24,25 DIHYDROXYCALCIFEROL: ASSAY IN NON-ANEPHRIC PATIENTS ON CHRONIC HAEMODIALYSIS AND ASSESSMENT OF ITS POSSIBLE PATHOPHYSIOLOGICAL ROLE IN RENAL OSTEODYSTROPHY

G Lambrey, T M Nguyen*, A Fournier, J L Sebert, J Cassouf, J F de Fremont, P Marie†, P Meunier‡, C Caillens¶, J Gueris†, M Garabedian*, S Balsan*

Service de Néphrologie-Hémodialyse du CHU d'Amiens, *Laboratoire des Tissus Calcifiés, Hôpital des Enfants Malades, †INSERM Unité André Lichwitz, Hôpital Lariboisière, Paris, ‡Laboratoire d'Histomorphométrie, Université Alexis Carrel, Lyon, ¶Laboratoire d'Exploration Fonctionnelle, Hôpital Tenon, Paris, France

Summary

Recent experimental data suggest that 24,25(OH)\textsubscript{2} vitamin D\textsubscript{3} (24,25(OH)\textsubscript{2}D\textsubscript{3}) may have a specific effect on bone mineralisation and resorption. To assess the possible pathophysiological role of this metabolite in renal osteodystrophy, we measured simultaneously plasma concentrations (P) of 25(OH)D\textsubscript{3}, 24,25(OH)\textsubscript{2}D\textsubscript{3}, parathyroid hormone (PTH), calcitonin, alkaline phosphatase, calcium and phosphate in 12 non-anephric patients on chronic haemodialysis before and during 6 months' administration of a combination of 25(OH)D\textsubscript{3} and 1a(OH)D\textsubscript{3}. Furthermore, two bone biopsies were performed before and after vitamin D therapy for histomorphometry.

Results and conclusions

1 Plasma 24,25(OH)\textsubscript{2}D\textsubscript{3} may be not only low but normal or even high before treatment

2 There is no correlation at any time between plasma 24,25(OH)\textsubscript{2}D\textsubscript{3} and 25(OH)D\textsubscript{3} suggesting a specific regulation of the 24 hydroxylase activity

3 A significant negative correlation was found between the peak of plasma 24,25(OH)\textsubscript{2}D\textsubscript{3}, the changes in active resorption surface, and in osteoclast numbers, in agreement with a possible inhibitory effect of 24,25(OH)\textsubscript{2}D\textsubscript{3} on bone resorption

4 The peak of plasma 24,25(OH)\textsubscript{2}D\textsubscript{3} was positively correlated with the changes in the mineralisation front (MF) in agreement with a possible beneficial effect of 24,25(OH)\textsubscript{2}D\textsubscript{3} on bone mineralisation

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The peak of plasma 24,25(OH)$_2$D$_3$ was negatively correlated with the initial state of the MF, suggesting a possible regulation of the 25(OH)D$_3$ 24 hydroxylase activity dependent on the state of bone mineralisation.

Introduction

The physiological role of 24,25 dihydroxycalciferol (24,25(OH)$_2$D$_3$) is still controversial. Recent experimental and clinical data suggest however that 24,25(OH)$_2$D$_3$ must no longer be considered as a degradation product since it may have specific effects such as: securing normal hatchability [1], stimulating sulphate incorporation into proteoglycans by chondrocytes in culture [2,3], inhibiting the PTH induced increase of bone resorption in vitro [4], inhibiting directly PTH secretion in normal and uraemic animals [5–7], stimulating calcium absorption without increase in urinary calcium in patients with and without kidneys [8], decreasing plasma calcium and increasing serum alkaline phosphatase in uraemic man [9]. Furthermore, it has an additive effect to that of 1,25(OH)$_2$D$_3$ in preventing parathyroid hyperplasia in D deficient chickens [10] and in promoting bone mineralisation in D deficient osteomalacic men [11]. Thus, 24,25 (OH)$_2$D$_3$ may have specific properties in bone formation, mineralisation and resorption and therefore be implicated in the pathogenesis of renal osteodystrophy. The only published data relating plasma concentrations (P) of 24,25(OH)$_2$D$_3$ and bone histology in uraemic patients are those of Kanis suggesting that osteomalacic patients have lower plasma 24,25(OH)$_2$D$_3$ than the patients with no osteomalacia and that the low levels of plasma 24,25(OH)$_2$D$_3$ found in uraemic patients may be increased by 1α hydroxylated D derivatives, and by 25(OH)D$_3$ [12]. Therefore, it seems to us interesting to report our data on plasma 24,25(OH)$_2$D$_3$ in non-anephric patients on chronic haemodialysis before and during 6 months’ treatment with a combination of 1α and 25(OH)D$_3$ and to correlate them with the histomorphometric data derived from two bone biopsies taken before and after vitamin D treatment.

