Phosphatemic effect of cinacalcet in kidney transplant recipients with persistent hyperparathyroidism

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Abstract

BACKGROUND: In kidney transplant recipients, persistent hyperparathyroidism leads to hypercalcemia and increased urinary phosphorus excretion. The calcimimetic drug cinacalcet effectively decreases parathyroid hormone (PTH) levels and corrects hypercalcemia in these patients. The purpose of the present study is to examine the effect of cinacalcet treatment on determinants of renal phosphorus reabsorption under steady-state conditions. STUDY DESIGN: Open-label prospective uncontrolled trial. SETTING & PARTICIPANTS: 10 stable kidney transplant recipients with persistent hyperparathyroidism. INTERVENTION: Cinacalcet, 30 and 60 mg/d, for 2 weeks. OUTCOMES & MEASURES: Changes in urinary phosphorus excretion in timed urine samples, intact and carboxy-terminal (C-term) fibroblast growth factor 23 (FGF-23), intact PTH, venous pH, and bicarbonate values at defined intervals over 24 hours. RESULTS: Cinacalcet decreased renal phosphorus excretion in the first 8 hours by 30% to 40%, but not from 8 to 24 hours after drug administration. Serum phosphorus levels normalized in all patients. Cinacalcet markedly decreased plasma intact PTH levels (60%; P < 0.001). Cinacalcet also decreased mean intact FGF-23 levels from 67 +/- 8 (SE) to 51 +/- 5 and to 54 +/- 6 pg/mL (P < 0.001) and mean C-term FGF-23 levels from 108 +/- 15 to 87 +/- 9 and to 101 +/- 9 RU/mL (P < 0.01), respectively. There was high correlation between intact FGF-23 and C-term FGF-23 levels (r = 0.598; P < 0.001). Acid-base status was unchanged. LIMITATIONS: This is a small study and does not examine the long-term effect of cinacalcet treatment. CONCLUSIONS: Cinacalcet effectively corrected urinary phosphate wasting in kidney transplant recipients, resulting in normalization of serum phosphorus levels. The phosphatemic effects of cinacalcet correlated with a marked decrease in the phosphaturic hormone PTH, rather than with a change in FGF-23 levels or acid-base status, highlighting the importance of PTH in posttransplantation hypophosphatemia.
Background: In kidney transplant recipients, persistent hyperparathyroidism causes hypercalcemia and increased urinary phosphorus excretion. The amount of renal phosphorus excretion is determined largely by glomerular filtration and tubular reabsorption. PTH decreases renal phosphorus reabsorption through internalization of the tubular type 2a sodium-phosphate transporter and by decreasing type 2a sodium-phosphate transporter gene transcription.1,2 The recently described bone hormone fibroblast growth factor 23 (FGF-23) decreases phosphorus reabsorption through changes in the type 2a sodium-phosphate transporter and suppresses 1α-hydroxylase activity in the kidney.3,4 Circulating FGF-23 levels are increased in patients with primary,5,6 secondary,7,9 and persistent hyperparathyroidism10,11 and contribute to the development of early posttransplantation hypophosphatemia.12

Calcimimetics decrease PTH production and secretion by increasing the sensitivity of the calcium-sensing receptor to calcium on chief cells of the parathyroid gland.13 Cinacalcet is a calcimimetic agent approved for treatment of patients with primary and secondary hyperparathyroidism. We and others have examined the effect of cinacalcet in kidney transplant recipients with persistent hyperparathyroidism and shown that cinacalcet treatment decreased serum calcium and PTH levels.14,15 Interestingly, most, but not all, studies reported that cinacalcet also...
increased phosphorus levels in such patients.\textsuperscript{14-18}

