The administration of an active vitamin D₃ analogue reduced the serum concentrations of 1-84 and truncated parathyroid hormone in pseudohypoparathyroidism type Ib patients

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Abstract. Serum calcium is one of major regulators of PTH amino-terminal (N-terminal) truncation and secretion of full-length (1–84)PTH from parathyroid glands. However, the effect of active vitamin D₃ on PTH truncations remains controversial. To determine whether active vitamin D₃ accelerates the truncation of PTH, the vitamin D₃ analogue alfalcaldiol was administered to patients with pseudohypoparathyroidism type Ib (PHP Ib). Both the (1–84)PTH molecule and N-terminally truncated fragments such as (7–84)PTH can be measured by commercially available two-site total PTH (T-PTH) assays. The development of whole PTH (W-PTH) assays specific for full-length (1–84)PTH has enabled us to distinguish between N-terminally truncated PTH and full-length (1–84)PTH. W-PTH/T-PTH ratios were calculated and used as an index of PTH N-terminal truncations. Both serum W-PTH and T-PTH levels were elevated in untreated PHP Ib patients. The administration of alfalcaldiol reduced both the W-PTH and T-PTH levels, but not with the W-PTH/T-PTH ratios. Thus, the administration of an active vitamin D₃ analogue did not seem to have a major effect on the rate of PTH N-terminal truncation, even though it did reduce the secretion of both full-length and truncated PTH. Possibly, active vitamin D₃ attenuates the effect of elevated calcium on PTH N-terminal truncation in PHP Ib patients.

Key words: Active vitamin D₃, Calcium-sensing receptor (CaR), N-terminal truncation, Parathyroid hormone (PTH), Whole PTH

PROTEOLYTIC modifications of parathyroid hormone (PTH) is one of mechanisms for regulating the secretion of full-length (1–84)PTH from parathyroid glands. Therefore, not only (1–84)PTH but also other PTH fragments, such as carboxy-terminal (C-terminal) PTH fragments [1, 2], have been observed in human serum. Extracellular Ca²⁺ induces PTH cleavage to generate C-terminal fragments such as (34–84)PTH, both in vivo [3] and in vitro [2, 4].

Both the (1–84)PTH molecule and amino-terminal (N-terminally) truncated fragments such as (7–84)PTH can be measured by commercially available two-site total PTH assays. The development of full-length (1–84)PTH-specific assays has enabled us to distinguish between N-terminally truncated PTH and full-length (1–84)PTH [5, 6]. Interestingly, the (1–84)PTH/ N-terminally truncated PTH ratio was negatively correlated with serum Ca²⁺ levels in patients on maintenance hemodialysis [7] and in predialysis patients with chronic kidney diseases [8, 9]. In addition, extracellular Ca²⁺ not only suppressed PTH secretion, but also accelerated the N-terminal truncation of PTH in primary cultured human parathyroid cells [10], indicating that the serum Ca²⁺ concentration regulates the degradation of (1–84)PTH by N-terminal truncation.

Pseudohypoparathyroidism (PHP) is a disorder characterized by isolated renal PTH resistance mani-
fested as hypocalcemia, hyperphosphatemia, and increased serum PTH concentrations. In PHP type I patients, the N-terminal truncation of PTH is accelerated even though their serum calcium levels are lower than those in normal subjects.[11] In addition, their PTH N-terminal truncation ratios remain unchanged when their serum calcium levels are normalized by calcitriol treatments.

In this study, we attempted to determine the precise roles of active vitamin D₃ and serum calcium levels in the N-terminal truncation of PTH in PHP type Ib (PHP Ib) patients. To address these questions we altered the patients’ serum calcium levels by treatments with the active vitamin D₃ analogue alfacalcidol.

**Materials and Methods**

**Patients**

This study included 4 patients with PHP Ib. All participants with PHP Ib were newly diagnosed and had not received any treatment for the disease. The diagnosis of PHP Ib was established by clinical symptoms along with hypocalcemia, hyperphosphatemia, low values of nephrogenous cAMP, high serum levels of PTH. In addition, reduced urinary cAMP and phosphate responses to iv infused synthetic human (1-34) PTH were observed. The diagnosis of PHP Ib was based on the absence of Albright’s hereditary osteodystrophy.

The study was performed between March 2005 and June 2009. All subjects provided written informed consent before participation, which was approved by the institutional ethics committee (Osaka City University Graduate School of Medicine) and was conducted in accordance with the principles of the Declaration of Helsinki.

