Changes in fibroblast growth factor 23 during treatment of secondary hyperparathyroidism with alfacalcidol or paricalcitol

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Abstract

Background. Fibroblast growth factor 23 (FGF23) increases renal phosphate excretion and decreases the formation of 1,25 dihydroxyvitamin D. In patients with chronic kidney disease, plasma FGF23 levels are markedly elevated by unknown mechanisms. We explored the changes in FGF23 during treatment of secondary hyperparathyroidism (SHPT) with alfacalcidol or paricalcitol in haemodialysis patients.

Methods. Intravenous alfacalcidol and paricalcitol were compared in haemodialysis patients with SHPT in a randomized 2 × 16-week cross-over study, with 6 weeks washout period preceding treatment and between treatment periods. In 57 of the enrolled patients, blood samples were frozen before and after each treatment period and available for measurement of FGF23.

Results. Treatment with both analogues increased FGF23 (P < 0.05 in all treatment periods). The magnitude of increase was similar for alfacalcidol and paricalcitol (Period 1: 223 versus 314%; P = 0.384 and Period 2: 174 versus 227%; P = 0.510) and the levels returned to pre-treatment levels during the washout period. Independent predictors of rise in FGF23 were baseline levels of FGF23 (P < 0.01), changes in ionized calcium (P < 0.01) and phosphate (P < 0.01) and cumulative dose of vitamin D analogues (P = 0.024).

Conclusion. Alfacalcidol and paricalcitol increase the plasma levels of FGF23 equally and substantially in haemodialysis patients.
Introduction

Fibroblast growth factor 23 (FGF23) is a phosphate and vitamin D regulating hormone. FGF23 is secreted by osteocytes and targets the FGF receptor–klotho complex in cell membranes [1]. FGF23 reduces the renal phosphate reabsorption through down-regulation of the sodium–phosphate co-transporter in the proximal tubule [2]. FGF23 decreases circulating 1,25 dihydroxyvitamin D by decreasing Cyp27b1, and thereby decreasing the renal 1α-hydroxylation of 25 hydroxyvitamin D to 1,25 dihydroxyvitamin D, and by increasing Cyp24, thereby increasing the degradation of 1,25 dihydroxyvitamin D through 24-hydroxylation [3]. Decreased 1,25 dihydroxyvitamin D in turn reduces plasma phosphate (p-phosphate) by decreasing the intestinal phosphate absorption.

Circulating FGF23 rises progressively as kidney function declines [4] and dialysis patients have remarkably high FGF23 levels up to 1000-fold higher than healthy individuals [5]. The precise mechanism responsible for the rise in FGF23 is unknown.

It is well known that patients with chronic kidney disease (CKD) have an increased risk of cardiovascular disease and mortality [6, 7]. Increased levels of FGF23 are associated with increased mortality in incident and prevalent haemodialysis patients, independently of p-phosphate and other risk factors [8, 9]. FGF23 elevation is associated with increased left ventricular mass index and left ventricular hypertrophy, vascular dysfunction, arterial stiffness and aortic calcification [10–13]. Whether FGF23 exerts a direct toxicity or whether FGF23 is a biomarker of harmful effects of the phosphate metabolism or vitamin D deficiency is unknown.

Exploration of factors influencing on FGF23 level provides information concerning the regulation of the mineral metabolism and may clarify the link between increased mortality and disturbances in mineral metabolism in patients with CKD.

Doxercalciferol and calcitriol have been shown to increase FGF23 during treatment of secondary hyperparathyroidism (SHPT) [14, 15]. Whether alfacalcidol and paricalcitol increase FGF23 during suppression of parathyroid hormone (PTH) has not been described.

The aim of this study was to evaluate and compare the changes in FGF23 during the treatment of SHPT with alfacalcidol and paricalcitol in haemodialysis patients in a randomized cross-over study. Furthermore, we explored whether FGF23 level predicted treatment response as suggested by other groups [16, 17].

Materials and methods

Subjects

Subjects in this study were a subset of participants in the SHPT study (57 of 86 enrolled patients), a multicentre, open-label, randomized cross-over study comparing alfacalcidol and paricalcitol. These subjects have been described elsewhere [18]; in brief, adults receiving chronic haemodialysis therapy for at least 3 months prior to screening were eligible if they had sufficiently regulated p-phosphate (<1.8 mmol/L) and ionized plasma calcium (ionized p-calcium) (<1.25 mmol/L) and if plasma intact PTH (p-PTH) was >350 pg/mL (37.1 pmol/L) after a period of minimum 6 weeks washout, without any kind of vitamin D supplement.