We recently studied the correlation of steady-state pharmacokinetics with the pharmacodynamic effects of cinacalcet in 10 kidney transplant recipients with persistent hyperparathyroidism and showed that cinacalcet dose-dependently decreased ionized serum calcium levels and increased urinary calcium excretion significantly in the first 8 hours after administration of 60 mg of cinacalcet. Kidney function assessed by using measured creatinine clearance (46 mL/min 1.73 m\textsuperscript{2}) was stable (creatinine clearance in mL/min/1.73 m\textsuperscript{2} may be converted to mL/s/1.73 m\textsuperscript{2} by multiplying by 0.01667). 1,25-Dihydroxyvitamin D\textsubscript{3} and 25-hydroxyvitamin D\textsubscript{2} levels remained in the normal range.\textsuperscript{17} The purpose of the present study is to examine the effect of cinacalcet treatment on determinants of renal phosphorous reabsorption in these 10 patients, in particular on the phosphatonin FGF-23. The improvement in urinary phosphate wasting appears to result from a cinacalcet-mediated decrease in PTH levels, rather than an effect of the phosphaturic hormone FGF-23 or acid-base status, highlighting the importance of PTH in posttransplantation hypophosphatemia.

**METHODS**

Ten kidney transplant recipients with persistent hyperparathyroidism and stable allograft function were studied. The local ethics committee approved the study, and all patients gave their written informed consent. The study design and parts of the results have been published elsewhere.\textsuperscript{17} Briefly, cinacalcet was administered at 30 mg/d for 2 weeks, followed by another 2 weeks with 60 mg/d. Patients were studied under steady-state conditions at the end of each 2-week treatment period. On the morning of the study days and after overnight fasting, blood samples were drawn from a forearm vein through an intravenous catheter at 8:00 AM (study time 0 hours) and at various times thereafter until 8:00 AM the following day. Cinacalcet was administered at 8:00 AM after the first blood sampling. Phosphorus excretion was measured in 2 timed urine samples collected between 8:00 AM and 4:00 PM (8-hour sample) and 4:00 PM and 8:00 AM (16-hour sample) the following day.

After the 8:00 AM blood collection, patients had a standardized breakfast (120 g of scrambled eggs, 2 slices of toasted white bread, 1 tablespoon of fruit preserve, 1 tablespoon of margarine, 60 g of melon, 120 g of apple juice) containing approximately 300 mg of phosphorus and 120 mg of calcium. At noon, they ingested a light meal, and supper was approximately 300 mg of phosphorus and 120 mg of calcium. At 8:00 AM the following day. Cinacalcet was administered at 30 mg/d for 2 weeks, followed by another 2 weeks with 60 mg/d. Patients were studied under steady-state conditions at the end of each 2-week treatment period. On the morning of the study days and after overnight fasting, blood samples were drawn from a forearm vein through an intravenous catheter at 8:00 AM (study time 0 hours) and at various times thereafter until 8:00 AM the following day. Cinacalcet was administered at 8:00 AM after the first blood sampling. Phosphorus excretion was measured in 2 timed urine samples collected between 8:00 AM and 4:00 PM (8-hour sample) and 4:00 PM and 8:00 AM (16-hour sample) the following day.

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**Laboratory Analysis**

Intact FGF-23 (Kainos Laboratories Inc, Tokyo, Japan) and carboxy-terminal (C-term) FGF-23 (Immutopics Inc, San Clemente, CA) were measured using specific enzyme-linked immunoassorbent assay kits according to the manufacturer’s protocol. Venous bicarbonate and pH were determined immediately after collection in electrolyte-balanced heparin tubes (1 mL) using automated blood gas analysis (ABL 815 Flex; Radiometer, Copenhagen, Denmark). Phosphorus and calcium were measured by using standard methods; creatinine, by using the Jaffé method; and plasma intact PTH (iPTH), by using a double-antibody chemiluminescence immunoassay (Roche Diagnostics, Basel, Switzerland). Deoxypyridinoline-creatinine ratio was determined in the morning spot urine sample. All biochemical analyses were performed at the Department of Clinical Chemistry of our institution.

**Statistical Analysis**

Mean values for continuous data were compared by using Student t-test. All P values were 2 sided for comparison with baseline values, and P less than 0.05 is considered statistically significant. Results are expressed as mean ± SE. Pearson correlation coefficient was used to test for associations between continuous variables. One-way analysis of variance for repeated measures with Bonferroni post hoc test was used when analyzing time course profiles of iPTH, intact FGF-23, and C-term FGF-23.

