Intact PTH assay overestimates true 1-84 PTH levels after maxacalcitol therapy in dialysis patients with secondary hyperparathyroidism

Junichiro J. Kazama1,2, Kentaro Omori1, Noboru Higuchi1, Naoki Takahashi1, Yumi Ito1, Hiroki Maruyama1, Ichiei Narita1, Thomas L. Cantor3, Ping Gao3 and Fumitake Gejyo1

1Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences and 2Division of Intensive Care Medicine, Niigata University Medical Hospital, Niigata, Japan and 3Scantibodies Laboratory, Inc., Santee, CA, USA

Abstract

Background. Although the so-called intact parathyroid hormone (iPTH) assay detects not only true 1-84 PTH (1-84PTH) but also large C-terminal PTH fragments, it remains inconclusive whether the 1-84PTH assay is more useful in clinical practice. Previous studies have shown that the results of these two PTH assays in dialysis patients are closely correlated.

Methods. Chronic dialysis patients whose plasma iPTH levels were >400 pg/ml were selected for inclusion in the present study. Following a 4 week wash-out time during which all vitamin D administration was halted, maxacalcitol was intravenously injected at the end of dialysis sessions three times per week for 24 weeks, at an initial dosage of 10 μg.

Results. Ninety-seven patients with secondary hyperparathyroidism were included in our analysis. Their serum calcium levels were elevated from the start levels while phosphate levels remained unchanged. The plasma 1-84PTH levels constantly declined throughout the 24 weeks. Although the patients’ plasma 1-84PTH and iPTH levels were closely correlated with each other both at the beginning of the study and after 24 weeks of maxacalcitol therapy, the ratio of 1-84PTH/iPTH consistently decreased throughout the study period (P < 0.01). The changes in the ratio were significantly correlated with changes in serum calcium levels.

Conclusions. Twenty-four weeks of intravenous maxacalcitol injection therapy significantly reduced the 1-84PTH/iPTH ratio. Estimated 1-84PTH levels from iPTH levels using a conversion formula obtained before the treatment were 21.0 ± 20.4% higher than measured 1-84PTH levels after the therapy. Thus, iPTH measurement has a potential risk to overestimate 1-84PTH levels when evaluating the efficacy of maxacalcitol therapy in dialysis patients with secondary hyperparathyroidism.

Keywords: estimation; intact parathyroid hormone assay; maxacalcitol; 1-84 parathyroid hormone; secondary hyperparathyroidism

Introduction

Parathyroid function is one of the most commonly used non-invasive indicators of bone metabolism in dialysis patients. Thus, in routine clinical nephrology practice today, the so-called 'intact parathyroid hormone' (iPTH) assay system is commonly applied to estimate both parathyroid function and bone turnover status [1]. This iPTH assay measures not only the true 1-84 PTH (1-84PTH) but also the large C-terminal PTH fragments. Moreover, 7-84PTH [2,3], which seems to be a component of the large C-terminal fragments, demonstrates antagonistic actions against 1-84PTH [4–6].

Thus, a novel assay system that detects only 1-84PTH has been developed [3]. However, whether the measurement of 1-84PTH is really useful in clinical practice remains inconclusive. A previous study reported that the ratio of 1-84PTH/(iPTH – 1-84PTH) accurately reflects bone metabolism [7], while other clinical studies provided negative results [8–11]. Since much larger-scale studies would be necessary to reach any final conclusion, we do not intend to argue the usefulness of the 1-84PTH/(iPTH–1-84PTH) ratio for the prediction of bone metabolism in this manuscript.

Apart from the question of the usefulness of the 1-84PTH/(iPTH – 1-84PTH) ratio in clinical practice, previous research has shown that it is the plasma
1-84PTH levels, and not the 1-84PTH + large C-terminal PTH fragment levels, that represent parathyroid function in target organs like bone. However, clinical studies have shown that 1-84PTH and iPTH levels are closely correlated with each other in dialysis patients [7,10,11]. In other words, although there are a few exceptional cases, the ratio of 1-84PTH/iPTH is generally distributed over a quite narrow range in dialysis patients. Therefore, the estimation of 1-84PTH levels from iPTH levels does not seem to pose serious problems in the majority of cases. In fact, the iPTH assay has been applied to estimate bone metabolism in dialysis patients and the obtained levels did indeed show a fairly good correlation [12,13]. If it is generally acceptable to estimate 1-84PTH levels from the conventional iPTH assay, the usefulness of the 1-84PTH assay will be quite limited in clinical practice.

