Parathyroid Hormone Regulates Fibroblast Growth Factor-23 in a Mouse Model of Primary Hyperparathyroidism

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ABSTRACT

The importance of fibroblast growth factor 23 (FGF-23) in the pathogenesis of phosphate wasting disorders has been established, but controversy remains about how parathyroid hormone (PTH), which also stimulates urinary phosphate excretion, regulates the circulating level of FGF-23. We found that the serum FGF-23 concentration was higher in PTH-cyclin D1 transgenic mice, a model of primary hyperparathyroidism, than in wild-type mice. The serum FGF-23 concentration was significantly and directly correlated with serum PTH and calcium, and inversely correlated with phosphate levels in 90- to 118-week-old mice (all \( P < 0.005 \)). Quantitative real-time reverse-transcriptase PCR revealed abundant expression of \( fgf23 \) in bone, especially in calvaria. The \( fgf23 \) expression in calvaria was significantly higher in the transgenic mice compared to the wild-type mice, and correlated well with serum FGF-23 levels. There was a direct correlation between the expression of \( fgf23 \) and the expression of osteocalcin and ALP, suggesting that activation of osteoblasts is important in the regulation of FGF-23. Serum FGF-23 levels decreased in the transgenic mice after parathyroidectomy. In conclusion, PTH plays a major role in the regulation of serum FGF-23 level in primary hyperparathyroidism, likely via activation of osteoblasts in bone.


An excess of fibroblast growth factor-23 (FGF-23), a member of the FGF family, is now known to be a major factor in the development of hypophosphatemic rickets/osteomalacia, including X-linked hypophosphatemic rickets and oncogenic osteomalacia.1–3 Autosomal dominant hypophosphatemic rickets, a similar disorder characterized by renal phosphate wasting, has been reported to be associated with mutations of the FGF-23 gene4 that prevent its cleavage.5 Administration of recombinant FGF-23 decreases serum phosphate levels in mice by increasing renal phosphate excretion.6 Implantation of Chinese hamster ovary cells stably expressing FGF-23 into mice led to hypophosphatemic rickets \textit{in vivo},6 indicating the importance of FGF-23 in the development of hypophosphatemic rickets.

Plasma FGF-23 levels are directly and significantly correlated with serum parathyroid hormone (PTH), calcium, and phosphate levels in uremic patients who are on maintenance hemodialysis.7,8 There is some evidence that plasma FGF-23 levels may be regulated or affected by dietary phosphate. Recently, dietary consumption of phosphate was...
shown to regulate serum FGF-23 levels in both uremic rats and nonuremic mice. In addition, 1,25 dihydroxyvitamin D3 [1,25(OH)2D3] upregulates serum FGF-23 level in mice and in thyroparathyroidectomized rats without a corresponding increase in serum phosphate levels, suggesting a role for both dietary phosphate and 1,25(OH)2D3 in FGF-23 secretion. Elevated FGF-23 levels have also been reported in patients with primary hyperparathyroidism (PHPT). FGF-23 concentrations are significantly correlated with serum calcium and intact PTH levels and inversely correlated with creatinine clearance and calcium independently associated factors. Although both of these clinical studies suggested the importance of PTH action in the regulation of FGF-23 in patients with PHPT, serum FGF-23 levels decreased significantly after parathyroidectomy (PTX) in one study, whereas the decrease was NS in the other.

Here we used PTH-cyclin D1 transgenic (PC2) mice, which exhibit parathyroid-targeted overexpression of the human cyclin D1 oncogene, as a model of PHPT. These mice develop not only abnormal parathyroid cell proliferation but also chronic biochemical hyperparathyroidism, with characteristic abnormalities in bone and, notably, a shift in the relationship between serum calcium and PTH. These mice eventually exhibited adenomatous-appearing parathyroid region with reduced calcium-sensing receptor expression. In this study, we attempted to determine the roles of PTH in the regulation of serum FGF-23 levels using PC2 mice. To investigate the sources of serum FGF-23 in mice, we analyzed fgf23 expression in various tissues by quantitative real-time reverse transcriptase–PCR (RT-PCR) and examined the correlation among fgf23 expression, other biochemical markers, and the circulating levels of the protein. We also performed PTX in PC2 mice to determine the influence of PTH oversecretion on elevated serum FGF-23 levels.

