in patients with postmenopausal osteoporosis [44].

effects they cannot be considered safe for a long-term treatment of postmenopausal osteoporosis [47].

Vitamin D metabolites in postmenopausal osteoporosis

We have studied a group of carefully selected women with severe, symptomatic postmenopausal osteoporosis. In each patient the diagnosis was confirmed histologically by microscopic inspection of bone biopsies, since we believe there is no other means to distinguish with certainty between patients with osteoporosis and patients with mixed patterns of osteoporosis and osteomalacia.

In our osteoporotic women we observed lower mean levels of serum 1,25(OH)2D than in age-matched controls (respectively 27.0 ± 9.9 pg/ml and 41.2 ± 19.4 pg/ml), the difference between osteoporotic patients and normal peers being statistically significant [48]. Similar findings were reported by others [42, 49].

The decrease in 1,25(OH)2D serum levels is not due to reduced substrate availability, as demonstrated by our data on serum 25OHD (which will be mentioned below), but, more probably, to decreased stimulation of 1αOHase.

In view of the stimulating effect of estrogens on 1αOHase activity, it is likely that after the menopause the decrease in serum 1,25(OH)2D is related to the estrogen deficiency.

1,25(OH)2D deficiency is in turn responsible for the impairment of intestinal calcium transport that results in chronic calcium deficiency leading to increased bone resorption as a homeostatic response.

In fact, a short-term treatment with physiological doses of 1,25(OH)2D3 (1 μg/day) resulted in a dramatic improvement of intestinal calcium absorption in osteoporotic women [50]. A similar treatment with 24,25(OH)2D3 was completely ineffective [51].

It is well known that vitamin D is completely useless in the therapy of postmenopausal osteoporosis [52]; 25OHD treatment has also been found to be ineffective in increasing intestinal calcium absorption in these patients [53]. These findings are in agreement with our observation that in postmenopausal osteoporotic women the "vitamin D status", as expressed by the serum concentration of 25OHD, is generally normal or even higher than normal [54].

Indeed, in our postmenopausal osteoporotic patients we observed higher mean levels of serum 25OHD than in age-matched normal women (Fig. 4). Our results were confirmed by others, although the difference was not significant [42].

We hypothesized that this finding was related to the low 1,25(OH)2D levels: the reduced concentration of 1,25(OH)2D was probably responsible for an impaired product-inhibition of liver 25OHase with consequent increase in 25OHD synthesis, on condition that enough substrate was available.

Our hypothesis was confirmed by the observation that long-term administration of physiological doses of 1,25(OH)2D3 to postmenopausal osteoporotic women caused the levels of 25OHD to decrease progressively toward the range of normal peers [55] (Fig. 4).

Further confirmation came from the study of the serum 25OHD response to whole-body artificial u.v. irradiation. Postmenopausal osteoporotic women showed a higher 25OHD and a lower 1,25(OH)2D response than age-matched controls. This observation was consistent with the hypothesis of an impairment of 1αOHase in these patients with consequent increase in 25OHase activity.

At present, we know that our hypothesis was correct. *In vitro* studies have demonstrated that 1,25(OH)2D is actually capable of exerting inhibitory effects on 25OHD synthesis [56] and a recent study in normal volunteers has confirmed that the oral administration of vitamin D in combination with 1,25(OH)2D3 results in lower increases in 25OHD serum levels [57].

The product-inhibition mechanism is common in endocrinology so that the finding of higher mean levels of serum 25OHD in postmenopausal osteoporotic women should not be seen as a surprising one.

Obviously, a high serum 25OHD is not a pathognomonic feature of postmenopausal osteoporosis; it only expresses an increased activity of the liver vitamin D-25-hydroxylation system in patients with relative 1,25(OH)2D deficiency. This increase in serum 25OHD has a limiting factor in the availability of the parent vitamin, so that it can be observed only in patients with a good dietary intake or endogenous synthesis of vitamin D.

Effect of long-term 1,25(OH)2D treatment in postmenopausal osteoporosis

The aforementioned data and the consequent pathogenetic hypothesis have important therapeutic implications: on that basis we have undertaken an open study of the effects of long-term treatment with synthetic 1,25(OH)2D3 in postmenopausal osteoporosis [58].

The design of the study did not include a control group to be treated with a placebo. In such a long-term study we felt it was not ethical to exclude a large number of patients from a treatment which, according to our previous experience [59], was likely to exert beneficial effects. On the other hand, it is known that postmenopausal osteoporotic women on placebo therapy show a significant annual bone loss, which is greater than that seen in premenopausal and healthy postmenopausal women [60].

