Influence of Glomerular Filtration Rate on Non-(1-84) Parathyroid Hormone (PTH) Detected by Intact PTH Assays

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Background: Commercial intact parathyroid hormone (I-PTH) assays detect molecular form(s) of human PTH, non-(1-84) PTH, different from the 84-amino acid native molecule. These molecular form(s) accumulate in hemodialyzed patients. We investigated the importance of non-(1-84) PTH in the interpretation of the increased I-PTH in progressive renal failure.

Methods: Five groups were studied: 26 healthy individuals, 12 hemodialyzed patients, and 31 patients with progressive renal failure subdivided according to their glomerular filtration rate (GFR) into 11 with a GFR between 60 and 100 mL·min⁻¹·1.73 m⁻², 12 with a GFR between 30 and 60 mL·min⁻¹·1.73 m⁻², and 8 with a GFR between 5 and 30 mL·min⁻¹·1.73 m⁻². We evaluated indicators of calcium and phosphorus metabolism and creatinine clearance (CrCl) in the progressive renal failure groups, and the HPLC profile of I-PTH and C-terminal PTH in all groups.

Results: Only patients with a GFR <30 mL·min⁻¹·1.73 m⁻² and hemodialyzed patients had decreased Ca²⁺ and 1,25-dihydroxyvitamin D, and increased phosphate. In patients with progressive renal failure, I-PTH was related to Ca²⁺ (r = −0.66; P <0.0001), CrCl (r = −0.61; P <0.001), 1,25-dihydroxyvitamin D (r = −0.40; P <0.05), and 25-hydroxyvitamin D (r = −0.49; P <0.01) by simple linear regression. The importance of non-(1-84) PTH in the composition of I-PTH increased with each GFR decrease, being 21% in healthy individuals, 32% in progressive renal failure patients with a GFR <30 mL·min⁻¹·1.73 m⁻², and 50% in hemodialyzed patients, with PTH(1-84) making up the difference.

Conclusions: As I-PTH increases progressively with GFR decrease, part of the increase is associated with the accumulation of non-(1-84) PTH, particularly when the GFR is <30 mL·min⁻¹·1.73 m⁻². Concentrations of I-PTH 1.6-fold higher than in healthy individuals are necessary in hemodialyzed patients to achieve PTH(1-84) concentrations similar to those in the absence of renal failure.

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Many commercial intact parathyroid hormone (I-PTH)5 assays react with circulating molecular form(s) of PTH other than PTH(1-84) (1–3). These molecular form(s) are not recognized by antibodies specific for the sequence 1-12 of the 84-amino acid structure of PTH (4, 5). This does not impair immunoreactivity with the reporter antibody in commercial I-PTH assays because most N-terminal antibodies react with an epitope distal to position 14 (6). In healthy individuals, these non-(1-84) molecular form(s) account for up to 20% of circulating I-PTH immunoreactivity (1) and behave as C-terminal fragment(s), increasing the non-(1-84) relative proportion with hypercalcemia and decreasing it with hypocalcemia (1, 7, 8). In hemodialyzed patients, the non-(1-84) form(s) may account for up to 55% of I-PTH immunoreactivity (2). This increased proportion in renal failure patients is probably best explained by decreased renal clearance of I-PTH molecular form(s) behaving like a C-terminal fragment (1, 2, 9, 10). This accumulation of non-(1-84) PTH in renal failure is pertinent to the increased nonsuppressible frac-

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tion of I-PTH found in that condition (2, 3, 11–16) and also to the fact that I-PTH values overestimated parathyroid-mediated osseous abnormalities in uremia (17). It is unclear whether non-(1-84) PTH could be important in explaining increases in I-PTH in early renal failure and as the condition progresses. This study was thus planned to study the relationship between glomerular filtration rate (GFR) and non-(1-84) PTH in the genesis and evolution of increasing I-PTH in renal failure.

**Subjects and Methods**

Thirty-one patients with progressive renal failure participated in the study. Twenty-six healthy subjects who had participated in parathyroid function and I-PTH HPLC composition studies (1, 8) served as controls. Twelve hemodialyzed patients who also participated in similar studies (2) were used as examples of end-stage renal failure.

**EXPERIMENTAL PROTOCOL**

The protocol was approved by a local ethics committee. All participants signed an informed consent form. All subjects were studied while maintaining their usual diet. Hemodialyzed patients were studied in October, at the end of summer, and progressive renal failure patients were studied in May, at the end of winter. Blood was obtained from all subjects after an overnight fast where water was permitted. A 24-h urine specimen was also obtained from patients with progressive renal failure. Ionized calcium (Ca²⁺), phosphate, alkaline phosphatase, creatinine, I-PTH and C-terminal PTH (C-PTH), 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D [1,25(OH)₂D], and creatinine clearance (CrCl) were measured in all patients with progressive renal failure. Most variables were also measured in the other two groups.

