Original Article

Effects of calcitriol on parathyroid function and on bone remodelling in secondary hyperparathyroidism

André Falcão Pedrosa Costa, Luciene Machado dos Reis, Melani Custódio Ribeiro, Rosa Maria Affonso Moysés and Vanda Jorgetti

Nephrology Department, School of Medicine, University of São Paulo, São Paulo, Brazil

Abstract

Background. Secondary hyperparathyroidism (2HPT) develops in chronic renal failure due to disturbances of calcium, phosphorus and vitamin D metabolism. It is characterized by high turnover bone disease and an altered calcium–parathyroid hormone (PTH) relationship. Calcitriol has been widely used for the treatment of 2HPT. However, it remains controversial whether calcitriol is capable of inducing changes of the calcium–PTH curve. The aim of the present study was to examine this issue and to determine the effect of calcitriol on bone remodelling in patients with severe 2HPT.

Methods. We evaluated 16 chronic haemodialysis patients with severe 2HPT (PTH 899±342 pg/ml). Each patient underwent a dynamic parathyroid function test (by infusion of calcium gluconate and sodium citrate) and a bone biopsy before and after a 6 month period of i.v. calcitriol therapy (CTx).

Results. After treatment, eight patients were identified as calcitriol responders and the other eight as non-responders, based on plasma PTH level (<300 pg/ml for responders and >300 pg/ml for non-responders). The first group had higher plasma 25OHD3 levels (39±8 vs 24±7 ng/ml, P<0.005). As to the calcium–PTH curve, we found differences in slope (−12.7±5.2 vs −21.7±11.4, P=0.05), basal:maximum PTH ratio (48.8±14.9 vs 71.05±20.1%, P=0.01) and time to achieve hypocalcaemia (79.0±13.5 vs 94.3±13.7 min, P<0.001). Initial histomorphometric parameters did not allow identification of the different groups. After the 6-month CTx, alterations in the calcium–PTH curve were clearly seen in responders [a drop in maximum PTH (from 1651±616 to 938±744 pg/ml, P<0.05) and minimum PTH (from 163±75.4 to 102.2±56.7 pg/ml, P<0.005)], associated with an increase in minimum/basal PTH ratio (from 23.3±11.6 to 34.5±20.4%, P<0.05) and maximum calcium (from 0.99±0.07 to 1.1±0.09 mmol/l, P<0.05). Set point and slope were not altered after calcitriol treatment, in responders (set point = 1.17±0.08 vs 1.15±0.1 mmol/l, ns; slope = −12.7±5.2 vs −12.9±9.3, ns) or non-responders (set point = 1.21±0.05 vs 1.21±0.2 mmol/l, ns; slope = −21.7±11.4 vs −17.3±8.4, ns). Bone formation parameters were reduced in all patients [osteoid surface (OS/BS)=from 57.1±21.6 to 41.6±26%, P<0.05 for responders, and from 76.7±12 to 47.1±15%, P<0.001 in non-responders], but non-responders had increased bone resorption [eroded surface (ES/BS)=7.1±3.4 vs 16.6±4.9, P<0.05].

Conclusion. Calcitriol had non-uniform effects on parathyroid function and bone remodelling in uraemic patients. Non-responders exhibited a decoupled remodelling process that could further influence mineral balance or possibly also bone structure. To avoid such undesirable effects, early identification of non-responder patients is crucial when using calcitriol for the treatment of 2HPT.

Keywords: bone biopsy; calcitriol; calcium; histomorphometry; PTH; secondary hyperparathyroidism

Introduction

Secondary hyperparathyroidism (2HPT) is the most frequent pattern of renal osteodystrophy, and is caused by several interrelated factors, such as hypocalcaemia, hyperphosphataemia and calcitriol deficiency [1]. Many disturbances, such as changes in parathyroid hormone (PTH) and vitamin D metabolism, a skeletal resistance to calcemic PTH action, altered calcium–PTH dynamic relationship and decreased levels of vitamin D receptor and calcium sensor receptor have also been implicated in the pathogenesis of 2HPT [1,2].

