Effects of new vitamin D analogues on parathyroid function in chronically uraemic rats with secondary hyperparathyroidism

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Abstract
Background. Treatment with calcitriol or its analogue, alfacalcidol often leads to hypercalcaemia, hyperphosphataemia or both in patients with chronic renal failure and advanced secondary hyperparathyroidism. We tested three new vitamin D analogues (CB 1093, EB 1213, GS 1725) in an attempt to identify potentially non hypercalcaemic compounds, capable of decreasing plasma parathyroid hormone (PTH) concentration.

Methods. Male Wistar AF rats aged 12–14 weeks were fed a synthetic, phosphate-rich diet and underwent either sham surgery (control) or a standard two-step 5/6th nephrectomy. Four weeks later, renal function was mildly decreased in the latter. Chronic renal failure rats were then divided into six groups, with 8–10 rats in each group. They received daily i.p. injections, from days 0 to 4, of either placebo, calcitriol, or one of the following three active vitamin D analogues: CB1093, 0.25 µg; EB1213, 0.25 or 1.25 µg; and GS1725, 0.025 µg/kg body weight per day, respectively. Sham-operated rats received no drug. On day 5, arterial blood was sampled and rats were sacrificed.

Results. At predefined dosage schedules, all three compounds significantly decreased plasma immunoreactive PTH levels (except EB1213 at low dose). The decrement was somewhat less marked than that obtained with calcitriol, at the dose of 0.25 µg/kg b.w. per day. However, calcitriol induced a marked increase in plasma calcium and phosphate concentrations at that dose, whereas vitamin D analogues led to a more modest increase in plasma calcium level, and none to a worsening of hyperphosphataemia. CB1093 treatment was even associated with a significant decrease in plasma phosphate level.

Conclusion. All three calcitriol analogues tested are promising as non-hypercalcaemic agents in the treatment of uraemic secondary hyperparathyroidism. However, more prolonged administration to uraemic rats of calcitriol analogues with slightly modified dosage schedules and of calcitriol with lower, non-hypercalcaemic dose is required for an optimal comparison before considering clinical trials.

Key words: calcitriol; vitamin D analogues; secondary hyperparathyroidism; uraemia; plasma calcium; plasma phosphorus

Introduction
The treatment of the secondary hyperparathyroidism of chronic renal failure patients with calcitriol or its analogue, alfacalcidol often leads to hypercalcaemia, hyperphosphataemia, or both [1]. Therefore, new vitamin D analogues have been developed, with the goal to keep the inhibitory effect on parathyroid hormone (PTH) synthesis similar to that of the parent hormone calcitriol, but to circumvent its propensity to increase plasma calcium and phosphorus.

Numerous vitamin D analogues have been synthesized by research laboratories during the past years, especially through side-chain modifications [2,3], for the purpose of immunomodulatory and antiproliferative actions on various types of cells, without exerting marked effects on intestinal mineral absorption and skeletal remodelling. This goal can theoretically be achieved by various pharmacological changes, including modified affinity for vitamin D binding protein and shorter plasma half-life, perturbed binding to the nuclear vitamin D receptor, and abnormal activity of the hormone-receptor complex at the target gene [2–4].

Among the analogues which have been examined in uraemic secondary hyperparathyroidism, 22-oxa-calcitriol has been studied to a large extent. It has been shown to inhibit PTH synthesis in vitro [5] and to decrease plasma PTH levels in experimental animals in vivo [6,7], without inducing significant hypercalcaemia [5]. However, our personal experience with that drug was not as favourable as that of others, since in our hands it did not offer any definite advantage over calcitriol in the treatment of uraemic rats with severe hyperparathyroidism [7]. To date, only preliminary clinical experience exists with 22-oxa-calcitriol in uraemic patients still [8]. However, in that study no
comparison has been made with the parent compound, calcitriol.