However, the ratio of these two PTH levels must be stable, regardless of the patient’s condition, in order to estimate 1-84PTH levels from iPTH levels. For example, PTH measurement is often performed to evaluate the efficacy of active vitamin D therapy in dialysis patients. If the ratio of 1-84PTH/iPTH changes when the patient undergoes active vitamin D therapy, parathyroid activity will not be accurately evaluated from plasma iPTH levels.

Thus, we attempted a prospective clinical study of treatment with maxacalcitol (22-oxa-calcitriol), a newly developed artificial active vitamin D analogue [14], for dialysis patients who suffer from progressive secondary hyperparathyroidism and who are refractory to oral vitamin D therapy. The aim of this study was to investigate the influence of maxacalcitol therapy in the estimation of 1-84PTH levels from iPTH levels.

**Subjects and methods**

**Patients**

The protocol of the present study was planned following the ethics guidelines of the Declaration of Helsinki. Patients who were undergoing chronic haemodialysis therapy at the facilities of the Niigata Society of Dialysis Complication Research and whose plasma iPTH levels were >400 pg/ml even while being treated with oral vitamin D were included in the study. Exclusion criteria were complication of malignancy or chronic inflammatory diseases, previous continuous ambulatory peritoneal dialysis treatment, kidney transplantation or parathyroidectomy. After approval of the study protocol by the ethics committees of the various medical facilities, the patients were duly informed about the study and its purposes in the majority of cases. In fact, the iPTH assay has been applied to estimate bone metabolism in dialysis patients and the obtained levels did indeed show a fairly good correlation [12,13]. If it is generally acceptable to estimate 1-84PTH levels from the conventional iPTH assay, the usefulness of the 1-84PTH assay will be quite limited in clinical practice.

**Methods**

The wash-out period, part of the preparation for participating in the study, consisted of ceasing the use of activated vitamin D agents ≥4 weeks prior to initiation of the study. Patients whose plasma iPTH levels fell to <400 pg/ml after the wash-out period were not excluded from the study. After completion of the wash-out period, 10 μg maxacalcitol was intravenously injected three times per week at the end of every dialysis session as an initial dosage. Thereafter, the amount of maxacalcitol per injection varied between 2.5 and 15.0 μg, so that the serum calcium concentration would not exceed 11.5 mg/dl. Haemodialysis conditions, including dialysers, dialysate compositions and anticoagulants, remained unchanged throughout the study period. Although we allowed phosphate binders to be used, the dosage for each patient remained unchanged throughout the study period. The goal of the treatment was to maintain iPTH levels of <250 pg/ml. The treatment was continued for the 24 weeks of the study period, unless the goal of the treatment was achieved or unless one of the following described events occurred. The use of other active vitamin D analogues, glucocorticoid agents, bisphosphonates or any other antiosteoporotic agents was prohibited during the study period and it was decided that those who had to use the above-mentioned drugs during the study period would be automatically withdrawn. Patients who underwent surgical thyroidectomy or parathyroidectomy were also withdrawn from the study. Maxacalcitol therapy was discontinued when the adjusted serum calcium levels exceeded 11.5 mg/dl. For evaluation, calcium levels (mg/dl) were adjusted by serum albumin levels (g/dl) when they were <4.0 g/dl:

\[
\text{Adjusted calcium level} = \text{Measured calcium level} + 0.8 \\
\times (4 – \text{Serum albumin levels})
\]

The treatment was also discontinued if any other intolerable events occurred.