Excessive PTH levels alter the bone remodelling process by increasing bone formation and resorption,
in association with various degrees of medullar fibrosis.

Calcitriol (1,25(OH)₂D₃) has been used in the treatment of 2HPT because of the well-established fact that it can suppress PTH secretion and influence bone remodelling [3]. However, what the effects of calcitriol on bone remodelling are, and whether modification in the calcium–PTH curve occurs, are both contentious issues [4,5].

The goal of the present study was to analyse, using a calcium–PTH curve and bone biopsy, respectively, the effects of calcitriol treatment on parathyroid function and on bone remodelling in end-stage renal failure patients with severe 2HPT.

Subjects and methods

Subjects

Sixteen chronic haemodialysis patients (seven male and nine female) from the renal osteodystrophy unit at Hospital das Clínicas, University of São Paulo Medical School, aged 36 ± 11 years, with symptomatic osteodystrophy (all had bone pain, articular pain and muscular weakness, and one had a hip fracture) were included in this study after informed consent. Haemodialysis duration was 8 ± 3.6 years. All patients were dialysed three times per week using low-flux polysulfone membrane and a dialysate containing 3.5 meq/l of calcium. After approval by the institution’s ethics committee, patients with a serum PTH >400 pg/ml were included in the study protocol. Exclusion criteria were: age <15 years, diabetes mellitus, previous transplantation, clinical or laboratory evidence of liver disease, alcohol abuse, oestrogen replacement therapy, a serum calcium or phosphorus >11.0 or 6.0 mg/dl, respectively, or use of calcitriol therapy (CTx) during the previous 6 months.

Biochemical analysis

All patients underwent the following analyses before and after i.v. CTx: total calcium [normal value (NV) 8.5–10.5 mg/dl], phosphorus (NV 2.3–4.6 mg/dl), total alkaline phosphatase (ALP) (NV 60–170 IU/l), serum albumin (NV 3.5–4.5 g/dl), intact PTH using immunoradiometric assay (NV 8–76 pg/ml, ELSA-PTH, Gif-Sur-Yvette, France), serum aluminium (NV <30 mg/l, by means of atomic absorption spectrometry in a graphite oven), bone-specific alkaline phosphatase (bAP) (NV women aged 25–44 years, 11.6–29.6 IU/l; women >45 years, 14.2–92.7 IU/l; men >25 years, 15.40.3 IU/l, using ELISA, Metra BioSystems, CA, USA), 25(OH)D₃ and 1,25(OH)₂D₃, with an immunoradiometric assay (DiaSorin, MN, USA; NV 8.9–46.7 ng/ml and 15.9–55.6 ng/ml, respectively) and deoxyxypyrindinoline (DPD), using ELISA, Metra BioSystems, CA, USA; NV 3.43 mmol/l for women and 3.25 mmol/l for men. Blood ionized calcium was measured using the Ciba-Corning Auto-Analyzer through a specific electrode.

Assessment of parathyroid function

Parathyroid function (calcium–PTH curve) was analysed before and after calcitriol treatment according to the protocol proposed by Ramirez et al. [6]. Hypercalcaemia was induced by i.v. 10% calcium gluconate in a 5% glucose solution. The initial infusion rate was 2 mg/kg/min, which was subsequently increased by 1 mg/kg every 20 min. Hypocalcaemia was induced using 0.7 g/l of a sodium citrate solution (Fórmula A, USP, Brazil). The initial infusion rate was 28 mg/kg/min, it was then increased every 10 min by 5 mg/kg in the first hour and by 10 mg/kg in the second hour. Blood samples were taken during the two phases at -15, 0 and every 10 min until completion of the test. Blood ionized calcium was promptly analysed with the Ciba-Corning Auto-Analyzer using a specific electrode. Serum intact PTH samples were frozen to allow for later measurement. A maximum 2-h period was always observed during induced hyper- and hypocalcaemia tests. In both phases, patients underwent the tests after an 8-h fast and a 2-h resting period, with vital signs being continuously monitored. Tests were terminated after 2 h had elapsed or if any of the following were detected: a 0.2 mmol/l variation in basal ionized calcium, a maximum ionized calcium concentration of 1.4 mmol/l and minimum of 0.9 mmol/l, or any clinical abnormalities. Plasma calcium was monitored subsequently and the patient was discharged when these levels had returned to basal values.