Besides its action at the level of the PTH gene transcription, calcitriol also exerts powerful genomic actions on cell growth and differentiation [9]. In various culture systems in vitro, it has been shown to depress cell proliferation and to enhance cell maturation at high medium concentrations. The hyperparathyroidism of chronic renal failure is characterized by parathyroid gland hyperplasia, in addition to increased PTH synthesis and secretion. The administration of calcitriol to rats at the time of the creation of experimental renal failure has been shown to prevent excessive parathyroid cell proliferation [10], possibly by interfering with c-myc early gene expression [11]. Several calcitriol analogues have been synthesized which have powerful antiproliferative properties without inducing hypercalcaemia, such as calcipotriol for the treatment of psoriasis, a hyperproliferative disorder [12]. Thus the control of parathyroid overfunction by calcitriol analogues in chronic renal failure could be beneficial both in terms of PTH synthesis and parathyroid cell proliferation.

The purpose of the present study was to find out new non-hypercalcaemic vitamin D analogues which might be capable of decreasing plasma PTH levels in the setting of experimental chronic renal failure. Our ultimate goal was to identify a calcitriol derivative capable of controlling secondary hyperparathyroidism in uraemic patients as well as or even better than the native hormone, without exerting its undesired side effects.

Subjects and Methods

Animals

Male Wistar AF rats, aged 12-14 weeks and weighing approximately 200 g, were purchased from IFFA CREDO, Lyon, France and housed in well-lit rooms, with a light/dark cycle of 12/12 h. They had free access to distilled water throughout. They were fed with a standard laboratory diet or a sham surgery, according to a previously described protocol [7]. Briefly, bipolar resection of the parenchyma of the left kidney was followed by total ablation of the right kidney 7 days later. In control rats, sham surgery was performed with decapsulation of the two kidneys. Thereafter, Nx rats were allowed to develop stable chronic renal failure during 4 weeks before entering study protocol.

Administration of vitamin D analogues

Dose-finding studies were previously performed by Leo Laboratories in normal rats (data not shown). Briefly, the vitamin D analogues were administered daily, for 7 days, at three dose levels. Serum calcium levels were measured on day 7. The highest dose that could be administered without a significant increase in serum calcium was chosen for our experiments in rats with chronic renal failure.

In the present study seven groups of 6-10 rats each were formed. Six groups of non fasted uraemic rats received during five subsequent days a daily intraperitoneal (i.p.) injection of either vehicle (group 1, uraemic control), calcitriol (group 2), or vitamin D analogues (groups 3 through 6). Another group of non-fasted rats with normal renal function, after sham surgery, received no injections (group 7, normal control). Table 1 shows the types and respective dosage schedules of the compounds administered. Calcitriol and vitamin D analogues (Figure 1) were kindly provided by Leo Laboratories, Denmark. Rats received i.p. injections of 340 μl propylene glycol, containing either no active compound (vehicle) or respective vitamin D derivatives.

Blood biochemistry

Blood was sampled at two time-points, first by jugular vein puncture 3 days before starting i.p. injections, and second by aortic puncture at the time of sacrifice, 24 h after the last injection. Rats underwent ether anaesthesia before each blood sampling. Plasma total calcium, phosphorus, total protein, urea and creatinine were determined by previously described routine techniques [7]. Blood ionized calcium and pH were measured with ICAL Ionised Calcium Analyzer (Radiometer, Copenhagen, Denmark). Plasma immunoreactive (i) PTH was measured using rat-specific radioimmunoassay recognizing the N-terminal portion of the hormone (Immutopics Inc., California, USA).

Figure 2 provides a schematic diagram of the time schedule used to create chronic renal failure, to inject calcitriol or new analogues, and to sample blood.