**Administration of alfacalcidol**

The participants were administered alfacalcidol in an initial dose of 0.25 µg/day. The doses were increased by 0.25–0.50 µg/day every 4–6 weeks. Once the serum calcium levels exceeded 2.625 mmol/L or the urinary calcium/creatinine ratios exceeded 0.300, the doses of alfacalcidol were decreased by 0.25–0.50 µg/day every 4–6 weeks. If the serum calcium levels fell below 1.750 mmol/L or the participants complained of tetanies, the doses of alfacalcidol were increased again by 0.25-0.50 µg/day every 4–6 weeks. For individual participants, the study was terminated between January and June 2009. The average period of participation was 27.3 months (range: 20.0–33.0 months).

**Measurement of serum parameters**

Serum samples were obtained from all participants after fasting overnight. The samples were stored at –80°C until assayed. The serum samples were assayed using two different methods of PTH measurement: the total PTH (T-PTH) assay and the whole PTH (W-PTH) assay. The T-PTH assay measures the sum of (1-84)PTH and N-terminally truncated PTH fragments, such as (7–84)PTH, and was performed using the Scantibodies Total Intact PTH Coated Bead Kit (Scantibodies Laboratory, Inc., Santee, CA). The W-PTH assay is a two-site immunoradiometric assay that measures (1–84)PTH exclusively, and was performed using the Scantibodies Whole PTH Kit (Scantibodies Laboratory, Inc.). Serum 1,25(OH)₂D was measured with 1,25(OH)₂D RIA kit (Immunodiagnostic Systems Limited, Boldon, England). Other serum parameters were measured using an autoanalyzer. The estimated glomerular filtration rates (eGFR) were estimated using the serum creatinine values and the abbreviated Modification of Diet in Renal Disease (MDRD) study equation, modified with the Japanese coefficient [12]. The eGFRs in all participants were normal, and were stable during the study.

**Statistical analysis**

Results are expressed as the means ± standard deviation (SD). Serum calcium levels were expressed as corrected calcium, after adjustment for serum albumin. Total calcium was adjusted for plasma albumin level using a Payne’s formula: corrected calcium (mg/dL) = total calcium (mg/dL) + (4 - albumin (g/dL)), when the serum albumin levels were below 4 g/dL. The factor to convert from conventional unit (mg/dL) to SI unit (mmol/L) is 0.2495. Differences in serum parameters were evaluated using the Mann–Whitney U test. Correlations between two variables were examined using the Spearman’s correlation coefficient. P values less than 0.05 were considered significant. Statistical analyses were performed using the R software (R Foundation for Statistical Computing, Vienna, Austria).
Reductions in PTH by alfacalcidol in PHP

Gradually increased and both the W-PTH and T-PTH levels gradually decreased with the increments in alfacalcidol doses (Fig. 1). The serum calcium levels gradually decreased while both the W-PTH and T-PTH levels gradually increased when the alfacalcidol doses were reduced. The W-PTH/T-PTH ratios were stable during the treatment. Data for one patient are shown in Fig. 1. The other three PHP Ib patients exhibited very similar clinical courses (data not shown).

Results

Profiles of the participants

Both the W-PTH and T-PTH levels were elevated in the PHP Ib patients compared with their normal ranges (Table 1).

Administration of alfacalcidol on PHP Ib

In the PHP Ib patients, the serum calcium levels gradually increased and both the W-PTH and T-PTH levels gradually decreased with the increments in alfacalcidol doses (Fig. 1). The serum calcium levels gradually decreased while both the W-PTH and T-PTH levels gradually increased when the alfacalcidol doses were reduced. The W-PTH/T-PTH ratios were stable during the treatment. Data for one patient are shown in Fig. 1. The other three PHP Ib patients exhibited very similar clinical courses (data not shown).
Correlations between the doses of alfalcacidol and other variables

The doses of alfalcacidol were significantly and positively correlated with the corrected calcium levels in the PHP Ib patients ($r = 0.762$, $P < 0.001$). The doses were significantly correlated in a negative manner with both the W-PTH (Fig. 2A) and T-PTH (Fig. 2B) levels, however, no relationships were observed between doses and W-PTH/T-PTH ratios (Fig. 2C).

Correlations between corrected-calcium level and other variables

The corrected calcium levels were significantly and negatively correlated with both the W-PTH (Fig. 3A) and T-PTH (Fig. 3B) serum levels in the PHP Ib patients, however, no relationship was observed between calcium levels and the W-PTH/T-PTH ratios (Fig. 3C).