Subjects in this study consisted of participants in whom blood samples had been successfully collected for the whole study period. Some participants in the SHPT study did not have a blood sample collection because of (i) improper collection at study sites, (ii) participants refusing to participate in biobank collection and (iii) withdrawal from the original SHPT study.

The study was in compliance with the Helsinki Declaration of 1975 and revised in 2000 and was approved by the Danish National Committee on Biomedical Research Ethics (SJ-27).

Design

The design has previously been described and discussed in detail [18]. The study took place in 10 public Danish dialysis departments, all being a part of a hospital nephrology department.

The randomization was performed after a 6-week washout period during which the patients were not allowed to receive any kind of vitamin D supplement. The first arm received alfacalcidol for 16 weeks and then went through another 6 weeks washout period before they received paricalcitol for 16 weeks. The second arm received paricalcitol for 16 weeks and then went through another 6 weeks washout period before they received alfacalcitols for 16 weeks. The drugs were given at the end of haemodialysis treatment two or three times a week depending on the frequency of haemodialysis treatment. The start dose of alfacalcidol (Etapharm®, LEO Pharma A/S, Ballerup, Denmark) was 3 μg a week and the start dose of paricalcitol (Zemplar®, Abbott Scandinavia AB, Solna, Sweden) was 9 μg a week. Every second week, the dose was titrated 50% according to p-phosphate, p-calcium and p-PTH. As long as p-phosphate <1.8 mmol/L and ionized p-calcium <1.35 mmol/L and p-PTH >150 pg/mL, the dose was increased. When p-PTH <150 pg/mL and p-phosphate <1.8 mmol/L and ionized p-calcium <1.35 mmol/L, the dose was retained. If at any time p-phosphate was >1.8 mmol/L or ionized p-calcium was >1.35 mmol/L in two repeated measurements, the dose was reduced. The minimum dose was alfacalcidol: 1.5 μg/week and paricalcitol: 4.5 μg/week. If further reduction was needed, the treatment was temporarily withdrawn.

After inclusion, the dose of calcium-containing phosphate binders could only be unchanged or reduced. Elevated p-phosphate was treated with calcium-free phosphate binders, dietary intervention and re-evaluation of the dialysis dose. Elevated p-calcium led to dietary intervention and reduction of calcium-containing phosphate binders. The calcium concentration of dialysate was fixed at 1.25 mmol/L.

FGF23 measurements

Serum samples were collected at the beginning (6 and 22 weeks) and at the end (28 and 44 weeks) of each treatment period, interrupted by a 6-week washout period. The blood sample was drawn from the blood lines of the dialyser before start of the dialysis. The samples were collected and frozen at Roskilde County Hospital. At the end of the study, the samples were aliquoted and shipped cold to Odense University Hospital for analysis. FGF23 was measured by a sandwich enzyme-linked immunosorbent assay (Kainos Laboratories Inc., Tokyo, Japan), which detects only the biologically active intact FGF23. The intra- and inter-assay coefficients of variation were <5.0%.

p-PTH, ionized p-calcium and p-phosphate were measured every second week during the treatment periods in order to guide the dose adjustment and analysed at the participating departments’ local laboratories.

Statistical analysis

The distribution of the variables and the changes in the variables from the start until the end were described. FGF23 and PTH were described by median (range) because of right skewness and presented as compared between treatment groups as log-transformed data in order to reach a normal distribution. Other continuous data were described as mean (SD) or (SEM) and categorical data as number and percentages. Comparison of log FGF23 changes between groups in cross-over design was performed according to Altman et al. [19]. Parametric tests were used if the distributions were normal (paired and unpaired t-tests) and non-parametric test (Fischer’s exact test, Mann–Whitney U and Wilcoxon signed-ranks test) for categorical or not normally distributed continuous data. Pearsons correlation and multiple linear regression were performed on baseline parameters.