The classical model described by Brown [7] was used for the determination of set point, slope and maximum and minimum PTH. Basal maximum PTH, minimum/basal PTH and minimum maximum PTH ratios were calculated. Maximum and minimum calcium were regarded as analogous to maximum and minimum PTH, respectively. The time, in minutes, required to obtain hyper- and hypocalcaemia matched the time for each test.

Bone biopsy

Bone biopsies were carried out in either the right or left iliac crest using a 7 mm inner diameter electrical trephine (Gauthier Medical, Rochester, MN, USA). Whenever a second biopsy was performed, it was always on the opposite side from the first. All patients were pre-labelled with oral tetracycline (20 mg/kg/day for 3 days) administered over two separate periods, 10 days apart.

Undecalcified bone fragments were submitted to the usual processing and histological studies [8]. Sections were stained with toluidine blue stain. To detect aluminium and iron bone tissue deposits, acid solochrome azurine [9] and Perls staining were employed, respectively.

Bone histomorphometry was conducted in a double-blind protocol, using the semi-automatic method contained in the software Osteomasure (Osteometrics Inc., Atlanta, GA, USA). The static and dynamic parameters were analysed following the American Society of Bone and Mineral Research standardization [10]. Patients were classified as having osteitis fibrosa (OF) or mixed bone disease (MBD), as described previously [11].

Calcitriol treatment

An i.v. calcitriol dose (1 μg after each haemodialysis session = 3 mg/week) was given to all patients immediately after the first bone biopsy. All individuals then had a serum monthly follow-up of laboratory tests. If serum calcium, phosphorus and calcium × phosphorus levels were within the acceptable range, the dose was increased incrementally up to a maximum of 12 mg/week. This schedule was maintained for 6 months and was interrupted in the case of a serum PTH reduction to a level of 300 pg/ml, a calcium × phosphorus
product > 60, a phosphorus level > 6.0 mg/dl, or a serum calcium level > 11 mg/dl.

Those patients who had a decrease of serum iPTH below 300 pg/ml by the end of the study were deemed responders (n = 8). The others were considered as non-responders (n = 8).

Statistical analysis

Results analysed were expressed as mean ± SD. Curve parameters were obtained using ‘Prism’ software (Serial number GPA-22319-201).

Data before and after calcitriol treatment in the same patient were analysed using Student’s paired t-test. Unpaired Student’s t-test was utilized to compare all calcium–PTH curves, histomorphometric results and clinical data between responders and non-responders. Pearson’s correlation was employed to analyse a possible overall correlation. P < 0.05 was considered to be significant.

Results

Clinical and biochemical aspects prior to calcitriol treatment

All patients had high serum PTH levels (899 ± 342 pg/ml). Overall, basal serum total calcium concentration was normal (8.7 ± 0.9 mg/dl) and the mean values for basal blood ionized calcium and serum phosphorus were 1.18 ± 0.06 mmol/l and 4.8 ± 1.0 mg/dl, respectively. Serum ALP and bAP were markedly increased (685 ± 765 and 339 ± 568 IU/l, respectively). Serum aluminium was 25.6 ± 11.7 μg/l, showing no correlation to any of the data studied.

None of the patients had evidence of undernutrition. The serum albumin level and the body mass index were both normal (4.0 ± 0.4 g/dl and 21.3 ± 3%, respectively), corroborating this finding.

None of the patients exhibited vitamin D deficiency with regard to basal serum 25OHD3 levels (28.6 ± 11.4 ng/ml), although responders exhibited higher levels than non-responders (39.7 ± 9.3 vs 23.9 ± 7.9 ng/ml, P < 0.05). Serum calcitriol levels were 26.5 ± 9.5 pg/ml. Serum DPD was measured in 10 patients and was 230 ± 222 and 244 ± 136 nmol/l for responders and non-responders, respectively (ns).