One-hundred-and-fifty-four women with severe, histologically proven postmenopausal osteoporosis, aged 49–78 yr, have been so far treated with calcitriol at the oral dose of 0.5 μg twice a day.

All the patients admitted to the study had: back

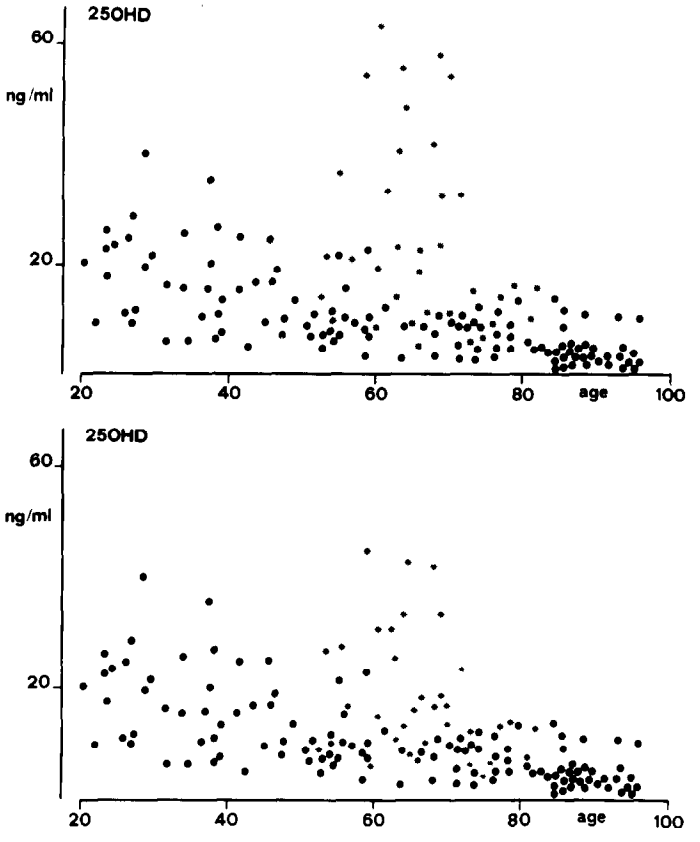

Fig. 4. Upper panel: 25OHD serum levels vs age in normal women (●) and postmenopausal osteoporotic women (*). Lower panel: effect of long-term calcitriol treatment on 25OHD serum levels in postmenopausal osteoporotic women.

pain and/or difficulty in walking; a radiographic appearance of vertebral translucency with one or more non-traumatic vertebral fractures (edge crushes with kyphosis and/or codfishing); decreased bone mineral content as determined by absorptiometry at the distal end of radius and ulna in comparison with age-matched, non-osteoporotic women; a typical histological pattern of osteoporosis as assessed by microscopic inspection of bone biopsies from the iliac crest (undecalcified bone specimens were prepared in order to exclude patients with osteoid seams); normal renal function; normal values of serum calcium, phosphate, alkaline phosphatase and 24-h urinary calcium, phosphate and hydroxyproline excretion; impaired intestinal radiocalcium transport, as assessed by the measurement of the circulating fraction of the ^{47}Ca oral dose administered.

The study began in 1980 and is still in progress, 7 patients having so far been treated for more than 5 yr, 3 for 4–5 yr, 19 for 3–4 yr, 16 for 2–3 yr, 42 for 1–2 yr, 67 for 6 months–1 yr.

The patients have been examined periodically and laboratory parameters determined every other month.

The following results have been obtained:

a dramatic and stable improvement of intestinal calcium absorption, beginning at the second month of treatment or earlier (mean basal value 0.125 ± 0.03 SD, mean value after 2 months 0.205 ± 0.05) (Fig. 5);

significant increases in 24-h urinary calcium excretion, which correlated directly with the changes in calcium absorption (mean basal value 146.5 mg/24 h ± 64.1, mean value after 2 months 313.5 mg/24 h ± 121.8, with similar mean values thereafter; $r = 0.45$, $P < 0.01$) (Fig. 5);

non-significant changes in 24-h urinary hydroxyproline excretion, so that a skeletal origin for the increased urinary calcium could be ruled out (Fig. 5);

an increase in osteocalcin serum levels, that in basal conditions averaged lower than in age-matched controls (respectively 3.8 ng/ml ± 1.4 in a group of 25 osteoporotic women and 6.8 ng/ml ± 2.0 in 25 controls);

no changes in renal function, as assessed by blood urea nitrogen, serum creatinine and creatinine clearance determinations (Fig. 5);