Explanatory factors for FGF23 changes were found by analysis of variance of repeated measurements. Treatment, period, per cent change

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**Keywords:** alfacalcidol; chronic kidney disease; fibroblast growth factor 23; paricalcitol; vitamin D
Changes in FGF23 during alfacalcidol and paricalcitol

in ionized calcium, per cent change in phosphate, baseline log FGF23, cumulative equipotent dose of vitamin D analogue, per cent change in PTH, 25 hydroxyvitamin D and alkaline phosphatase were entered as explanatory variables and removed by backwards selection.

Equi-potent dose of vitamin D analogues was calculated by multiplying alfacalcidol dose by 3, as the alfacalcidol:paricalcitol dose ratio is 1:3.

Explanatory factors at baseline (Week 6) for PTH response after first treatment period (Week 22) were explored for PTH (Week 22) <300 pg/mL by logistic regression and for log PTH (Week 22) by multiple linear regression. Baseline levels of calcium, phosphate, FGF23, PTH and treatment group entered the models. Non-significant variables were removed by backwards selection.

All tests were two-sided tests (α = 0.05). Analysis was performed in SAS 9.1 software package (SAS Institute Inc., Cary, BC).

Results

Patient characteristics

In 57 patients (22 females and 35 males), blood samples from all four visits were collected. There was no difference in baseline characteristics between groups (Table 1). There was no difference between this subgroup and the enrolled patients of the SHPT study. The alfacalcidol–paricalcitol (AP) group contained three previously transplanted patients, of whom one received immunosuppression (prednisolone). No patients in the paricalcitol–alfacalcidol (PA) group had been transplanted. None were using bisphosphonate or antiosteoporotic agents during the study. Baseline 25-hydroxy vitamin D <50 nmol/L were present in 75% of the patients (AP: n = 18; PA: n = 24; P = 0.367).

Baseline parameters

Median initial FGF23 was 2480 pg/mL (range 95 to 32 650 pg/mL). The FGF23 distribution was highly skewed to the right but was normally distributed after a logarithmic transformation. Log FGF23 at baseline showed significant correlation with ionized calcium (r = 0.457; P = 0.0003), phosphate (r = 0.397; P = 0.0022) and PTH (r = 0.345; P = 0.0086). No correlation between log FGF23 and 25 hydroxyvitamin D (r = –0.010; P = 0.940), 1,25 dihydroxyvitamin D [r = 0.015; P = 0.955 (only measured for n = 16)] or alkaline phosphatase (r = –0.118; P = 0.382) was found.

Multivariate analysis revealed baseline calcium (β = 3.54; P = 0.0006), baseline phosphate (β = 0.91; P = 0.0034) and baseline PTH (β = 0.0001; P = 0.0262) as independent predictors of baseline log FGF23, whereas 25 hydroxyvitamin D, 1,25 dihydroxyvitamin D and alkaline phosphatase were not significantly related to baseline log FGF23 and were removed during backwards selection.

Vitamin D analogue treatment and changes in FGF23

Levels of log FGF23 did not differ between groups throughout the course (Figure 1). No period (P = 0.601) or period × treatment interaction (P = 0.431) was seen. No difference in log FGF23 changes between treatment groups was found (P = 0.275). FGF23 levels increased in both treatment groups by 272% (SD 390) during the first treatment period (Week 6 to Week 22) and 198% (SD 305) during the second treatment period (Week 28 to Week 44). Median final FGF23 in the first period was 7323 pg/mL (range 91 to 62 640 pg/mL). In the second period, the initial FGF23 was median 3384 (range 97 to 26 343 pg/mL) increasing to a final FGF23 median of 9124 pg/mL (range 175 to 63 000 pg/mL). The concomitant changes in PTH, ionized calcium and phosphate are shown in Figure 1.

Per cent change in log FGF23 was normally distributed when inspecting the histogram and probability plot. The factors significantly associated with log FGF23 changes were per cent change in ionized calcium (β = 0.3135; P = 0.0051), per cent change in phosphate (β = 0.1680; P < 0.0001), baseline log FGF23 (β = −0.1191; P < 0.0001), baseline log 1,25 dihydroxyvitamin D (β = −0.281; P = 0.0022) and PTH (β = −0.345; P = 0.0086). No correlation between log FGF23 and 25 hydroxyvitamin D (r = –0.010; P = 0.940), 1,25 dihydroxyvitamin D [r = 0.015; P = 0.955 (only measured for n = 16)] or alkaline phosphatase (r = –0.118; P = 0.382) was found.