It was not possible to distinguish responders from non-responders using basal PTH values (831 ± 269, 473–1320 pg/ml for responders, and 961 ± 409, 450–1647 pg/ml for non-responders). Table 1 summarizes basal clinical and biochemical data in responders and non-responders, respectively.

Parathyroid function prior to calcitriol treatment

Overall, mean set points were normal (1.18 ± 0.06 mmol/l). The set point was correlated with serum total calcium concentration (r = 0.57, P = 0.01). The slope was –15.3 ± 7.4, maximum PTH value was 1516 ± 505 pg/ml, minimum PTH was 198 ± 82.6 pg/ml; maximum and minimum calcium levels were 0.97 ± 0.06 and 1.38 ± 0.04 mmol/l, respectively.

Basal/magnitude PTH ratio was 60 ± 19.6%, minimum/basal PTH 23 ± 7.2%, and minimum/magnitude PTH 14.6 ± 6.1%. Time to attain hypocalcaemia was 90 ± 10 min, compared to 80 ± 10 min for hypercalcaemia.

Comparing the two groups, we found that responders presented lower slope values (<12.7 ± 5.2 vs –21.7 ± 11.4, P < 0.05) a lower basal/magnitude PTH ratio (48.8 ± 14.9% vs 71.05 ± 20.1, P < 0.01) and less time to reach hypocalcaemia (79.0 ± 13.5 vs 94.3 ± 13.7 min, P < 0.001). Set point values were not altered before CTx, although they were slightly higher in non-responders. The data are shown in Table 2.

Table 1. Basal clinical and biochemical data in responders and non-responders

<table>
<thead>
<tr>
<th></th>
<th>Before CTx</th>
<th>After CTx</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Responders (n = 8)</td>
<td>Non-responders (n = 8)</td>
</tr>
<tr>
<td>Age (year)</td>
<td>36 ± 2.9</td>
<td>35 ± 14</td>
</tr>
<tr>
<td>Dialysis time (year)</td>
<td>8.5 ± 1.4</td>
<td>7.6 ± 3.4</td>
</tr>
<tr>
<td>Basal PTH (pg/ml)</td>
<td>831 ± 269a</td>
<td>961 ± 409</td>
</tr>
<tr>
<td>Total calcium (mg/dl)</td>
<td>8.83 ± 0.21</td>
<td>8.7 ± 1.2</td>
</tr>
<tr>
<td>Basal ionized calcium (mmol/l)</td>
<td>1.21 ± 0.02b</td>
<td>1.16 ± 0.07</td>
</tr>
<tr>
<td>Basal phosphorus (mmol/l)</td>
<td>4.67 ± 0.36</td>
<td>4.8 ± 0.9c</td>
</tr>
<tr>
<td>Total ALP (IU/l)</td>
<td>428 ± 201b</td>
<td>850 ± 1035c</td>
</tr>
<tr>
<td>bAP (IU/l)</td>
<td>223 ± 273b</td>
<td>397 ± 677</td>
</tr>
<tr>
<td>25OHD3 (ng/ml)</td>
<td>39.1 ± 8.2</td>
<td>23.8 ± 7.9</td>
</tr>
<tr>
<td>1,25(OH)2D3 (pg/ml)</td>
<td>33.6 ± 9.2</td>
<td>26.7 ± 7.8</td>
</tr>
<tr>
<td>DPD (nmol/l)</td>
<td>230 ± 222</td>
<td>244 ± 136</td>
</tr>
<tr>
<td>Aluminium (μl)</td>
<td>27.2 ± 15.6</td>
<td>25.2 ± 9.4</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.07 ± 0.1</td>
<td>4.15 ± 0.06</td>
</tr>
</tbody>
</table>

Data are mean ± SD.

P values match t-tests between responders and non-responders. Letters mean t-tests before and after CTx.