Material and methods

Patients and treatment protocol

Twelve non-anephric patients (7 men, 5 women; age range 22–58 years) on chronic haemodialysis for at least 18 months were selected for this study on the basis of their willingness to cooperate by having two bone biopsies at 6 months interval. Throughout the study, they were dialysed according to the same schedule (3 times 4 hours a week) with a dialysate calcium of 7.0mg/dl. Before the first bone biopsy, performed in June 1976, they were treated with no vitamin D supplement but had an oral calcium carbonate supplement of 3–12g/day and Al(OH)$_3$ in order to keep their plasma phosphate below 6.0mg/dl. They then received a
mixture of 25(OH)D$_3$ and 1α(OH)D$_3$ at the initial daily dose of 50 and 1μg respectively, while the CaCO$_3$ was decreased to 3g. Later on, vitamin D metabolites were transiently discontinued and resumed at half doses when plasma calcium (PCa) increased above 11.0mg/dl and plasma phosphate (PPO$_4$) increased above 7.0mg/dl, in spite of the increase in Al(OH)$_3$.

Design of the study

An iliac bone biopsy was performed in June 1976 in all patients and repeated in December.

Besides the weekly determination of predialytic PCa and PPO$_4$, simultaneous determinations of plasma concentrations of 25(OH)D$_3$, 24,25(OH)$_2$D$_3$, PTH, calcitonin and serum alkaline phosphatase were performed at the time of, and between the two bone biopsies.

Analytical methods

Bone biopsies were performed after tetracycline labelling and assessed histomorphometrically as previously described [13]. Furthermore, the osteoid thickness index was calculated according to Meunier [14].

The vitamin D metabolites were measured by radiocompetition according to the method of Preece [15] after the following procedures: (1) lipidic extraction of the plasma by a solution of chloroform-methanol; (2) a first chromatography of the chloroformic phase on a Sephadex LH 20 column and (3) a second chromatography with a high pressure liquid chromatograph to purify 24,25(OH)$_2$D$_3$ and with a silicic acid column to purify 25(OH)D$_3$. The normal level of plasma 25(OH)D$_3$ (± SD) is 20 ± 7ng/ml depending on sun exposure (maxima at the end of the Summer, minima at the end of Winter). The normal level of plasma 24,25(OH)$_2$D$_3$ (± SD) is 1.4 ± 0.8ng/ml. The detection level for these two metabolites is 0.2ng/ml [16].

PTH was measured with a C terminal specific antibody according to the method of Gueris [17]. The normal range is 4–8ng/ml of protein of parathyroid adenoma culture medium.

Calcitonin was measured according to the method of Heynen [18]. The normal values are below 200pg/ml.

Statistical methods

Comparison of groups was made with the Wilcoxon test. Linear correlation calculations were made with a programmed computer.

Results

Analysis of biochemical parameters according to changes in bone resorption

Although the osteoid volume was increased in most of our patients, their osteoid surface was also increased so that their osteoid thickness index was either normal
Figure 1. Individual data of P 24,25(OH)₂D₃ and P 25(OH)D₃ according to the bone resorption and the treatment. The hatched area along the ordinates indicates the normal range. In each group, each individual is represented by the same special symbol for the three parameters. Each point represents a single value.
or low but never increased, indicating the absence of true osteomalacia, according
to Meunier [14], in spite of the fact that the mineralisation front was decreased
in all patients before vitamin D treatment. Serum alkaline phosphatase was nor-
mal in all patients and remained normal during treatment. The main histological
abnormality was osteitis fibrosa as assessed by increased active resorption surface
(ARS) i.e. the surface with Howship lacunae occupied by an osteoclast. Initial
and subsequent changes in ARS allowed to distinguish three groups of patients:
Group 1 (3 patients) with normal initial ARS which stayed normal; Group 2 (4
patients) with increased initial ARS which did not significantly decrease or even
increased; Group 3 (5 patients) with also increased initial ARS which however
decreased to normal values.