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Table 1. Baseline characteristicsa

<table>
<thead>
<tr>
<th>All randomized N = 86</th>
<th>Enrolled in FGF23 study N = 57</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfacalcidol–paricalcitol n = 41</td>
<td>Paricalcitol–alfacalcitol n = 45</td>
</tr>
<tr>
<td>Alfacalcidol–paricalcitol n = 26</td>
<td>Paricalcitol–alfacalcitol n = 31</td>
</tr>
<tr>
<td>Age (years ± SD)</td>
<td>63.6 ± 13.7</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>27/14</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>41 (100%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0</td>
</tr>
<tr>
<td>Diabetes</td>
<td>6 (15%)</td>
</tr>
<tr>
<td>Time on dialysis [month; median (range)]</td>
<td>38 (3–236)</td>
</tr>
<tr>
<td>Aetiology of ESRD</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>5 (12%)</td>
</tr>
<tr>
<td>Nephrosclerosis</td>
<td>7 (17%)</td>
</tr>
<tr>
<td>Polycystic</td>
<td>9 (22%)</td>
</tr>
<tr>
<td>Chronic glomerulonephritis</td>
<td>3 (8%)</td>
</tr>
<tr>
<td>Chronic interstitial</td>
<td>5 (12%)</td>
</tr>
<tr>
<td>Post-renal</td>
<td>7 (17%)</td>
</tr>
<tr>
<td>s-FGF23 (pg/mL) median (IQR)</td>
<td>n.a.</td>
</tr>
<tr>
<td>p-intact PTH (pg/mL) median (IQR)</td>
<td>525 (417–659)</td>
</tr>
<tr>
<td>p-phosphate (mmol/L ± SD)</td>
<td>1.48 ± 0.27</td>
</tr>
<tr>
<td>p-calcium ion (mmol/L ± SD)</td>
<td>1.16 ± 0.07</td>
</tr>
<tr>
<td>p-25-hydroxyvitamin D2 +D3 (mmol/L ± SD)</td>
<td>42.1 ± 21.3</td>
</tr>
</tbody>
</table>

a n.a., not applicable; IQR, interquartile range; s, serum; p, plasma.
Relationship between baseline factors and PTH suppression when treated with vitamin D analogues

Patients with PTH level >300 pg/mL after 16 weeks of treatment were defined as non-responders to vitamin D analogue treatment. A total of 14/57 non-responders after Period 1 and 22/57 non-responders after Period 2 were present.

Log FGF23 was significantly higher at baseline in the non-responders than in the responders after Period 1 (P = 0.016) and Period 2 (P < 0.001). However, a logistic regression analysis of explanatory factors for reaching a PTH level <300 pg/mL left only phosphate statistically significant (P = 0.028). Multiple linear regression showed a significant relation between baseline levels of FGF23 (β = 0.00001324; P = 0.0158) and phosphate (β = 0.456; P = 0.0133) and the final level of log PTH (Week 22) (R² = 0.238).

Discussion

Circulating levels of FGF23 increase with declining kidney function [4] and we found several fold increased levels of FGF23 in our haemodialysis population compared to reported levels in healthy adults [20]. The reason for this increased level of FGF23 in haemodialysis patients is not fully understood [21]. We clearly demonstrate a substantial increase in FGF23 induced by alfacalcidol and paricalcitol during 16 weeks of treatment of SHPT. This is most probably a general effect of vitamin D analogues as we demonstrate equal changes during alfacalcidol and paricalcitol treatment. This is in accordance with previous findings: FGF23 increases during treatment of SHPT in paediatric peritoneal dialysis patients with calcitriol or doxercalciferol [15] and during calcitriol treatment of SHPT in adult haemodialysis patients [14]. In contrast, Wetmore et al. [22] found that paricalcitol and doxercalciferol left FGF23 unchanged in a comparative study with calcimimetics plus low-dose calcitriol analogue. This could indicate differences between vitamin D analogues and their induction of FGF23 increase. However, in the study by Wetmore et al., unchanged levels of PTH, calcium and phosphate were present in the vitamin D analogue treated group, which as shown in the present study plays an important role in the vitamin D induced increase of FGF23.

Vitamin D is part of a complex interaction between factors of the mineral metabolism including calcium, phosphate, PTH and the FGF23–klotho system. The observed FGF23 increase may be a direct stimulatory effect of vitamin D and/or an indirect effect through changes in the other parameters such as increased phosphate and calcium levels.