Serum calcium, inorganic phosphate, albumin and iPTH levels were monitored at least once every 4 weeks in each dialysis unit. Each physician in charge decided on the appropriate treatment plan based on the data and clinical symptoms of the patient in question. The iPTH levels measured in each dialysis unit were used by each physician in charge to determine the treatment plans; however, they are not shown in this paper. In addition to routine laboratory examinations, blood samples were collected at 0, 6, 12 and 24 weeks after the initiation of the maxacalcitol therapy. These samples were separated into plasma and sera and stored at –80°C. The 1-84PTH, iPTH, bone-specific alkaline phosphatase (bAP) and intact osteocalcin (OC) levels were measured simultaneously from the stored samples for the purposes of this research. The plasma 1-84PTH and iPTH levels were assayed by immunoradiometric assay methods (Scantibodies Laboratory, Inc., Santee, CA, USA) [4]. The intra-assay coefficient of variation (CV%) for 1-84PTH was <5.0% and the interassay CV% for 1-84PTH was <8.0%. The intra-assay CV% for iPTH was <5.0% and the interassay CV% for iPTH was <7.0%. The iPTH data included in this manuscript were obtained from the stored samples only. Serum bAP levels were determined by an enzyme immunoassay (EIA) method (Kokusai Shiyouku, Kobe, Japan) and the intra-assay CV% was <5.0%. The serum OC levels were measured by EIA (Sumitomo Seiyaku Biomedical Co., Ltd, Osaka, Japan) and the intra-assay CV% was found to be <6.0%. All the blood samples mentioned above were collected at 9.00 a.m. on the third day after the last dialysis session.
Statistical analysis

All values are expressed as means±SD and $P<0.05$ was considered to be statistically significant. A paired $t$-test was applied to compare the results. Statistical computations were performed using an Apple Macintosh computer type G4 with software Stat View 5.0 (SAS Institute Inc., Cary, NC, USA).

Results

The initial participants in the study included 140 patients. During the study period, seven patients withdrew of their own will. No patient began to use glucocorticoid agents, bisphosphonates or antiosteoporotic agents during the study. The dosage of phosphate binders for each patient was not changed throughout the study. Surgical parathyroidectomy was performed in two patients, who were consequently withdrawn from the study. The treatment was discontinued in one patient because of increased itching and in nine patients because of hypercalcemia that exceeded 11.5 mg/dl. No other complications, including the development of ectopic calcification, were documented. The treatment goal was achieved in 24 patients and maxacalcitol therapy was therefore discontinued prior to completion of the 24 week observation period. The remaining 97 patients received maxacalcitol therapy three times per week for 24 weeks and were periodically evaluated during the study period. The dosage of maxacalcitol remained unchanged from the initial dosage of 10 $\mu$g throughout the study period in 51 of these 97 patients. Table 1 summarizes the clinical features of all 97 patients included in the study.

Figure 1 shows the effects of maxacalcitol therapy on plasma iPTH levels, which decreased in a time-dependent manner. The decline in the first 6 weeks of the study period was the most remarkable, after which the iPTH levels continued to decline steadily. Serum bAP levels decreased consistently throughout the study. In contrast, serum OC levels decreased after a transient elevation in the first 6 weeks. Serum calcium levels were obviously elevated while phosphate levels remained unchanged (Table 2).

Table 1. Clinical features of the 97 patients who received maxacalcitol therapy three times per week throughout the 24 week study period

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.9±11.8</td>
</tr>
<tr>
<td>Gender</td>
<td>Male 67, Female 30</td>
</tr>
<tr>
<td>Duration of haemodialysis therapy (months)</td>
<td>130.2±86.9</td>
</tr>
<tr>
<td>Primary disease</td>
<td>Chronic glomerulonephritis 79, Nephrosclerosis 4, Diabetic glomerulosclerosis 6, Polycystic kidney 4, Other 1, Unknown 3</td>
</tr>
<tr>
<td>Phosphate binder</td>
<td>Yes 70, No 27</td>
</tr>
<tr>
<td>Previous treatment</td>
<td>Alfacalcidol 0.25 $\mu$g/day 63, Alfacalcidol 0.5 $\mu$g/day 18, Calcitriol 0.25 $\mu$g/day 9, Calcitriol 0.5 $\mu$g/day 5, Calcitriol 4.0 $\mu$g/week 1, Calcitriol 6.0 $\mu$g/week 1</td>
</tr>
</tbody>
</table>

Table 2. The effect of maxacalcitol therapy. The serum calcium levels were significantly elevated while the phosphate levels remained unchanged. The serum OC levels decreased after a transient elevation in the first 6 weeks. The serum bAP levels consistently decreased