RESULTS

Serum FGF-23 in PC2 Mice

At 27 to 33 wk, the PC2 mice already showed significantly higher serum calcium levels than age-matched wild-type (WT) mice, and, at older ages, the mice exhibited typical signs of biochemical hyperparathyroidism, such as hypercalcemia, hypophosphatemia, and elevated PTH levels (Table 1). PC2 mice had significantly higher levels of serum FGF-23 than WT mice at all ages examined, and the levels increased significantly with age, reaching three times those of WT mice in 90- to 118-wk-old mice (Figure 1). Serum FGF-23 levels were significantly directly correlated with serum PTH and calcium levels and inversely correlated with serum phosphate levels in 90- to 118-wk-old mice (Figure 2). Serum 1,25(OH)2D levels were directly correlated with serum FGF-23 levels but not significantly. Serum urea nitrogen levels were not significantly different between PC2 mice and age-matched WT mice at any age (Table 1).

Tissue Expression of fgf23 and Osteoblastic Markers

For investigation of the sources of serum FGF-23 in mice, fgf23 expression was analyzed in various tissues from 90- to 118-wk-old PC2 and WT mice using quantitative real-time PCR. High fgf23 expression was observed in femur and calvaria and was 1.8 and 14.4 times higher than in the thymus of WT mice, respectively (Figure 3A). In PC2 mice, fgf23 expression was 15.2- and 54.6-fold in the femur and calvaria, respectively, compared with that in the thymus of WT mice (Figure 3A). When fgf23 expression was examined in more detail in bone, it was found to be 20.2-fold higher in calvaria and 2.7-fold in the femur of PC2 mice than in the same sites of WT mice (Figure 3B). The highest expression was observed in PC2 calvaria. The fgf23 expression levels in calvaria were significantly directly correlated with serum FGF-23 levels (Figure 4). fgf23 expression levels in calvaria were also significantly directly correlated with ALP and osteocalcin expression levels (Figure 5).

Effect of PTX on Serum FGF-23 Levels

For investigation of the effect of PTH on serum FGF-23 levels, PTX was performed on 60- to 75-wk-old PC2 mice. The success of the PTX was confirmed by the presence of significant decreases in serum calcium and PTH levels and a significant

Table 1. Serum biochemistries of experimental mice

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>Genotype</th>
<th>n</th>
<th>Calcium (mg/dl)</th>
<th>Phosphate (mg/dl)</th>
<th>PTH (pg/ml)</th>
<th>1,25(OH)2D (pg/ml)</th>
<th>SUN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27 to 33</td>
<td>WT</td>
<td>8</td>
<td>8.1 ± 0.1</td>
<td>7.0 ± 0.6</td>
<td>52 ± 7</td>
<td>138 ± 18</td>
<td>30 ± 4</td>
</tr>
<tr>
<td></td>
<td>PC2</td>
<td>11</td>
<td>9.2 ± 0.1b</td>
<td>8.3 ± 0.5</td>
<td>54 ± 4</td>
<td>159 ± 8</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>60 to 75</td>
<td>WT</td>
<td>8</td>
<td>8.0 ± 0.1b</td>
<td>8.0 ± 0.5</td>
<td>81 ± 22</td>
<td>141 ± 14</td>
<td>24 ± 1</td>
</tr>
<tr>
<td></td>
<td>PC2</td>
<td>10</td>
<td>9.5 ± 0.2bc</td>
<td>5.6 ± 0.4b,c</td>
<td>153 ± 21c</td>
<td>113 ± 22c</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>90 to 118</td>
<td>WT</td>
<td>6</td>
<td>8.9 ± 0.2c</td>
<td>6.3 ± 0.3</td>
<td>45 ± 9</td>
<td>119 ± 13</td>
<td>24 ± 1</td>
</tr>
<tr>
<td></td>
<td>PC2</td>
<td>18</td>
<td>11.8 ± 0.4b,c,d</td>
<td>3.7 ± 0.2b,c,d</td>
<td>158 ± 24b,c</td>
<td>190 ± 13b,c</td>
<td>23 ± 1</td>
</tr>
</tbody>
</table>

*P = 0.048*  
*P = 0.001*  
*P = 0.023*  
*P = 0.760*

aMale/female ratios were similar between PC2 and WT mice. Data are means ± SEM. P values of changes in serum parameters were calculated by two-way ANOVA. SUN, serum urea nitrogen.  
bP < 0.05 versus same-age WT mice.  
cP < 0.05 versus PC2 mice at 27 to 33 wk.  
dP < 0.05 versus PC2 mice at 60 to 75 wk by post hoc analysis (Games-Howell).
increase in serum phosphate level 72 h after the PTX compared with pre-PTX levels (Table 2). By 72 h after PTX, serum FGF-23 levels had significantly increased from 358 ± 35 to 196 ± 19 pg/ml (P < 0.001; Figure 6), reaching the levels of age-matched WT mice (193 ± 23 pg/ml; P = 0.938 versus PC2 mice 72 h after PTX).