From a clinical point of view:

dramatic relief from pain and improvement of motility;

significant reduction in the occurrence of new non-traumatic, clinically and radiologically relevant fractures as compared with the period between the

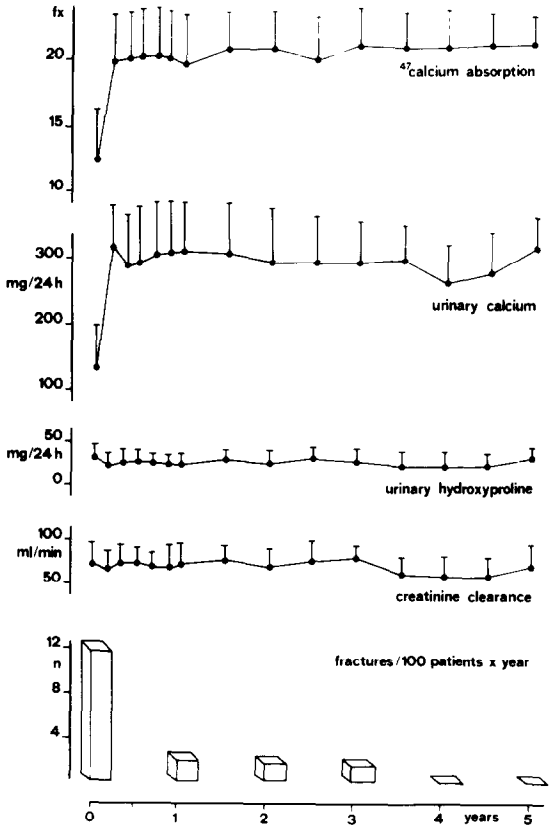

Fig. 5. Effects of long-term treatment with calcitriol in postmenopausal osteoporosis.

menopause and the initiation of therapy (12.2 fractures/100 patients × year before the treatment, 2.6 during the first year of treatment, 2.3 in the second, 2.2 in the third, no fractures during the fourth and fifth year of treatment) (Fig. 5);

small but significant increases in bone mineral content beginning after 10 months of therapy;

no untoward effects.

Long-term treatment with calcitriol has been shown not only to restore a normal calcium absorption but also to result in relief from pain, improvement of ambulancy, reduction in fracture occurrence, increase in bone mineral content, without untoward effects on renal function despite the hypercalciuria. The latter was exclusively of absorptive origin; it was not due to increased bone resorption, as demonstrated by the absence of any significant change in urinary hydroxyproline excretion [61]. Thus, 1,25(OH)2D3 administration did not result in osteoclast stimulation, at least in humans.

On the contrary, our results concerning serum osteocalcin are a good indication of a maintained sensitivity of osteoblasts to 1,25(OH)2D3 stimulation in postmenopausal osteoporosis [62]. In fact, it is known that osteocalcin synthesis is stimulated by 1,25(OH)2D3 and that serum osteocalcin is a specific marker of bone formation [63]; therefore it is possible that the increase in osteocalcin levels observed during calcitriol treatment was a consequence of osteoblast stimulation.

The role of 1,25(OH)2D3 in the treatment of postmenopausal osteoporosis has recently been confirmed [64, 65].

On the basis of the above data the conclusion can be drawn that the administration of 1 μg/day of 1,25(OH)2D3 is an effective and well-tolerated substitution treatment of postmenopausal osteoporosis.

A decrease in the renal 25OHD-1αOHase activity plays a key role in postmenopausal osteoporosis.

CONCLUSION

To sum up, it appears that substantial analogies exist among different pathological conditions sharing, as a common feature, a direct or indirect derangement of 1,25(OH)2D production.

In chronic liver disease 25OHD synthesis is not severely impaired, thanks to the great functional reserve of the liver.

In chronic renal failure 1,25(OH)2D synthesis is impaired, due to the decrease in the functioning renal mass. Kidney transplantation represents the etiologic therapy of this condition, whereas a substitution treatment with synthetic 1,25(OH)2D results in improvement of bone lesions.

In hypoparathyroidism 1,25(OH)2D synthesis is impaired due to reduced PTH stimulation: in these patients the subcutaneous administration of synthetic human PTH restores normal 1,25(OH)2D levels, whereas 1,25(OH)2D itself given orally exerts beneficial effects.

In postmenopausal osteoporosis 1,25(OH)2D synthesis is impaired due to reduced estrogen stimulation. Estrogens could be considered as an etiologic therapy of this condition despite some possible untoward effects, whereas the administration of synthetic 1,25(OH)2D3 represents an effective and safe substitution treatment.

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