**LABORATORY METHODS**

Ca²⁺ was measured immediately after blood collection with an ICA ionized calcium analyzer (Radiometer); the interassay CVs for 38 determinations at 0.77 and 1.75 mmol/L were 3.3% and 2.7%, respectively. Serum phosphate, creatinine, and alkaline phosphatase were measured by automated colorimetry (Baxter Paramax). Serum 25(OH)D and 1,25(OH)₂D were measured by RIA (Diasorin) after extraction with acetonitrile and, for 1,25(OH)₂D, after chromatography was performed on C₁₈ and silica cartridges. The within-assay CV for duplicate determinations was 6% for the 25(OH)D assay and 10–14% for the 1,25(OH)₂D assay. Serum PTH was measured by means of two different PTH assays. The first was a commercial immunoradiometric assay (Allegro Intact PTH; Nichols Institute) initially reported to react only with PTH(1-84) because synthetic PTH(1-34) was not retained by the C-terminal-directed solid-phase antibody and synthetic PTH(39-84) and (39-68) were not recognized by the labeled N-terminal reporter antibody (18). Nonetheless, this and other commercial I-PTH assays (19) have been demonstrated to react with molecular form(s) of PTH other than PTH(1-84) in humans (1–3) and dogs (20, 21) when sera obtained under various calcium concentrations were fractionated by HPLC. In the company’s brochure, the stated detection limit of the assay is 0.1 pmol/L. The intraassay CV for duplicates was 3.1%. Serum C-PTH was measured by an in-house C-PTH assay described previously (7, 8). This assay detects predominantly large C-terminal fragments of the hormone, PTH(1-84), being four- to sixfold less reactive on a molar basis than PTH(39-84). The antigenic determinant in this assay is in the region (65-84) of the PTH molecule; therefore, PTH(1-34), PTH(39-68), and PTH(44-68) are nonreactive. The detection limit was 1 pmol/L using 3 SD from the zero calibrator run in quadruplicate in 10 different assays. The intraassay CV at 50% binding was 3.3%. For I- and C-PTH measurements, all patients with progressive renal failure were measured in the same assay.

To analyze individual molecular forms of PTH detected by both assays, one pool was prepared from equal volumes of serum from each of the individuals in each of the three subgroups of patients with progressive renal failure, and fractionated once by HPLC. Results from the healthy subjects and patients with end-stage renal failure have been presented previously (1, 2, 8). Serum PTH was first extracted on Sep-Pack Plus C₁₈ cartridges (Waters Chromatography Division) (22). Samples were eluted with 3 mL of 800 mL/L acetonitrile in 1 g/L trifluoroacetic acid. After evaporation with nitrogen, the residual volume was freeze-dried and reconstituted in 2 mL of 1 g/L trifluoroacetic acid for HPLC analysis. Each sample was then loaded on a C₁₈ µBondapak analytical column (3.9 × 300 mm; Waters), and eluted with a noncontinuous 15–50% linear gradient of acetonitrile in 1 g/L trifluoroacetic acid, delivered at 1.5 mL/min for 65 min by a Bio-Rad Model 2700 HPLC (Bio-Rad). The 1.5-mL fractions were collected in polypropylene tubes precoated with 1 g/L bovine serum albumin in water. After evaporation with nitrogen, each fraction was freeze-dried and reconstituted to 1 mL with 7 g/L bovine serum albumin in water, and appropriate volumes were assayed for I-PTH. Recovery during all these procedures was >85%. Furthermore, PTH(1-84) and PTH(7-84) calibrators, added to hypoparathyroid serum and processed as described, eluted as single peaks at the expected positions, showing that PTH was not degraded during the above procedures.

**MATHEMATICAL AND STATISTICAL ANALYSIS**

Results are presented as means ± SD. Comparisons between groups were performed by a one-way ANOVA and the Student-Newman-Keuls comparison test for 2 by 2 comparisons. Standard methods were used for simple and multivariate regression analysis. HPLC profiles were corrected to 100% recovery, and the surface under each peak was evaluated by planimetry using the peak-fitting module of Origin 3.5 (Microcal Software).
Results

The characteristics of the groups studied are summarized in Table 1. To better outline differences in relation to GFR, patients with progressive renal failure were subdivided into three groups: GFR between 60 and 100 mL·min⁻¹·1.73 m⁻², between 30 and 60 mL·min⁻¹·1.73 m⁻², and <30 mL·min⁻¹·1.73 m⁻². Sex distribution varied among the groups, and healthy individuals were at least 12 years younger than those with renal failure. As expected from our stratification of patients, each group differed significantly from the others with respect to creatinine or CrCl. Only patients with a CrCl <30 mL·min⁻¹·1.73 m⁻² and hemodialyzed patients had lower mean Ca²⁺ and 1,25(OH)₂D values and a higher mean phosphate value. Alkaline phosphatase was higher in all renal failure groups. Patients with CrCl <30 mL·min⁻¹·1.73 m⁻² had a lower 25(OH)D value than hemodialyzed patients. I- and C-PTH increased progressively with each step in GFR decrease. When only patients with progressive renal failure (n = 31) were considered (Fig. 1), a positive correlation was found between the GFR and Ca²⁺ or 1,25(OH)₂D value, whereas a negative correlation was found between GFR and phosphate, I-PTH, or C-PTH. When only patients with GFR >30 mL·min⁻¹·1.73 m⁻² were considered (n = 23), only the negative correlation between the GFR and I-PTH (r = −0.469; P = 0.024) or C-PTH (r = −0.578; P = 0.004) values remained significant.