<table>
<thead>
<tr>
<th>Time</th>
<th>Ca (mg/dl)</th>
<th>Pi (mg/dl)</th>
<th>OC (ng/dl)</th>
<th>bAP (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0W</td>
<td>9.3±1.0</td>
<td>6.4±1.5</td>
<td>106.2±68.6</td>
<td>47.8±34.3</td>
</tr>
<tr>
<td>6W</td>
<td>9.7±1.0$^a$</td>
<td>6.4±1.5</td>
<td>117.9±79.3</td>
<td>37.9±29.4$^b$</td>
</tr>
<tr>
<td>12W</td>
<td>9.8±0.9$^a$</td>
<td>6.4±1.5</td>
<td>90.1±61.3$^b$</td>
<td>32.0±19.9$^a$</td>
</tr>
<tr>
<td>24W</td>
<td>10.1±0.9$^a$</td>
<td>6.5±1.5</td>
<td>75.1±55.7$^a$</td>
<td>25.6±12.1$^a$</td>
</tr>
</tbody>
</table>

$^a$P<0.001 vs 0W; $^b$P<0.01 vs 0W.
each other both at the beginning of the study and after 24 weeks of maxacalcitol therapy (Figure 2); however, 1-84PTH showed a significantly larger decrement than iPTH in this 24 week period (51.8±26.5% vs 46.2±29.2%; P<0.0001). The plasma 1-84PTH levels declined consistently throughout the 24 week study period. The plasma levels of (iPTH / C0 1-84PTH) declined rapidly in the first 6 weeks, then remained almost unchanged up to the 24th week (Figure 3). The ratio of 1-84PTH/iPTH levels decreased consistently throughout the study period (Figure 4). Finally, the change in the ratio significantly correlated with the change in serum calcium levels before and after maxacalcitol treatment (Figure 5).

**Discussion**

High dose vitamin D treatment is used in the treatment of uraemic hyperparathyroidism. PTH measurement is necessary to evaluate the efficacy of active vitamin D
therapy and the assay system must be reliable for impartial judgment. Plasma 1-84PTH and iPTH levels have been found to be closely correlated in dialysis patients with a relatively homogeneous background [7,10,11]. Moreover, previous studies revealed that the correlations between iPTH and bone metabolic marker or bone histomorphometry were as close as those between 1-84PTH and bone metabolic markers [11] or bone histomorphometry [9]. In the present study, the close correlation between iPTH and 1-84PTH was confirmed to exist both before and after the administration of maxacalcitol therapy (Figure 2). Therefore, it seems acceptable to apply the iPTH assay to estimate 1-84PTH levels in a cross-sectional study.

However, the regression line differed between measurements taken before and after maxacalcitol therapy. This can be attributed to the fact that the ratio of 1-84PTH/iPTH decreased in a time-dependent manner with the progression of maxacalcitol therapy (Figure 4). Therefore, we cannot estimate 1-84PTH levels from serum calcium levels. Estimated 1-84PTH levels did not appear to be correlated with 1-84PTH levels or 1-84PTH/iPTH ratios in this study. Thus, the influence of serum calcium levels seemed to be not quite crucial. Further investigations might reveal other factors that also affect the ratio.

It was previously assumed that maxacalcitol suppressed parathyroid function without hypercalcaemic action, however, some studies have reported that maxacalcitol shows a calcemic effect on dialysis patients [18,19]. Our data confirm these earlier clinical findings that maxacalcitol appears not to differ from conventional active vitamin D analogues with regard to calcium metabolism in dialysis patients. A rather interesting finding was that no remarkable change was induced in serum phosphate levels by the maxacalcitol therapy. Note that the use of phosphate binder was unchanged in all 97 patients and the dosage of maxacalcitol injected was unchanged in 51 subjects. An additional study is necessary to determine why maxacalcitol therapy did not raise serum phosphate levels.

In conclusion, intravenous maxacalcitol injection therapy was performed in patients with uraemic secondary hyperparathyroidism. The drop in plasma PTH levels was predominantly caused by a decrease in plasma 1-84PTH levels, while the levels of iPTH – 1-84PTH were less changed. The ratio of 1-84PTH/iPTH decreased with the progression of the maxacalcitol therapy. This change might partly implicate elevated serum calcium levels. Estimated 1-84PTH levels
from iPTH were significantly higher than measured 1-84PTH levels after the maxacalcitol therapy. Thus, iPTH assay has a potential risk to overestimate 1-84PTH levels when evaluating the efficacy of maxacalcitol therapy in dialysis patients with secondary hyperparathyroidism.

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