Statistical analysis

Results have been expressed as means ± SD, and the statistical significance determined by non-parametric tests (Mann

Table 1. Rat groups and doses of vitamin D compounds

<table>
<thead>
<tr>
<th>Rat group</th>
<th>n</th>
<th>Renal function</th>
<th>Compound</th>
<th>Dosage regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>9</td>
<td>decreased</td>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>9</td>
<td>decreased</td>
<td>Calcitriol</td>
<td>0.25 μg/kg bd wt x day</td>
</tr>
<tr>
<td>Group 3</td>
<td>9</td>
<td>decreased</td>
<td>CB1093</td>
<td>0.25 μg/kg bd wt x day</td>
</tr>
<tr>
<td>Group 4</td>
<td>10</td>
<td>decreased</td>
<td>EB1213</td>
<td>0.25 μg/kg bd wt x day</td>
</tr>
<tr>
<td>Group 5</td>
<td>10</td>
<td>decreased</td>
<td>EB1213</td>
<td>1.25 μg/kg bd wt x day</td>
</tr>
<tr>
<td>Group 6</td>
<td>9</td>
<td>decreased</td>
<td>GS1725</td>
<td>0.025 μg/kg bd wt x day</td>
</tr>
<tr>
<td>Group 7</td>
<td>6</td>
<td>normal</td>
<td>nil</td>
<td></td>
</tr>
</tbody>
</table>

n = number of rats in each group.
Effect of calcitriol or analogs in CRF rats

Study design

Fig. 1. Schematic representation of the experimental design.

Fig. 2. Chemical structure of calcitriol and the three vitamin D analogues studied.

Whitney U test or Wilcoxon test) and by ANOVA test, as appropriate. P values <0.05 were considered statistically significant.

Results

Body weight and renal failure progression (Table 2)
The six groups of chronic renal failure rats had comparable body weight three days before (day −3) and 1 day after the last of the daily i.p. injections over 5 days of vitamin D compounds or vehicle (day +5), whereas the group of sham-operated rats with normal renal function (group 7) had a significantly greater body weight at same time points. At day −3 mean plasma creatinine concentrations were increased to a similar extent in group 1 to group 6 rats, compared with group 7 rats. However, at day +5 calcitriol-treated group 2 rats had significantly higher mean plasma creatinine levels than all other groups. Group 6 rats had an intermediate increase, compared with the other groups in which the slight increase in plasma creatinine was comparable to that observed in placebo-treated uremic rats. Plasma urea concentrations were comparable in all groups at day −3. They increased to a similar extent as that of plasma creatinine, except in group 7 where the mean level remained unchanged.

Effect of vitamin D analogues on plasma parameters of calcium metabolism

Table 3 shows mean ±SD values of plasma total calcium, ionized calcium, phosphorus, total protein, and iPTH concentrations for all rat groups studied, at day −3 and day +5. Before starting i.p. injections, the concentrations of all plasma parameters were comparable in the six chronic renal failure rat groups. As expected, however, most of them were different from those of group 7 rats with normal renal function. Plasma total protein was the same in all uremic rat groups before start of treatment. It decreased significantly in all of them at day +5, most certainly as a consequence of blood sampling at day −3.

The administration of calcitriol (group 2) led to a significant decrease of mean plasma iPTH at day +5, compared with that of placebo-treated uremic rats (group 1). Since the distribution of initial iPTH values was widespread in several rat groups, individual plasma iPTH concentrations at day −3 and day +5 have been depicted in Figure 3. In group 2, the two rats that exhibited the highest initial iPTH levels (833 and 637 pg/ml, respectively) had a decrease of similar magnitude (to 316 and 260 pg/ml respectively) as that of the other seven rats. However, in the latter plasma iPTH levels were diminished to values below the lower limit of normal range (group 7) whereas they remained
Circulating parameters reflecting calcium metabolism

