Hyperphosphatemia Accelerates Parathyroid Cell Proliferation and Parathyroid Hormone Secretion in Severe Secondary Parathyroid Hyperplasia

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Abstract. We studied the role of phosphorus retention in parathyroid cell proliferation and parathyroid hormone (PTH) oversecretion in severe secondary parathyroid hyperplasia. Mice transplanted with human parathyroid tissue from a patient who had undergone parathyroidectomy for severe secondary hyperparathyroidism were divided into four groups; each group was given a diet with a different phosphorus content (0.4, 0.7, 1.0, and 1.2%) to alter serum phosphorus concentrations. Histologic examinations of grafts by hematoxylin-eosin or by bromodeoxyuridine (BrdU) immunohistochemical staining were performed to assess parathyroid cell proliferation. Changes in serum phosphorus concentrations unidirectionally affected PTH secretion from the graft, because human PTH did not cross-react with mouse PTH. Serum phosphorus concentrations of 1.0P and 1.2P groups were significantly higher than those of 0.4P and 0.7P groups (p<0.05). Serum phosphorus concentrations were significantly correlated with the gradient of human PTH elevation with a coefficient of 0.48 and a p<0.05. Furthermore, serum phosphorus concentrations and the gradient of human PTH elevation were significantly higher in mice with BrdU-immunoreactive cells in the parathyroid graft than in mice without immunoreactive cells in the graft. These results indicate that uncontrolled hyperphosphatemia may accelerate the proliferation of parathyroid cells, exacerbating PTH oversecretion.

Key words: Secondary hyperparathyroidism, Hyperphosphatemia, Transplantation, Nude mouse, Bromodeoxyuridine

THE main factors involved in the pathogenesis of secondary hyperparathyroidism include phosphorus retention, decreased concentrations of calcitriol, and hypocalcemia [1–5]. Because these factors are interrelated, it is difficult to analyze the individual effects on parathyroid disease. Several investigators have provided evidence for a direct role for phosphorus retention in the pathogenesis of secondary hyperparathyroidism [6–9]. These in vivo and in vitro studies were conducted in the setting of mild parathyroid hyperplasia associated with moderate chronic renal failure which did not require hemodialysis. Therefore, a direct effect of phosphorus retention on parathyroid hormone (PTH) oversecretion and parathyroid gland growth has never been reported in severe secondary parathyroid hyperplasia associated with end-stage chronic renal failure requiring long-term hemodialysis. The present study was designed to further understand the role of phosphorus retention in the parathyroid cell proliferation and PTH oversecretion of severe secondary parathyroid hyperplasia. Important
characteristics of this study are as follows: (a) Parathyroid tissue from a patient with severe secondary parathyroid hyperplasia was transplanted into nude mice [10]. (b) The calcium and phosphorus metabolism in mice unidirectionally affected PTH secretion and cell proliferation of the graft [11, 12]. (c) Mice received a longer period of stimulation with hypo- or hyperphosphatemia compared to previous studies.

Materials and Methods

Human Parathyroid Tissue Transplantation and Administration of Diets

Specimens were obtained from the nodular hyperplastic gland of a patient with severe secondary hyperparathyroidism who was receiving long-term hemodialysis. Informed consent was obtained from this patient. The patient received vitamin D pulse therapy and became refractory to this therapy [13]. The weight of the nodular hyperplastic gland was 3418 mg. Serum chemistries before surgery showed a calcium concentration of 10.0 mg/dL and a phosphorus concentration of 6.8 mg/dL. A serum intact PTH assay showed a PTH concentration of 1200 pg/mL. Tissue specimens were cut into 1-mm³ pieces and inserted into pockets prepared in the gluteus muscles of recipient mice (female nude mice (KSNnu/nu)). Each mouse received one piece of the tissue (n=20). Animals were maintained in sterile cages with free access to water and food pellets for 12 weeks following transplantation.

Because graft function becomes stable 4 weeks following transplantation [14], all animals were given a normal diet for 4 weeks. Thereafter, animals were randomly divided into four groups after blood sampling for baseline measurement of intact human PTH (hPTH) concentrations. Four diets of different phosphorus contents (0.4, 0.7, 1.0, and 1.2% phosphate content; denoted as 0.4, 0.7, 1.0, and 1.2P-diet, respectively) were prepared in order to alter serum concentrations of phosphorus in the mice. Vitamin D was adequately contained in all diets (240 IU/100 g). Each group of mice was fed one of these diets for 8 weeks beginning at the 5th week after transplantation. Serum concentrations of intact hPTH were measured again 12 weeks following transplantation. Changes in hPTH secretion were evaluated by calculating the PTH gradient (the ratio of serum hPTH concentrations at 4 and 12 weeks following transplantation). Serum calcium and phosphorus concentrations were determined 12 weeks following transplantation.