**RESULTS**

Ten kidney transplant recipients (6 men and 4 women aged 58.3 ± 2.6 years) with stable graft function (glomerular filtration rate [GFR] > 40 mL/min/1.73 m\textsuperscript{2}, estimated by using the Modification of Diet in Renal Disease Study equation) were included in the study a median of 3.3 years (range, 0.8 to 31.3 years) after transplantation. Kidney function was stable throughout the study. The individual immunosuppressive regimens included cyclosporine (8 patients), mycophenolate mofetil (6 patients), azathioprine (3 patients), prednisone (3 patients, all ≤ 10 mg/d), and tacrolimus (1 patient) and were unchanged during the study. Table 1 lists baseline laboratory data.

As shown in Fig 1, cinacalcet treatment increased serum phosphorus levels dose dependently and decreased the inappropriate increased fractional excretion of phosphorus by approximately one-third in the first 8 hours after cinacalcet administration. Fractional excretion of phosphorus from 4:00 PM to 8:00 AM on the following day did not change. Urinary total phosphorus excretion in first 8 hours (17.5 ± 3.2 to 10.9 ± 2.0 to 16.5 ± 2.9 mg; P = 0.2) and the subsequent 16 hours (46.6 ± 6.1 to 43.8 ± 9.8 to 44.6 ± 9.6 mg; P = 0.5) was not changed by cinacal-
Cinacalcet treatment. Serum phosphorus levels remained less than 4.5 mg/dL in all patients and at any sampling time. To elucidate whether the cinacalcet-induced decrease in phosphorus excretion was associated with changes in hormones that influence phosphorus levels, we measured PTH and FGF-23 during the course of 24 hours under steady-state conditions. As previously shown, at baseline, iPTH levels were increased about 4 times the upper normal level and cinacalcet treatment decreased iPTH levels in the first 8 hours after dosing significantly by approximately 60% at nadir (Fig 2). Intact and C-term FGF-23 levels amounted to approximately twice the upper limit of the normal range at baseline at time 0 hour. Treatment with 30 and 60 mg/d of cinacalcet for 2 weeks decreased intact FGF-23 levels from 67 ± 8.5 to 51 ± 4.5 and to 54.1 ± 7.7 pg/mL (P < 0.001) and C-term FGF-23 levels from 108 ± 14.6 to 87.3 ± 8.5 and to 100.8 ± 13.9 RU/mL (P < 0.01), respectively (Fig 3). Intact FGF-23 levels had a tendency to increase after the sampling at 8:00 AM. This kind of circadian rhythm of intact FGF-23 levels also was seen during cinacalcet treatment and was less pronounced.

Table 1. Baseline Laboratory Data for the 10 Study Patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Range</th>
<th>Baseline</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.4-10.2</td>
<td>10.1 ± 0.2</td>
<td>9.3 ± 0.3</td>
<td>8.5 ± 0.4</td>
</tr>
<tr>
<td>Intact FGF-23 (pg/mL)</td>
<td>29.7 ± 20.7*</td>
<td>67.2 ± 8.5</td>
<td>51.4 ± 4.5</td>
<td>54.1 ± 7.7</td>
</tr>
<tr>
<td>Carboxy-terminal FGF-23 (RU/mL)</td>
<td>72.9 ± 38.2*</td>
<td>108.1 ± 14.6</td>
<td>87.3 ± 8.5</td>
<td>100.8 ± 13.9</td>
</tr>
<tr>
<td>Serum bicarbonate (mEq/L)</td>
<td>20-25</td>
<td>22.2 ± 0.2</td>
<td>21.9 ± 0.8</td>
<td>22.4 ± 0.8</td>
</tr>
<tr>
<td>pH</td>
<td>7.36-7.40</td>
<td>7.33 ± 0.04</td>
<td>7.33 ± 0.01</td>
<td>7.34 ± 0.01</td>
</tr>
<tr>
<td>Urinary deoxypyridinoline-creatinine ratio (nmol/mmol)</td>
<td>2.5-9.5</td>
<td>11.1 ± 2.5</td>
<td>10.6 ± 2.2</td>
<td>10.5 ± 1.4</td>
</tr>
</tbody>
</table>

*Assay mean ± SD value derived from 118 normophosphatemic individuals.27

Note: Calcium in mg/dL may be converted to mmol/L by multiplying by 0.2495. Serum bicarbonate levels expressed in mEq/L and mmol/L are equivalent.