P < 0.001; *P < 0.05.
Table 2. Parathyroid function data before and after calcitrol treatment in responders and non-responders

<table>
<thead>
<tr>
<th></th>
<th>Before CTx</th>
<th>After CTx</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Responders (n=8)</td>
<td>Non-responders (n=8)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Set point (mmol/l)</td>
<td>1.17 ± 0.08</td>
<td>1.21 ± 0.05</td>
</tr>
<tr>
<td>Slope</td>
<td>-12.7 ± 5.2</td>
<td>-21.7 ± 11.4</td>
</tr>
<tr>
<td>Maximum PTH (pg/ml)</td>
<td>1651 ± 616a</td>
<td>1350 ± 327</td>
</tr>
<tr>
<td>Minimum PTH (pg/ml)</td>
<td>163 ± 75.4b</td>
<td>277 ± 142</td>
</tr>
<tr>
<td>Maximum calcium (mmol/l)</td>
<td>0.99 ± 0.07a</td>
<td>1.0 ± 0.08</td>
</tr>
<tr>
<td>Minimum calcium (mmol/l)</td>
<td>1.39 ± 0.05</td>
<td>1.37 ± 0.06</td>
</tr>
<tr>
<td>Basal/maximum PTH (%)</td>
<td>48.8 ± 14.9</td>
<td>71.05 ± 20.1</td>
</tr>
<tr>
<td>Minimum/basal PTH (%)</td>
<td>23.3 ± 11.6a</td>
<td>30.2 ± 17a</td>
</tr>
<tr>
<td>Time to achieve hypocalcaemia (min)</td>
<td>79 ± 13.5</td>
<td>94.3 ± 13.7</td>
</tr>
<tr>
<td>Time to achieve hypercalcaemia (min)</td>
<td>88.6 ± 15.7a</td>
<td>90 ± 28.7</td>
</tr>
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</table>

Data are mean ± SD.

* a,b,c P <0.05; ** P <0.001; ** P = 0.06.

**Bone biopsy findings before CTx**

Eight patients had OF whereas the other eight had MBD. We observed increased bone resorption surface (ES/BS) (9.9 ± 5.1%), and peritrabecular medullar fibrosis (FB.V) (10 ± 9.7%). In OF patients, the bone formation rate (BFR/BS) was high (0.23 ± 0.09 mm³/mm²/day).

Positive aluminium staining (Al.S/BS) was detected in three patients (100% of the trabecular surface in one and 50% in the two others). Iron staining was not significant: it was detected in three patients, but did not show any correlation with the other parameters analysed and was not altered after CTx. We found no significant differences between responders and non-responders in any of the histomorphometric parameters studied (Table 3).

**Clinical and biochemical aspects after CTx**

All the patients experienced a significant improvement in their symptoms, even those who had later been identified as non-responders.

Although there was a slight variance between the two groups, the difference in total calcitriol dose used throughout the treatment was not statistically significant (114 ± 39 vs 85 ± 45 mg for responders and non-responders, respectively).

Three patients (one responder and two non-responders) with aluminium intoxication received a weekly deferoxamine administration for 6 months following diagnosis.

Blood ionized calcium increased considerably in both groups, whereas serum phosphorus rose significantly only in non-responders (from 4.8 ± 0.9 to 9.8 ± 2.2).