Histologic Examination

Twelve weeks following transplantation, all animals were given intraperitoneal bromodeoxyuridine (BrdU) in saline (40 μg/g body weight) 4 hours before being killed. The grafts were resected and fixed in 70% ethanol for 16 hours, embedded in paraffin, and cut into sections 3 μm thick. Continuous sections were stained with hematoxin and eosin (H-E), and by a BrdU immunohistochemical procedure described elsewhere [15]. A negative control was prepared by replacing the primary antibody with nonimmune serum.

Determination of Intact hPTH, Calcium and Phosphorus Concentrations

Human PTH concentrations were determined in aliquots of serum that had been stored at −80°C. A double-antibody immunoradiometric assay (Allegro Intact PTH; Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) was used that did not cross-react with mouse PTH. Serum concentrations of total calcium and phosphorus were measured with an automatic analyzer (736-60; Hitachi, Tokyo, Japan).

Statistical Analysis

Data were expressed as means±SD. Correlation coefficients were calculated by linear regression analysis. PTH gradient, serum phosphorus and calcium concentrations, and calcium-phosphorus solubility products (Ca × P) were analyzed by the Cox proportional hazard regression model (univariate and multivariate). A level of p<0.05 was accepted as statistically significant.

Results

One of 20 mice died because of weakness 9 weeks following transplantation. Therefore, only the data
from 19 mice could be analyzed.

Use of four diets which differed in phosphorus content led to remarkable variation in serum phosphorus concentrations (Fig. 1). Serum phosphorus concentrations in 0.4P, 0.7P, 1.0P, and 1.2P groups were 4.6±0.9, 5.2±1.1, 7.1±0.5, and 7.2±0.5 mg/dL, respectively. Those of 1.0P and 1.2P groups were significantly higher than those of 0.4P and 0.7P groups (p<0.05). Serum phosphorus concentrations varied from 3.5 to 7.8 mg/dL (mean±SD, 6.0±1.4 mg/dL). Serum calcium concentrations in 0.4P, 0.7P, 1.0P, and 1.2P groups were 8.5±0.7, 8.5±0.2, 7.7±0.4, and 8.1±0.9 mg/dL, respectively. There were no significant differences in serum calcium concentrations of the four groups. Serum calcium concentrations varied from 7.1 to 9.4 mg/dL (mean±SD, 8.2±0.6 mg/dL) with a smaller range than phosphorus concentrations. Serum phosphorus concentrations were not correlated with serum calcium concentrations (r = -0.38, p=0.1, Fig. 2). Calcium-phosphorus solubility products (Ca×P) varied from 27 to 65 (mean±SD, 49.2±10.2). Although serum calcium concentrations were not correlated with Ca×P (r = -0.05, p=0.85), serum phosphorus concentrations were significantly correlated with Ca×P (r=0.94, p<0.001).

Because serum hPTH concentrations reached a plateau 4 weeks after transplantation in mice fed with a normal diet in our previous study [10, 14], they were evaluated at 4 and 12 weeks following transplantation. Serum hPTH concentrations in the four
groups were shown in Fig. 3. PTH gradients (the ratio of serum hPTH concentrations at 4 and 12 weeks following transplantation) were determined. PTH gradients in 0.4P, 0.7P, 1.0P, and 1.2P groups were 1.7, 1.9, 3.1, and 3.7, respectively. Those of 1.0P and 1.2P groups were significantly higher than those of 0.4P and 0.7P groups (p<0.05). PTH gradients varied from 0.37 to 4.98. Because serum phosphorus and calcium concentrations are closely related to PTH secretion, multivariate analysis of variance was used to analyze the relationship of serum calcium and phosphorus concentrations to PTH gradients. Serum phosphorus concentrations were correlated with PTH gradients with a coefficient of 0.48 and a p<0.05. On the other hand, the correlation between PTH gradients and serum calcium concentrations was not statistically significant (p=0.07) (Table 1).

Table 1. Multiple regression analysis of serum calcium (Ca), and phosphorus (P) concentrations to PTH gradients.

| Variable | Parameter Estimate | Prob > |T| | Standardized Estimate |
|----------|--------------------|--------|--------|----------------------|
| Ca       | -66.983            | 0.070  | -0.37  |
| P        | 39.214             | 0.022  | 0.48   |

The proliferative activity of grafts was evaluated by detecting the incorporation of BrdU into parathyroid cells. BrdU incorporation was measured by visualizing DNA-synthesizing cells using an anti-BrdU monoclonal antibody. Staining was not observed in control sections incubated with non-immune serum. BrdU-immunoreactive cells were observed in 7 of 19 grafts. Mice with BrdU-immunoreactive cells were hyperphosphatemic, ranging