Table 2. Body weight and plasma parameters reflecting renal function

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day</th>
<th>Group 1 placebo (vehicle)</th>
<th>Group 2 calcitriol (0.25 μg/kg.d)</th>
<th>Group 3 CB 1093 (0.25 μg/kg.d)</th>
<th>Group 4 EB 1213 (0.25 μg/kg.d)</th>
<th>Group 5 EB 1213 (1.25 μg/kg.d)</th>
<th>Group 6 GS 1725 (0.025 μg/kg.d)</th>
<th>Group 7 sham-op. (no injections)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (g)</td>
<td>D−3</td>
<td>263 ± 19</td>
<td>263 ± 32</td>
<td>268 ± 19</td>
<td>275 ± 14</td>
<td>254 ± 26</td>
<td>265 ± 19</td>
<td>353 ± 31</td>
</tr>
<tr>
<td></td>
<td>D+5</td>
<td>264 ± 23</td>
<td>247 ± 68</td>
<td>278 ± 24</td>
<td>296 ± 14</td>
<td>265 ± 38</td>
<td>272 ± 26</td>
<td>389 ± 32</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>D−3</td>
<td>7.56 ± 1.18</td>
<td>7.68 ± 1.86</td>
<td>8.37 ± 2.28</td>
<td>7.41 ± 0.91</td>
<td>5.98 ± 2.08</td>
<td>9.44 ± 1.02</td>
<td>7.90 ± 1.02</td>
</tr>
<tr>
<td></td>
<td>D+5</td>
<td>11.2 ± 1.52</td>
<td>28.2 ± 11.8</td>
<td>14.1 ± 5.93</td>
<td>13.3 ± 1.97</td>
<td>17.6 ± 9.46</td>
<td>17.8 ± 7.24</td>
<td>7.63 ± 0.62</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>D−3</td>
<td>78.2 ± 7.2</td>
<td>74.7 ± 11.3</td>
<td>77.1 ± 16.6</td>
<td>80.8 ± 6.68</td>
<td>87.0 ± 11.9</td>
<td>91.4 ± 13.5</td>
<td>47.5 ± 4.46</td>
</tr>
<tr>
<td></td>
<td>D+5</td>
<td>87.9 ± 9.7</td>
<td>155 ± 54.1</td>
<td>101 ± 32.3</td>
<td>90.5 ± 10.1</td>
<td>119 ± 47.9</td>
<td>125 ± 47.5</td>
<td>45.7 ± 3.27</td>
</tr>
</tbody>
</table>

All values are means ± SD. Number of plasma samples for each column were 7–10 in groups 1 through 6, and 6 in group 7.