Abbreviation: FGF-23, fibroblast growth factor 23.

Figure 1. Serum phosphorus levels and renal excretion of phosphorus. (A) Serum phosphorus levels increased 8 and 24 hours after cinacalcet administration. (B) Urinary phosphorus excretion in the first 8 hours was decreased by cinacalcet. Values shown as mean ± SE. Two-sided t-test. *P < 0.05, **P < 0.01. Note: Serum phosphorus in mg/dL may be converted to mmol/L by multiplying by 0.3229. Abbreviation: FEPO4, fractional excretion of filtered phosphate.

Figure 2. Time course profiles of percentage of change in intact parathyroid plasma (△iPTH) levels. Cinacalcet decreased iPTH levels, expressed as mean percentage of baseline values (dotted line, values at day 0 = 0%) in the first 8 hours after administration of 30 (open triangle) and 60 mg/d (closed square) of cinacalcet (P < 0.001). Values shown as mean ± SE.
with C-term FGF-23. The variation in intact FGF-23 levels was smaller compared with C-term FGF-23, as shown by a tighter SD. Correlation between intact FGF-23 and C-term FGF-23 levels was high ($r = 0.598; P < 0.001$; Fig 4).

Cinacalcet treatment did not change acid-base status, assessed by means of venous pH and bicarbonate level or urinary deoxypyridinolincreatinine ratio (Table 1).

**DISCUSSION**

Cinacalcet represents a promising therapeutic agent to treat renal allograft recipients with persistent posttransplantation hyperparathyroidism with hypercalcemia. We and others have shown that cinacalcet effectively decreases levels of the phosphaturic hormone PTH in such patients. The aim of the present study is to gain insight into the effect of cinacalcet on determinants of renal phosphorus reabsorption in kidney transplant patients with persistent hyperparathyroidism.

Renal allograft recipients with persistent hyperparathyroidism frequently show hypophosphatemia and increased rates of fractional excretion of phosphorus in the setting of stable graft function. The kidney is one of the major phosphorus-handling organs, and administration of cinacalcet for 2 weeks significantly decreased renal phosphorus excretion and increased serum phosphorus levels in our kidney transplant recipients with persistent hyperparathyroidism. A similar phosphatemic action of cinacalcet was seen in patients with stages 3 and 4 chronic kidney disease (CKD) with secondary hyperparathyroidism. In these patients, treatment with cinacalcet decreased urinary phosphorus excretion by approximately 10%, resulting in a significant increase in serum phosphorus levels from 4 to 5 mg/dL. In our study, levels of both phosphaturic hormones, PTH and FGF-23, were markedly increased at baseline. Cinacalcet treatment was very effective in decreasing iPTH levels, whereas intact and C-term FGF-23 levels were only moderately decreased. When PTH levels were lowest, urinary phosphorus excretion was decreased, although FGF-23 levels remained increased. Our finding that renal phosphorus absorption significantly decreased in the presence of increased
FGF-23 levels indicates that the effect of these phosphaturic hormones in renal phosphorus absorption is dissociated and highlights the importance of PTH reduction in increasing serum phosphorus levels in our patients. A similar situation is present in patients with tumor-induced osteomalacia (TIO). TIO, a rare acquired disease of renal phosphate wasting, is caused by mesenchymal tumors that produce FGF-23. In these patients, cinacalcet treatment decreased PTH and renal phosphorus excretion despite unchanged high FGF-23 levels. In our study, the better correction of serum phosphorus level with the higher cinacalcet dose did not result in a further decrease in FGF-23 levels, a finding that further supports our hypothesis that PTH reduction has a major role in the cinacalcet-mediated increase in serum phosphorus concentration. Hence, the phosphatemic action of cinacalcet appears to be mediated mainly by the decrease in PTH levels, and the decrease in FGF-23 levels contributes less to the cinacalcet-mediated decrease in urinary phosphorus excretion in kidney transplant recipients.