The upper panel of Figure 1 shows the individual variations of ARS in each of
these groups. The middle panel shows the P 24,25(OH)₂D₃ variations: in Group 1
with normal bone resorption, P 24,25(OH)₂D₃ is initially normal and stays nor-
mal; in Group 2, with non healing increased resorption, P 24,25(OH)₂D₃ is
initially subnormal in all cases, stays subnormal in two cases, increases slightly in
one case and increases above normal in only one case; in sharp contrast to Group
2, in Group 3, with healing increased bone resorption, P 24,25(OH)₂D₃ is
markedly increased above normal either initially in three cases or only after vita-
mim D treatment in two cases. At the time of the second biopsy, P 24,25(OH)₂D₃
has however returned to normal levels.

The lower panel of Figure 1 shows the variations of P 25(OH)D₃. Initial P
25(OH)D₃ is normal except in three patients of Group 3. After vitamin D admin-
istration, there is an increase of P 25(OH)D₃ in August–September and then a
decrease in December to initial values, whereas the oral dose of 25(OH)D₃ has
also been decreased.

To investigate the reason for the different evolution of bone resorption between
Groups 2 and 3, we have compared in these two groups the parameters which
may influence bone resorption. We have found no significant difference between
the two groups as regards the peak of P 25(OH)D₃ i.e. the highest value of P
25(OH)D₃, the cumulative doses of 25(OH)D₃ and 1α(OH)D₃, the changes in
PPO₄, in PPTH and P calcitonin. However, there was a significant difference in
the peak values of 24,25(OH)₂D₃ (i.e. the highest value of P 24,25(OH)₂D₃
observed either before or during vitamin D treatment), which was significantly
higher in Group 3 with healing bone resorption than in Group 2 with non healing
bone resorption (p = 0.05).

Correlation studies

Correlations between P 24,25(OH)₂D₃ and other simultaneously measured param-
eters No correlation was found at any time between P 24,25(OH)₂D₃ and the
other biochemical or histomorphometric parameters when these latter were
measured at the same time. In particular, there was no correlation between P
24,25(OH)₂D₃ and P 25(OH)D₃, nor between P 24,25(OH)₂D₃ and PPTH
measured simultaneously or between their changes, even when PCA and PPO₄
were considered constant by the method of partial correlations.
Figure 2. Correlation of the peak of P 24,25(OH)₂D₃ with the absolute change of active resorption surface (on the left) and with the absolute change of the number of osteoclasts (on the right) during vitamin D treatment.

Figure 3. Correlation of the peak of 24,25(OH)₂D₃ with the initial mineralisation front (on the left) and with the percent change of the mineralisation front (on the right), during vitamin D treatment.
Correlations of the peak of P 24,25(OH)$_2$D$_3$ with biochemical and histomorphometric parameters The peak of P 24,25(OH)$_2$D$_3$, i.e. the highest value of P 24,25(OH)$_2$D$_3$ measured either before or during treatment was found to be correlated as follows:

It is negatively correlated with the changes in ARS ($r = -0.65$, $n=12$, $p<0.05$) and with the changes in the osteoclast numbers ($r = -0.59$, $n=12$, $p<0.05$) (Figure 2).

It is negatively correlated with the initial mineralisation front (MF) ($r = -0.065$, $n=12$, $p<0.05$) (Figure 3).

It is positively correlated with the changes in the MF ($r = -0.63$, $n=12$, $p<0.05$) (Figure 3).

No other correlation was found. In particular, no correlation was found with simultaneously measured PTH nor with the changes in PTH during treatment.

Correlations of bone resorption with PTH, calcitonin and phosphate Active resorption surface and osteoclast numbers were positively correlated with PTH values at the time of the two biopsies. However, their changes between the two biopsies were not correlated to the changes of PTH, calcitonin and phosphate.

Discussion

24,25(OH)$_2$D$_3$ and bone resorption

Our data do not support the hypothesis that 24,25(OH)$_2$D$_3$ decreases PTH secretion as has been reported in animals [5–7] but not in uraemic men [9]. They favour however the hypothesis of a direct inhibiting effect on bone resorption as suggested by the in vitro data of Lieberherr [4]. As a matter of fact, there is a striking association between normal P 24,25(OH)$_2$D$_3$ and normal resorption (Group 1), low P 24,25(OH)$_2$D$_3$ which does not significantly increase (except in one case) and non healing bone resorption (Group 2), and finally between high P 24,25(OH)$_2$D$_3$ decreasing to normal levels and subsequent healing bone resorption (Group 3).