**DISCUSSION**

FGF-23 is a key regulator of serum phosphate and 1,25(OH)₂D, but how it is regulated by PTH is still unclear. Our observations suggest that, in a mouse model of PHPT, the calvaria is a major source of circulating FGF-23, likely via osteoblast activation. Serum PTH and calcium levels highly correlated with serum FGF-23 levels, whereas serum phosphate seemed to have minor roles in FGF-23 regulation in PHPT mice with normal kidney function. The decrement in serum FGF-23 levels in PC2 mice after PTX suggests that PTH functions to stimulate FGF-23 secretion.
The expression of bone, seemed to be a major determinant of serum FGF-23 in healthy human cortical bones. Expression of FGF-23 by PTH.

In previous studies, 1,25(OH)2D3 was found to upregulate serum FGF-23 levels in mice12,13 and in rats.9 Overexpression of a dominant negative vitamin D receptor inhibited 1,25(OH)2D3 stimulation of fgf23 promoter activity in vitro in osteoblasts,26 which suggested that 1,25(OH)2D3 is an important regulator of FGF-23 production in bone. Elevated serum calcium levels induced by oversecretion of PTH were observed in patients with PHPT, and elevated 1,25(OH)2D levels may enhance FGF-23 production in bone. In this study, serum 1,25(OH)2D levels were directly correlated with serum FGF-23 levels but not significantly. In addition, a decrease in circulating FGF-23 was observed after PTX. In contrast, the decrease in the level of 1,25(OH)2D after PTX was not statistically significant. Similar results have been obtained in patients with PHPT.15 These observations suggest that 1,25(OH)2D is a positive regulator of serum FGF-23 levels but that its role is limited in PHPT.

FGF-23 has a phosphaturic effect, and its circulating levels may also be regulated or affected by serum phosphate levels. In uremic patients who are on maintenance hemodialysis, plasma FGF-23 levels are elevated and are correlated with inorganic phosphate, PTH, and corrected calcium.7,8 Serum FGF-23 levels are also correlated with serum phosphate levels in uremic rats.9 Recently, a direct correlation between serum FGF-23 and serum phosphate levels was observed in nonuremic mice consuming dramatically changed amounts of dietary phosphate.11 In contrast, no correlation between FGF-23 and serum phosphate levels was observed in nonuremic healthy humans,8,27 and a inverse correlation was seen in patients with PHPT.14,15 Even when alimentary intake of phosphorus is excessive, surplus phosphate is excreted very quickly by the kidneys, and hyperphosphatemia is not usually observed in healthy humans. In addition, hyperphosphatemia would be expected to occur in patients with PHPT secondary to elevated PTH levels. In this study, the inverse correlation between FGF-23 and phosphate levels was confirmed in vivo in a model of PHPT. Elevated FGF-23 levels in PHPT would presumably enhance the phosphaturia that is already accelerated by elevated PTH levels, resulting in hypophosphatemia.

Our observations suggest that FGF-23 secreted from bone, especially cortical bone such as calvaria, may contribute to elevated serum FGF-23 levels in hyperparathyroidism. PTH is a potential stimulator of the production of FGF-23. The combined reduction in serum phosphate by FGF-23 and PTH may prevent tissue damage, for example by preventing ectopic calcification by lowering the serum calcium-phosphate product in the presence of hypercalcemia caused by oversecretion of PTH in PHPT.
Table 2. Changes in serum parameters after PTX in 60- to 75-wk-old PC2 micea

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before PTX</th>
<th>After PTX</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH (pg/ml)</td>
<td>153 ± 21</td>
<td>18 ± 3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>calcium (pg/ml)</td>
<td>9.5 ± 0.2</td>
<td>7.5 ± 0.3</td>
<td>0.0035</td>
</tr>
<tr>
<td>phosphate (pg/ml)</td>
<td>5.6 ± 0.4</td>
<td>7.9 ± 0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1,25(OH)2D (pg/ml)</td>
<td>113 ± 22</td>
<td>88 ± 12</td>
<td>0.3348</td>
</tr>
</tbody>
</table>

aData are means ± SEM of 10 mice. Blood samples were obtained before and 72 h after PTX. P values were calculated by the paired t test.

Figure 6. PTX decreases serum FGF-23 levels in PC2 mice. PTX was performed on 60- to 75-wk-old PC2 mice. Blood samples were obtained before and 72 h after PTX. Values for each mouse are represented (○), along with the means ± SE of 10 mice (●). ¶P = 0.0012 by paired t test.
uative School of Pharmaceutical Sciences) for providing us with the m-fgf23 cDNA and Dr. Mitsuru Fukui (Osaka City University Graduate School of Medicine) for assistance with statistical analyses.

DISCLOSURES
None.

REFERENCES