FGF-23 concentrations are increased in patients with CKD even before hyperphosphatemia appears, but the stimulus driving this increase is unclear. It is attractive to postulate that FGF-23 synthesis is induced by changes in serum phosphorus levels in response to changes in dietary phosphorus intake. However, in a recent study by Isakova et al, urinary calcium and phosphorus excretion increased postprandial in normal healthy volunteers and patients with CKD, whereas PTH levels increased only in patients with CKD and thus may represent a novel mechanism of secondary hyperparathyroidism. In our patients, cinacalcet decreased PTH levels and urinary phosphorus excretion postprandial and may blunt this suggested novel renal compensatory mechanism. We previously reported that in our patients, 30 and 60 mg of cinacalcet decreased serum calcium levels and increased urinary calcium excretion in the first 8 hours after cinacalcet administration. Lower serum calcium levels in patients with decreased GFR could increase serum phosphorus levels through the combination of stimulated PTH secretion and a decrease in calcium-phosphate product. However, the phosphorus homeostasis in our cinacalcet-treated patients was influenced less likely by the normalization of serum calcium levels because PTH levels decreased and calcium-phosphate product was unchanged.

The relationship between different FGF-23 assays was assessed in normophosphatemic individuals and patients with TIO. Similar to our results, high correlation was found between the C-term FGF-23 assay from Immutopics and intact FGF-23 assay from Kainos. However, in patients with suspected and confirmed TIO, the greatest sensitivity was found for the Kainos intact FGF-23 assay. Interestingly, all our patients had increased intact FGF-23 levels at any sampling time comparing with the normal mean of the assay (29.7 pg/mL), whereas C-term FGF-23 levels were not greater than the mean of the assay (72.9 RU/mL) in 3, 5, and 4 patients at study days 0, 14, and 28, respectively. The Immutopics FGF-23 assay detects C-term fragments along with the biologically active molecule, and these fragments may accumulate in patients with decreased GFR, resulting in increased variability. Although kidney function was stable in our patients and despite the high correlation between the 2 assays, the greater variability of the Immu-
topics assay may explain its decreased assay sensitivity compared with the Kainos FGF-23 assay in our patients with GFR less than 60 mL/min 1.73 m². Overall, our results confirm the excellent sensitivity of the Kainos intact FGF-23 assay.

Once-daily administration of cinacalcet was sufficient to normalize serum phosphorus levels. Interestingly, patients on dialysis therapy with kidney function that is completely abolished show a decrease in serum phosphorus levels in response to cinacalcet, suggesting a kidney-independent effect of cinacalcet on serum phosphorus levels. Serum phosphorus concentration is regulated not only by renal reabsorption, but also by the interaction between renal absorption and exchange with the bone. Release of phosphorus from the bone occurs as a consequence of increased osteoclast activity that is regulated by several hormones, including PTH and calcitonin. We previously showed that cinacalcet treatment decreased PTH and increased calcitonin levels, with both changes in favor of increased bone formation. Furthermore, given the calcium-lowering effect of cinacalcet and the unchanged marker for bone resorption (deoxypyridinoline-creatinine ratio), the phosphatemic action of cinacalcet in kidney transplant recipients is less likely to depend on a substantial release of phosphorus from the bone.

A limitation of our study is that intestinal phosphorus absorption was not determined. Patients were fasting 12 hours before study visits, and phosphorus intake was kept constant on the study morning, but we cannot exclude that a cinacalcet-mediated change in intestinal phosphorus absorption contributed to the phosphatemic action of cinacalcet. Second, factors regulating cellular phosphorus uptake are not well defined. Acid-base changes can cause profound changes in serum phosphorus levels by translocation of phosphorus in and out of cells. We could show that acid-base status in our patients was unchanged. In addition to FGF-23, other phosphatases, such as frizzled-related protein 4 and FGF-7, could have influenced the phosphatemic action of cinacalcet.\textsuperscript{10,29}

In conclusion, cinacalcet effectively corrected inappropriate urinary phosphate wasting and normalized serum phosphorus levels in renal allograft recipients with hypophosphatemia caused by persistent hyperparathyroidism. Overall, the cinacalcet-induced increase in renal phosphorus reabsorption appears to result predominantly through decreasing PTH levels.

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\textbf{REFERENCES}