The fact that in this latter group, high P 24,25(OH)$_2$D$_3$ was seen before vitamin D while resorption was high, may be explained by the fact that this high P 24,25(OH)$_2$D$_3$ has occurred too recently to improve bone resorption.

Since normalisation of bone resorption is preceded by an increase of P 24,25 (OH)$_2$D$_3$ above the normal value and is associated with normal simultaneously measured P 24,25(OH)$_2$D$_3$, one cannot expect to find any correlations between the changes in ARS or osteoclast numbers and the changes of P 24,25(OH)$_2$D$_3$ or between simultaneously measured P 24,25(OH)$_2$D$_3$ and bone resorption parameters. The prior occurrence of a transient increase of P 24,25(OH)$_2$D$_3$ above the normal value seems the determining factor in improving bone resorption. Therefore, it appears warranted to correlate the peak of P 24,25(OH)$_2$D$_3$, i.e. the highest value observed before the second biopsy and the changes in bone resorption parameters. The fact that a negative correlation was found between this
peak and the changes in bone resorption parameters is a further support to the hypothesis that 24,25(OH)_2 D_3 contributes to decreased bone resorption.

This effect appears independent of PTH, calcitonin and phosphate since no correlation was found between the changes of the resorption parameters and the changes in PTH, calcitonin and phosphate which may interfere with bone resorption [19].

24,25(OH)_2 D_3 and bone mineralisation

The fact that in uraemic men 24,25(OH)_2 D_3 increases calcium retention [8], decreases plasma calcium and increases serum alkaline phosphatase [9] suggests that it promotes bone mineralisation. The positive correlation we have found between the peak of P 24,25(OH)_2 D_3 and the changes in the mineralisation front also supports this hypothesis.

Synthesis of 24,25(OH)_2 D_3 in uraemic patients

In non anephric uraemic patients, P 24,25(OH)_2 D_3 may be not only low but normal or even high. These data differ from those of Taylor [20] who reported low values in four non anephric uraemic patients. Furthermore, in contrast to the positive correlation found between P 24,25(OH)_2 D_3 and P 25(OH)D_3 in normal individuals with normal bone [20,21], no such correlation was found in agreement with the results of Kanis [12] in uraemic patients with osteodystrophy and Nguyen [16] in children with D deficiency or pseudodeficiency rickets. Thus, P 24,25(OH)_2 D_3 is not dependent only on the quantity of substrate, i.e. of 25(OH)D_3. Since high P 24,25(OH)_2 D_3 may be observed with low P 25(OH)D_3 deficiency of 24 hydroxylase activity due to the reduction of the nephron mass cannot be incriminated in all cases. Therefore, a specific regulation of 24 hydroxy-

No correlation was observed between the peak of P 24,25(OH)_2 D_3 and simultaneously measured plasma concentrations of Ca, PO_4 and PTH, but a negative one was observed between peak of P 24,25(OH)_2 D_3 and the initial state of the mineralisation front. This suggests that the 24 hydroxylase activity may depend on the mineralisation state of the bone. If 24 hydroxylase activity is only located in the kidney, an undetermined mechanism controlling this is implied. If 24 hydroxylase activity is also located in bone, as suggested by Garabedian [3], this regulation would be strictly local. The fact that the initial mineralisation front is not correlated with the initial P 24,25(OH)_2 D_3 but with the peak of P 24,25 (OH)_2 D_3 might mean that in most patients the regulation of the 24 hydroxylase activity would be impossible in the absence of 25(OH)D_3 or 1,25(OH)_2 D_3 and that the administration of these metabolites might allow the expression of this regulation.

Administration of 25(OH)D_3 and/or 1α hydroxylated metabolites does not always however increase P 24,25(OH)_2 D_3 as reported by Kanis [12], since it occurred in only 3 out of our 6 patients with initially low P 24,25(OH)_2 D_3. The unpredictability of P 24,25(OH)_2 D_3 before and under treatment will justify the
determination of P 24,25(OH)$_2$D$_3$ whenever a therapeutic administration of 24,25(OH)$_2$D$_3$ is considered, since its use would a priori be appropriate only in those patients in whom other vitamin D metabolites cannot induce an increase of P 24,25(OH)$_2$D$_3$.

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