Fig. 4. Histologic findings (×200). A and C, HE stain; B and D, BrdU immunohistochemical stain. A and B were made from a graft in the mouse of serum phosphorus concentration, 7.8 mg/dL, serum calcium concentration, 7.8 mg/dL, and PTH gradient, 3.25. BrdU-immunoreactive cells were observed in B. C and D were made from a graft in the mouse of serum phosphorus concentration, 5.2 mg/dL, serum calcium concentration, 8.6 mg/dL, and PTH gradient, 1.78. BrdU-immunoreactive cells were not observed in D.
from 6.6 to 7.9 mg/dL. Serum phosphorus concentrations were significantly higher in the mice with BrdU-immunoreactive cells than in the mice without BrdU-immunoreactive cells (7.31 ± 0.35 versus 5.27 ± 0.27 mg/dL, respectively, p < 0.01) (Fig. 4). Furthermore, the PTH gradient was significantly higher in the mice with BrdU-immunoreactive cells than in the mice without BrdU-immunoreactive cells (3.54 ± 0.28 versus 1.85 ± 0.28, respectively, p < 0.001). On the other hand, serum calcium concentrations were not significantly different in the mice with and without BrdU-immunoreactive cells (Table 2).

**Table 2.** Differences in the mice with and without BrdU-immunoreactive cells.

<table>
<thead>
<tr>
<th></th>
<th>BrdU immunoreactive cell</th>
<th>- (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (mg/dL)</td>
<td>8.00±0.22</td>
<td>8.37±0.17</td>
</tr>
<tr>
<td>P (mg/dL)</td>
<td>7.31±0.35</td>
<td>5.27±0.27</td>
</tr>
<tr>
<td>Ca×P</td>
<td>58.3±2.8</td>
<td>43.9±2.2</td>
</tr>
<tr>
<td>PTH gradient</td>
<td>3.54±0.28</td>
<td>1.82±0.21</td>
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Ca, serum calcium concentration; P, serum phosphorus concentration; Ca×P, calcium-phosphorus solubility product

Discussion

This study consisted of functional and morphometric investigations concerning the effect of hyperphosphatemia on PTH oversecretion due to severe secondary parathyroid hyperplasia. We transplanted human parathyroid tissue obtained from a patient with severe secondary hyperparathyroidism into nude mice, and studied graft function and cell proliferation of the graft in mice fed with different amounts of dietary phosphorus for 2 months. Human PTH secreted from the parathyroid grafts did not cross-react with mouse PTH, and the elevated concentration of the human PTH did not elevate the serum calcium concentration in the mice. Therefore, the remarkably elevated human PTH in serum did not affect calcium and phosphorus metabolism in the mice. On the other hand, increased or decreased concentrations of serum phosphorus in the mice due to different amounts of dietary phosphorus unidirectionally influenced human PTH secretion from the graft. This experimental model can be used to separately analyze the relation between PTH secretion and hyperphosphatemia.

Four diets differing in phosphorus content led to remarkable variation in serum phosphorus concentrations of mice, irrespective of serum calcium concentrations. These results indicate that the diets disrupted calcium and phosphorus homeostasis in the mice. In addition, serum phosphorus concentrations strongly influenced changes in Ca×P products in this study. These changes influenced hPTH secretion from human parathyroid grafts. PTH gradients positively correlated with serum phosphorus concentrations. Therefore, hyperphosphatemia may directly affect PTH oversecretion irrespective of hypocalcemia. It has been suggested that persistent hyperphosphatemia increases the rate of parathyroid cell growth, resulting in acceleration of PTH oversecretion [7]. Recently, significant increases in parathyroid gland weight and parathyroid gland DNA demonstrated by phosphorus stimulation in uremic rats [6, 9, 16]. However, these results were obtained in experiments with mild parathyroid hyperplasia. In the present study, we did not find the weight increases of grafts during the experimental period of two months. Therefore, we investigated the effect of phosphorus retention on cell proliferation in severe secondary hyperplasia by BrdU-immunohistochemical staining. Serum phosphorus concentrations and PTH gradient were significantly higher in the mice with BrdU-immunoreactive cells than in the mice without BrdU-immunoreactive cells (3.54±0.28 versus 1.85±0.28, respectively, p<0.001). These results indicate that hyperphosphatemia accelerates PTH oversecretion through its influence on parathyroid cell proliferation in severe secondary parathyroid hyperplasia.

Hypocalcemia is also a prominent characteristic of secondary hyperparathyroidism at the early stages of chronic renal failure. However, hypocalcemia is not common in patients who undergo maintenance hemodialysis [17]. Therefore, hypocalcemia may not be an important factor in the progression of secondary hyperparathyroidism in long-term hemodialysis patients [18]. The effect of hyperphosphatemia is not as acute as that of hypocalcemia. Therefore, the effect of hyperphosphatemia and hypocalcemia on PTH oversecretion may be different. Uncontrollable hyperphosphatemia may accelerate the proliferation of parathyroid cells, resulting in increased PTH oversecretion.
In conclusion, we have demonstrated that hyperphosphatemia gradually induces parathyroid cell proliferation in human severe secondary hyperplastic tissue. The mechanism of phosphorus action should be analyzed in detail in severe secondary parathyroid hyperplasia of humans.

References