**Table 3. CTx effects on histomorphometric parameters in responders and non-responders**

<table>
<thead>
<tr>
<th>Histomorphometric parameters</th>
<th>Before CTx</th>
<th>After CTx</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Responders (n=8)</td>
<td>Non-responders (n=8)</td>
</tr>
<tr>
<td>BV/TV (%)</td>
<td>35.8 ± 9.9</td>
<td>26.5 ± 3.7</td>
</tr>
<tr>
<td>OV/BV (%)</td>
<td>23.9 ± 14.4a</td>
<td>34.5 ± 19a</td>
</tr>
<tr>
<td>O.Th (µm)</td>
<td>21.7 ± 3.4</td>
<td>21.2 ± 3.4</td>
</tr>
<tr>
<td>OS/BS (%)</td>
<td>57.1 ± 21.6</td>
<td>76.7 ± 12a</td>
</tr>
<tr>
<td>Ob.S/BS (%)</td>
<td>15.2 ± 9.5a</td>
<td>31.3 ± 20</td>
</tr>
<tr>
<td>ES/BS (%)</td>
<td>10.2 ± 6.2</td>
<td>7.5 ± 2.4a</td>
</tr>
<tr>
<td>Oc.S/BS (%)</td>
<td>1.5 ± 1.1</td>
<td>2.0 ± 1.2</td>
</tr>
<tr>
<td>Tb.Th (µm)</td>
<td>141 ± 36</td>
<td>128 ± 47</td>
</tr>
<tr>
<td>Tb.Sp (µm)</td>
<td>272 ± 107</td>
<td>358 ± 139</td>
</tr>
<tr>
<td>Tb.N (mm)</td>
<td>2.6 ± 0.9</td>
<td>2.3 ± 0.7</td>
</tr>
<tr>
<td>FB.V (%)</td>
<td>6.3 ± 4.1a</td>
<td>13.6 ± 9.8</td>
</tr>
<tr>
<td>Al.S/BS (%)</td>
<td>17.2 ± 37.2</td>
<td>20 ± 27.4</td>
</tr>
</tbody>
</table>

Data are mean ± SD.

* a,b,c P <0.05; ** P <0.001; ** P = 0.06.
underwent a second biopsy, seven were responders (from 428 ± 201.5 to 192 ± 112 IU/l, P < 0.05) and in non-responders (982 ± 962 to 738 ± 791 IU/l, P < 0.05). No statistically significant difference was found in serum bAP after CTx in non-responders, but there was a significant decrease in responders (from 223 ± 273 to 34 ± 32 IU/l, P < 0.05).

Serum PTH was significantly reduced only in responders (from 831 ± 269 to 221 ± 88 pg/ml, P < 0.0001). In non-responders, serum PTH remained unchanged (from 961 ± 409 to 904 ± 271 pg/ml, ns). For all patients together, serum 25OHD3 was not significantly in responders (from 831 to 221, P < 0.05). In non-responders, serum PTH remained unchanged (from 961 ± 409 to 904 ± 271 pg/ml, ns). For all patients together, serum 25OHD3 was not significantly reduced in resorption parameters.

Non-responders (6.3 ± 4.1 to 2.2 ± 1.8, P < 0.05). Responders exhibited a non-significant reduction in resorption parameters. Non-responders presented an increase in ES/BS (from 7.5 ± 2.4 to 16.6 ± 4.9%, P < 0.05).

None of the patients developed adynamic bone disease in response to CTx. Of the patients who underwent a second biopsy, seven were responders (four suffering from MBD and three from OF) and five were non-responders (two suffering from OF and three from MBD). After CTx, two of the four responders affected by MBD had improved mineralization, while the other two did not. Two of the three responders affected by OF had a reduction in bone turnover, while one showed no change in the histological pattern. Two of the three non-responders with MBD had no change in mineralization parameters, whereas one showed partial improvement. The two non-responders who suffered from OF did not have their BFR/BS significantly altered. After CTx, we found significant differences between responders and non-responders in O.S/BS (6.0 ± 4.4 vs 14.8 ± 6.1, P < 0.05), Es/BS (7.1 ± 3.4 vs 16.6 ± 4.9, P < 0.005), Oc.S/BS (0.7 ± 0.5 vs 2.2 ± 0.9, P < 0.005) and FB.V (2.2 ± 1.8 vs 9.5 ± 6.3, P < 0.05). The data are shown in Table 3.

Parathyroid function after CTx

Overall, CTx produced a reduction in maximum PTH levels (from 1500 ± 501 to 1088 ± 580 pg/ml, P < 0.05) and basal/maximum PTH ratio (from 60.0 to 23.4, P < 0.05), and an increase in maximum calcium values (from 0.99 ± 0.07 to 1.07 ± 0.09 mmol/l, P < 0.05).