Table 3. Circulating parameters reflecting calcium metabolism

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day</th>
<th>Group 1 placebo (vehicle)</th>
<th>Group 2 calcitriol (0.25 μg/kg.d)</th>
<th>Group 3 CB 1093 (0.25 μg/kg.d)</th>
<th>Group 4 EB 1213 (0.25 μg/kg.d)</th>
<th>Group 5 EB 1213 (1.25 μg/kg.d)</th>
<th>Group 6 GS 1725 (0.025 μg/kg.d)</th>
<th>Group 7 sham-op. (no injections)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Ca (mmol/l)</td>
<td>D−3</td>
<td>2.37 ± 0.16</td>
<td>2.40 ± 0.23</td>
<td>2.51 ± 0.09</td>
<td>2.50 ± 0.21</td>
<td>2.28 ± 0.19</td>
<td>2.34 ± 0.22</td>
<td>2.70 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>D+5</td>
<td>2.46 ± 0.18</td>
<td>3.16 ± 0.33</td>
<td>2.66 ± 0.15</td>
<td>2.42 ± 0.12</td>
<td>2.72 ± 0.22</td>
<td>2.93 ± 0.13</td>
<td>2.55 ± 0.18</td>
</tr>
<tr>
<td>Ca²⁺ (mmol/l)</td>
<td>D−3</td>
<td>1.18 ± 0.06</td>
<td>1.17 ± 0.04</td>
<td>1.21 ± 0.09</td>
<td>1.21 ± 0.09</td>
<td>1.14 ± 0.09</td>
<td>1.13 ± 0.05</td>
<td>1.23 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>D+5</td>
<td>1.34 ± 0.09</td>
<td>1.55 ± 0.14</td>
<td>1.39 ± 0.06</td>
<td>1.24 ± 0.07</td>
<td>1.19 ± 0.16</td>
<td>1.33 ± 0.05</td>
<td>1.33 ± 0.03</td>
</tr>
<tr>
<td>PO₄ (mg/dl)</td>
<td>D−3</td>
<td>3.10 ± 0.52</td>
<td>3.15 ± 0.47</td>
<td>3.72 ± 0.65</td>
<td>3.39 ± 0.68</td>
<td>3.89 ± 0.80</td>
<td>4.02 ± 0.89</td>
<td>2.41 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>D+5</td>
<td>2.25 ± 0.41</td>
<td>4.21 ± 1.32</td>
<td>2.95 ± 0.98</td>
<td>3.62 ± 0.73</td>
<td>3.92 ± 1.53</td>
<td>3.68 ± 0.84</td>
<td>2.25 ± 0.17</td>
</tr>
<tr>
<td>Protein (g/l)</td>
<td>D−3</td>
<td>57.1 ± 3.1</td>
<td>56.4 ± 3.8</td>
<td>57.6 ± 2.4</td>
<td>57.9 ± 3.4</td>
<td>58.2 ± 4.5</td>
<td>57.4 ± 2.5</td>
<td>61.0 ± 2.83</td>
</tr>
<tr>
<td></td>
<td>D+5</td>
<td>51.7 ± 5.3b</td>
<td>53.3 ± 3.5</td>
<td>52.9 ± 2.6b</td>
<td>51.7 ± 2.5b</td>
<td>55.0 ± 2.3b</td>
<td>53.8 ± 2.6b</td>
<td>N.D.</td>
</tr>
<tr>
<td>iPTH (pg/ml)</td>
<td>D−3</td>
<td>301 ± 64</td>
<td>375 ± 224</td>
<td>268 ± 149</td>
<td>298 ± 135</td>
<td>297 ± 165</td>
<td>234 ± 35</td>
<td>31 ± 16</td>
</tr>
<tr>
<td></td>
<td>D+5</td>
<td>224 ± 103</td>
<td>67 ± 126</td>
<td>73 ± 47b</td>
<td>343 ± 169</td>
<td>116 ± 140b</td>
<td>50 ± 44b</td>
<td>27 ± 9</td>
</tr>
</tbody>
</table>

All values are means ± SD. Number of plasma samples for each column were 7–10 in groups 1 through 6, and 6 in group 7. However, numbers of plasma samples for column of ionized calcium were more limited due to technical reasons (n = 4–7). *P < 0.05, b P < 0.01, and c P < 0.001, as compared to D−3.

Abbreviations: total Ca, plasma total Ca; Ca²⁺, blood ionised Ca; PO₄, plasma phosphorus; protein, plasma total protein; iPTH, plasma immunoreactive parathyroid hormone.

Correlation between changes of plasma calcium and changes of plasma iPTH.

We tested the hypothesis that the observed changes of plasma iPTH could be mainly due to changes of the plasma calcium concentration. We found a highly significant correlation between the changes of these two variables from day −3 to day +5 when considering data of all rat groups together, except those of sham-operated rats (Figure 4). However, no correlation existed within any single group.

Discussion

The present study in rats with experimental chronic renal failure and secondary hyperparathyroidism has been designed to identify new vitamin D analogues relatively high in the former. The effect of calcitriol was, however, associated with a marked increment of mean plasma total calcium, ionised calcium, and phosphorus whereas in placebo-treated uraemic rats (group 1) mean plasma calcium and phosphorus did not change significantly.

The vitamin D analogues CB1093 (group 3), EB1213 (only the dose of 1.25 μg/kg b.w. per day, group 5) and GS1725 (group 6) were able to induce significant decreases in mean plasma iPTH levels as well. The decrement obtained with the active analogues in terms of parathyroid gland inhibition was comparable to that of calcitriol (Figure 3). The effect of each active analogue was associated with a less marked increase in mean plasma total calcium or ionized calcium level, and by either no change (groups 5 and 6) or even a significant decrease (group 3) in median plasma phosphorus level.
that would be able to decrease plasma PTH levels, but that unlike calcitriol would not induce hypercalcaemia or hyperphosphataemia. The results show that with the dosage schedules and experimental conditions chosen, all three vitamin D analogues (EB1213 only at high dose) decreased circulating iPTH levels markedly. Moreover, they induced either only a modest or no increase in plasma calcium and phosphorus. Even though a correlation was found between the change of plasma calcium and that of plasma iPTH when considering all experimental groups together, such a correlation did not exist for any single vitamin D compound alone. This could either indicate that the role of increased calcium in the observed inhibition of iPTH was not more important than the direct action of vitamin D derivatives at the level of the parathyroid cell or, more probably, that the number of animals in each group was too small to allow a firm statement with respect to the role of hypercalcaemia.