Calcitriol increases the expression of FGF23 in vitro in bone-derived cell cultures [23–25], and the calcitriol-induced expression of FGF23 messenger RNA in bone culture derived from uraemic rats is even more pronounced than in bone culture derived from normal rats [25]. Therefore, a direct effect of paricalcitol and alfacalcidol seems plausible. Indeed, we found the cumulative dose of vitamin D analogues to be an independent predictor of increase in FGF23.

The positive association in the present study between increase in phosphate levels and the increase in FGF23
suggests that vitamin D analogues may increase FGF23 through increased circulating phosphate levels [26]. Whereas short-term changes in dietary phosphate load does not influence on FGF23 levels in CKD [27] and non-CKD patients [4], a prolonged phosphate exposure has been shown to regulate FGF23 [28]. Likewise, regulation of phosphate load by other treatment modalities also influences FGF23 levels. Treatment with non-calcium-containing phosphate binders has been shown to decrease FGF23 levels in CKD 3–4 and 5D patients [29–32], and a vegetarian diet with less absorbable phosphate decreases FGF23 compared to a meat diet in CKD 3–4 patients [33].

The positive association between baseline calcium and FGF23 and between changes in ionized calcium and changes in FGF23 may indicate a regulatory function of calcium on FGF23. Other clinical interventional trials indicate that calcium may have a regulatory function on FGF23 levels. In dialysis patients, calcimimetics reduced levels of FGF23 significantly while reducing levels of calcium [22, 34]. Sevelamer, a non-calcium-containing phosphate binder, decreased FGF23, whereas phosphate

![Fig. 2. Explanatory factors for changes in FGF23 during alfacalcidol and paricalcitol treatment. Univariate analysis with % phosphate changes, % changes in ionized calcium, baseline log FGF23 or cumulative equivalent dose of vitamin D analogue as independent variable versus % change in log FGF23 during 16 weeks of treatment with paricalcitol and alfacalcidol.](https://academic.oup.com/ndt/article-abstract/27/6/2263/1942711)
binding with calcium carbonate did not change levels of FGF23 in CKD 3–4 patients [31], and increasing levels of calcium in dialysate are positively associated with levels of FGF23 [32]. The regulatory mechanism of calcium on FGF23 remains to be further explored but is supported by Shimada et al. [35] who described a calcium-induced increase in FGF23 expression and circulating levels of FGF23 in vitamin D receptor-deficient mice.

FGF23 has been found to predict future refractory SHPT in dialysis patients [16, 17]. We did not find FGF23 levels to predict a PTH <300 pg/mL after 16 weeks of vitamin D analogue treatment. But a linear relation between baseline 25(OH)D levels and paricalcitol treatment. No intermediate blood samples were collected. Secondly, a selection bias of the enrolled subjects is possible as we only included patients who completed the study and had available blood samples. Thirdly, the small sample size may limit the ability to describe all factors that influence on the changes in FGF23. Fourthly, a control group was lacking, although the reversibility during the washout period supports that the FGF23 increase is mediated by the vitamin D analogues.

In conclusion, the vitamin D analogues alfalcacidol and paricalcitol increase FGF23 equally during 16 weeks of treatment. Treatment with vitamin D analogues may play a substantial role for the elevated levels of FGF23 in haemodialysis patients, both through a direct increase of FGF23 expression and through an increased level of phosphate and ionized calcium. The level of FGF23 did not predict but was associated with the responsiveness of SHPT to treatment with vitamin D analogues.

Acknowledgements. The authors thank Abbott Laboratories A/S for funding this study. The funding source has no influence on study design; in the collection, analysis and interpretation of data; in the writing of the manuscript or in the decision to submit the manuscript for publication. We thank Morten Aagaard Petersen MSc for statistical advice and James Heat D MSc for critical comments on the manuscript.

Conflict of interest statement. Abbott Laboratories A/S provided a grant for the present study. L.B. has received consulting fees from LEO Pharmaceuticals and Amgen and lecture fees from Genzyme and Swedish Orphan.

(See related article by Fish and Cunningham. FGF-23 and vitamin D: don’t shoot the messenger? Nephrol Dial Transplant 2012; 27: 2137–2139.)

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Received for publication: 5.6.11; Accepted in revised form: 20.10.11