When we considered the two groups separately, we found that responders exhibited a decrease in maximum PTH (from 1651 ± 616 to 938 ± 744 pg/ml, P < 0.05) and minimum PTH (from 163 ± 75.4 to 102.2 ± 56.7 pg/ml, P < 0.005) and an increase in maximum calcium (from 0.99 ± 0.07 to 1.1 ± 0.09 mmol/l, P < 0.05) and minimum/basal PTH ratio (from 23.3 ± 11.6 to 34.5 ± 20.4%, P < 0.05). The only significant effect in non-responders was a decrease in their minimum/basal PTH ratio (30.2 ± 17 vs 21.7 ± 11.8%, P < 0.05). The data are shown in Table 2.

Bone biopsy findings after CTx

Second bone biopsies could be performed in only 12 patients as the other four patients refused a second biopsy. Bone formation parameters showed significant changes in both groups after CTx. Thus, in responders, a significant reduction occurred in osteoid thickness (O.Th) (from 21.7 ± 7.3 to 12.9 ± 5.7 µm, P < 0.001), osteoid surface (OS/BS) (from 57.1 ± 21.6 to 41.6 ± 26.4%, P < 0.05) and osteoblastic surface (Ob.S/BS) (from 15.2 ± 9.5 to 6.0 ± 4.4%, P < 0.05). In non-responders, we also found a reduction in OS/BS (from 76.7 ± 12.1 to 47.1 ± 15.5%, P < 0.001) and a tendency towards reduction in O.Th (from 21.2 ± 3.4 to 14.4 ± 3.1 µm, P = 0.06). A significant reduction was observed for fibrosis, mainly in responders (6.3 ± 4.1 to 2.2 ± 1.8, P < 0.05). Responders exhibited a non-significant reduction in resorption parameters. Non-responders presented an increase in ES/BS (from 7.5 ± 2.4 to 16.6 ± 4.9%, P < 0.05).

Discussion

In the present study, we evaluated dynamic parathyroid function and bone remodelling changes in end-stage renal disease patients affected by severe 2HPT and receiving intermittent CTx. The clinical presentation and biochemical features of the patients were relatively homogeneous, not allowing us to distinguish early on between responders and non-responders.

Normal serum albumin levels and body mass index suggested that undernutrition probably did not significantly interfere with most of the parameters studied.

Although serum 25OHD3 levels were within the normal range, low values seen predominantly in non-responders might have been involved in the stimulation of parathyroid gland secretion. Moreover, values that are considered normal in the general population might not be adequate for uraemic patients, according to Ghazali et al. [12]. Our findings also suggest that, besides stimulating PTH secretion, low 25OHD3 levels may interfere with parathyroid response to CTx. Additional studies are necessary to confirm this hypothesis.

The set point value was normal in these patients, in accordance with earlier studies [13]. We also found a positive correlation between set point and basal calcium levels, as described previously [3]. However, a complete analysis of the curve showed that it was actually abnormal. The basal maximum PTH ratio was deranged, revealing gland secretion capacity to be, in basal conditions, excessively utilized. Actually, patients included in this study were utilizing 60% of this capacity.

Some curve parameters allowed for distinction between responders and non-responders. The slope was higher in non-responders, consistent with weaker gland sensitivity. Non-responders needed more time to achieve hypocalcaemia, probably because gland autonomy in this group was driving serum calcium in a manner different from what is seen under physiological conditions.
conditions. The basal/maximum PTH ratio was different in the two groups. Diminished calcium-sensing receptor density, as observed in larger glands, is a factor known to account for gland resistance to calcitriol [14]. Diminished calcium-sensing receptor density was also shown to be correlated with basal/maximum PTH [15], which might explain our findings.

Initial histomorphometric data were not useful for the distinction between responders and non-responders. In both groups, patterns of, respectively, severe OF and MBD were seen. Some patients exhibited mineralization defects. Particularly in this group, such a finding could mean an actual involvement of low 25OHD3 levels in bone disease, as also suggested by Ghazali et al. [12]. ALS/BS and serum aluminium were not correlated with any parameter of the curve, as shown by Felsenfeld et al. [16] as well. They found some curve differences while comparing patients with high and low turnover disease, but did not find aluminium to have any influence on the calcium–PTH curve. In our group of patients, calcitriol response was neither related to serum aluminium nor to bone aluminium deposition.