Calcitriol, at the dosage chosen on the basis of previous studies, exerted the greatest inhibitory effect on plasma PTH concentration, but this effect was accompanied by a marked increase in both plasma calcium and phosphorus. The decrease of plasma PTH obtained with that dose of calcitriol administered for 5 days resulted in a mean PTH level which was even lower than that of the sham-operated rats with normal renal function. This dramatic decrement probably was due to both the high calcitriol dose and the marked elevation of plasma calcium. Interestingly, the concomitant increase in plasma phosphorus was unable to offset calcitriol inhibitory effect on the PTH secretion of the parathyroid cells.

At the dosage chosen in this experiment, calcitriol led to a worsening of renal function, as testified by a doubling of plasma creatinine and an even fourfold increase of plasma urea level. This observation is in accord with an observation in uraemic patients at a time when calcitriol first became available for clinical treatment [13]. However, it has been well established since that the use of less pharmacological doses of calcitriol, avoiding severe hypercalcaemic episodes, is not associated with a deterioration of renal function [14]. Obviously, the daily calcitriol dose given to the rats in the present study was too high for the degree of renal failure experimentally achieved and the associated high degree of vitamin D responsiveness. At more severe stages of chronic renal failure, rats are more resistant to calcitriol and do not respond with a comparable increase in plasma calcium and phosphorus levels [7].

The possibly beneficial effect of another vitamin D analogue, namely 22-oxa-calcitriol, in the control of parathyroid overfunction has been closely scrutinized by two groups of authors [5,6]. They claimed that this drug allowed to counteract the secondary hyperparathyroidism of chronic renal failure in a manner as efficient as the parent hormone, calcitriol, in the
absence of significant calcæmic activity. However, we were unable to confirm such a superiority of 22-oxacalcitriol [7]. In fact, it proves rather difficult to define experimental conditions in such a way that they would allow to compare the action of novel analogues on the parathyroid gland with that of calcitriol, by finding out precisely the dosage required for each vitamin D compound to induce a comparable increase (or absence of increase) in plasma calcium and phosphorus. This difficulty is further aggravated by our incapacity to achieve strictly comparable degrees of chronic renal failure in rats from one experimental series to another.

The number of vitamin D receptors in the parathyroid tissue of uraemic patients is downregulated, at least in nodular areas of the hyperplastic glands [15]. It would be of particular interest to find out, if possible, vitamin D analogues with a relatively high affinity for the vitamin D receptor in the parathyroid cell, but a low affinity for the receptor in the osteoblast and the enterocyte. This would allow to overcome the relative resistance of uraemic hyperparathyroid patients to the action of calcitriol without inducing hypercalcæmia [16]. It remains to be seen however, whether it will be possible to obtain regression of parathyroid hyperplasia in case of severe secondary hyperparathyroidism after its transformation from a polyclonal to a monoclonal type of growth [17].

It would be premature to deduce from the findings made in the present study that any one of the new vitamin D analogues might be better suited than calcitriol for the treatment of secondary uraemic hyperparathyroidism. Obviously, we will have to compare the effects of vitamin D analogues with that of a calcitriol dosage regimen that would lead to a similar small increase or no increase at all in plasma calcium or phosphorus, maintaining however an inhibitory effect on parathyroid gland function. Therefore, further study is clearly needed to clarify this issue which is of great potential interest to patients with chronic renal failure.

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References


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