Discussion

This study was designed to evaluate the effects of alfalcacidol and serum calcium levels on the production of N-terminal truncations of PTH. Both the W-PTH and T-PTH serum levels were elevated in untreated PHP Ib patients compared with their normal ranges. In the treated patients, the alfalcacidol doses and corrected serum calcium levels were significantly and negatively correlated with both the W-PTH and T-PTH levels, however, no relationships were observed between the W-PTH/T-PTH ratios and alfalcacidol doses or calcium levels. Therefore, the active vitamin D$_3$ and serum calcium levels do not seem to affect the ratio of N-terminally truncated PTH, even though they affected the levels of both full-length (1–84)PTH and truncated PTH in the PHP Ib patients.

Serum calcium or extracellular Ca$^{2+}$ concentrations are known to affect the production of N-terminal truncations of PTH in patients with chronic kidney disease [7-9]. In vitro studies have also revealed that extracellular Ca$^{2+}$ can accelerate the production of truncations [10], suggesting that the stimulation of the calcium-sensing receptor (CaR) might contribute to the production of truncations. If serum calcium is a major factor in the production of truncations, the W-PTH/T-PTH ratios in untreated PHP patients should be higher than those in normal subjects because of hypocalcemia in the PHP patients. However, a previous study also revealed significantly reduced ratios in PHP I patients [11], even though their serum calcium levels were lower than those of normal subjects.

Extracellular Ca$^{2+}$ is known to be the most important regulator of PTH secretion, and this action is mediated by the CaR [13]. The relationship between serum calcium and PTH concentration can be modeled, and it fits a symmetrical sigmoidal curve with a four-parameter model [14]. The setpoint is the calcium level at which one-half of the maximal inhibition of PTH release occurs, and this was higher than normal in the patients with primary hyperparathyroidism [15]. The expression of CaR in parathyroid adenomas reduced in the patients with primary hyperparathyroidism [16]. A mouse model for primary hyperparathyroidism with hypo-expression of CaR on their parathyroid glands also had higher setpoint [17], indicating the importance of CaR expression in parathyroid glands on the setpoint.

The administrations of cinacalcet, a type 2 calcimetics, shifted to the left not only the inverse sigmoidal curve of PTH secretion, but also that of the PTH N-terminal truncation ratio [18], suggesting that both PTH secretion and N-terminal truncation were mediated by the CaR. It is not yet clear whether the intracellular signal transduction pathways controlling PTH N-terminal truncation and PTH secretion are the same, even though they both appear to be regulated by extracellular Ca$^{2+}$. If parts of these pathways are same, the truncation may be accelerated even with low serum calcium levels, because an abnormal setpoint could enhance the truncation.

The setpoint for PTH secretion was lower than normal in untreated PHP patients [11, 19], suggesting the over function of CaR in their parathyroid glands. Again, if the pathways of PTH secretion and N-terminal truncation in the abnormal parathyroid glands are same, the truncation could enhance as well as the secretion.

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Fig. 2 Correlations between doses of alfacalcidol and serum PTH levels in PHP Ib patients.
For all four PHP Ib patients, the alfacalcidol doses were plotted against the serum levels of W-PTH (A) and T-PTH (B), and against the W-PTH/T-PTH ratios (C). Both the W-PTH and T-PTH levels decreased significantly with higher doses of alfacalcidol, however, the W-PTH/T-PTH ratios were stable.

Fig. 3 Correlations between serum calcium levels and serum PTH levels in PHP Ib patients.
For all four PHP Ib patients, the corrected serum calcium levels were plotted against the serum levels of W-PTH (A) and T-PTH (B) and the W-PTH/T-PTH ratios (C). Both the W-PTH and T-PTH levels decreased significantly with higher serum calcium levels. No correlation was observed between the serum calcium levels and the W-PTH/T-PTH ratios, suggesting that serum calcium did not affect the N-terminal truncation of (1-84)PTH.

In addition to the direct effects of alfacalcidol on the parathyroid cells. The elevated serum calcium levels might have enhanced the PTH N-terminal truncations in these patients, however, the W-PTH/T-PTH ratios remained unchanged. In patients with PHP and idiopathic hypoparathyroidism, the administration of active vitamin D₃ normalized their reduced setpoints for PTH secretion [11, 19]. It is possible that the effects of the elevated serum calcium levels were counteracted by an alteration in setpoints for the truncation induced by the active vitamin D₃ analogue, resulting in stable truncation rates.

In conclusion, the administration of an active vitamin D₃ analogue elevated serum calcium levels and suppressed the secretion of both intact and truncated PTH in PHP Ib patients. However, these administrations did not accelerate the N-terminal truncation of PTH. A decreased sensitivity to calcium in the parathyroid cells might have attenuated the PTH N-terminal truncation induced by calcium in these patients. Further study is desirable to measure the actual setpoint for both PTH secretion and PTH N-terminal truncation.

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