Serum ALP dropped significantly in both groups. However, we found a significant decrease in bAP only in responders. Concomitantly, the decrease in bone formation parameters detected in biopsies was most pronounced in responders, suggesting that bAP is more specific than ALP [17].

After CTX, we observed no alteration in set point, although blood ionized calcium levels increased. This finding suggests to us that other factors are involved in set point determination, as changes in serum calcium were neither concomitant nor correlated to the set point [18].

Some calcium PTH curve parameters allowed us to distinguish responders from non-responders also after CTX. In responders, we found a decrease in maximum PTH, minimum PTH and maximum calcium, as well as an increase in minimum/basal PTH ratio. All these findings are in accordance with a better response of PTH secretion to variations in serum calcium. In non-responders, only a decrease in minimum/basal PTH ratio was detected, showing that CTX produced only a weak change in parathyroid function in these patients. The calcitriol effect on the calcium–PTH curve has been widely investigated. However, findings have not been uniform [19–21]. The issue remains in fact unresolved, probably due to different methods being employed to construct the curve [22] or authors studying groups consisting of both responders and non-responders. Our findings suggest that although set point and slope remain unaltered, CTX actually induced changes in the calcium–PTH relationship, predominantly in responders. Post-CTX histomorphometric analysis again disclosed differences between responders and non-responders. Both groups showed improvement in bone formation parameters, but the amelioration was more pronounced in responders. Fibrosis was considerably reduced in responders, but less markedly so in non-responders. Regarding bone resorption, non-responders had increased ES/BS. In those patients whose parathyroid glands were not suppressed by calcitriol, a true decoupling of bone remodelling occurred. These patients experienced an increase in bone resorption (as suggested by serum DPD levels and confirmed by an increase in ES/BS) and a concomitant decrease in bone formation parameters. This modification produced no changes in bone volume or in connectivity parameters, probably due to a too short follow-up time. The effect of calcitriol on bone cells is entirely understood. The hormone has important and well-known effects on osteoblasts, mediated by the large number of VDRs expressed in these cells [23]. An increase in a number of circulating compounds as a result of high osteoblastic activity, such as ALP, osteocalcin, osteopontin and Gla-proteins, occurs in response to calcitriol stimulation. Some studies, the majority of which were performed in vitro or using animal models, evaluated the role of calcitriol in bone resorption [24]. It could only be indirect through an enhancement of osteoblastic activity, promoting an increase in IL-6 and IL-6 receptor expression in these cells [25]. Recently, Holliday et al. [26] showed that calcitriol is capable of inducing osteoclastic differentiation and activation, also through a modification in the RANKL/OPG ratio [26].

In responders, taking parathyroid function and bone biopsy findings together, we found an improvement in parathyroid function, including a substantial reduction in basal PTH secretion. Lower PTH levels were associated with a decrease in bone remodelling, that is both formation and resorption. Therefore, in this group, the decrease in PTH secretion induced by CTX led to a reduction of osteoblast and osteoclast activity, improving histomorphometric features. In non-responders, CTX induced no significant reduction in PTH secretion. However, its effect was sufficient to reduce bone formation parameters. Hence, persistently high PTH levels associated with continuous CTX and low 25OHD3 in bone tissue could lead to undesirable disturbances of bone remodelling.

Bone changes in uraemic patients with 2HPT are complex. They are not solely linked to the action of PTH. Calcitriol clearly does not always produce uniform changes in parathyroid function and bone remodelling. Other clinical reports using CTX for 2HPT have come to similar conclusions [5,27]. There is probably a contraindication for calcitriol administration to a subgroup of uraemic patients with advanced 2HPT, reflected by the non-respondents of our present study. In such non-responders, disturbances of bone remodelling may result in dangerous secondary effects, such as profound alterations in ionic balance, which could have negative implications with respect to extra-skeletal calcification or for the bone structure itself. Therefore, accurate means of identifying non-responders should be found.
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