Fig. 2 illustrates that I-PTH values were related to Ca²⁺, CrCl, 1,25(OH)₂D, and 25(OH)D values by simple linear regression. Similar results were obtained with C-PTH (data not shown). With multivariate analysis, only Ca²⁺ (r = 0.549) and CrCl (r = 0.742) remained significantly correlated with I- and C-PTH values when all patients with progressive renal failure were considered. If those with a CrCl <30 mL·min⁻¹·1.73 m⁻² were excluded from the analysis, only CrCl and 25(OH)D values remained correlated with I- and C-PTH concentrations.

The HPLC profiles of I-PTH in healthy individuals, the three groups of patients with GFRs from 100 to <30 mL·min⁻¹·1.73 m⁻², and in hemodialyzed patients are illustrated in Fig. 3. The results are expressed as a percentage of total immunoreactivity to outline differences more clearly. Two peaks are identified with the two PTH assays, one comigrating with PTH(1-84) at 53 min, and another one at 49–51 min in front of PTH(1-84). This latter peak represents 21% of I-PTH immunoreactivity in healthy individuals, 32% in patients with a GFR <30 mL·min⁻¹·1.73 m⁻², and 50% in hemodialyzed patients. The percentage of non-(1-84) PTH correlated with serum creatinine (r² = 0.99; P < 0.0001) even when the group of hemodialyzed patients was excluded (r² = 0.94; P = 0.007). With each decrease in GFR (or increase in serum creatinine), non-(1-84) PTH represented a greater proportion of the I-PTH immunoreactivity measured. For a 5-fold increase in PTH(1-84) between healthy subjects and hemodialyzed patients, non-(1-84) PTH increased 18-fold, i.e., a 3.6-fold difference.

Discussion

This study was designed to evaluate the role of non-(1-84) PTH, detected by commercial intact PTH assays (3), in the evaluation of the increased I-PTH observed in progressive renal failure. Increased I-PTH concentrations have been shown to be closely associated with the osseous abnormalities of secondary hyperparathyroidism in end-stage renal failure (17,23–26). We demonstrated previously that non-(1-84) PTH behaves as a C-terminal fragment (1)

Table 1. Characteristics of groups studied.ᵃ

<table>
<thead>
<tr>
<th>Group characteristics</th>
<th>Healthy individuals</th>
<th>Progressive renal failure, CrCl (mL·min⁻¹·1.73 m⁻²)</th>
<th>Hemodialyzed patients</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60–100</td>
<td>30–60</td>
<td>&lt;30</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>26</td>
<td>11</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Sex, F/M</td>
<td>17/9</td>
<td>2/9</td>
<td>6/6</td>
<td>5/3</td>
</tr>
<tr>
<td>Age, years</td>
<td>36.2 ± 1.0</td>
<td>49.9 ± 17.8ᵃ Multiply</td>
<td>62.2 ± 9.0ᵃ Multiply</td>
<td>59.9 ± 18.8ᵃ Multiply</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>64.9 ± 12.9</td>
<td>71.9 ± 14.5</td>
<td>74.2 ± 15.1</td>
<td>74.7 ± 15.8</td>
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<tr>
<td>Ca²⁺ (1.19–1.34 mmol/L)</td>
<td>1.23 ± 0.03</td>
<td>1.26 ± 0.06</td>
<td>1.23 ± 0.02</td>
<td>1.19 ± 0.09ᵇ,ᵇ</td>
</tr>
<tr>
<td>PO₄ (0.70–1.30 mmol/L)</td>
<td>0.99 ± 0.10</td>
<td>1.08 ± 0.24</td>
<td>0.96 ± 0.19</td>
<td>1.35 ± 0.32ᵈ,ᵇ,ᵇ</td>
</tr>
<tr>
<td>Creatinine (55–112 µmol/L)</td>
<td>67 ± 16</td>
<td>113 ± 44ᵃ Multiply</td>
<td>160 ± 54ᵃ Multiply</td>
<td>343 ± 83ᵃ,ᵇ Multiply</td>
</tr>
<tr>
<td>CrCl (80–120 mL·min⁻¹·1.73 m⁻²)</td>
<td>81.6 ± 9.5</td>
<td>44.6 ± 8.5François</td>
<td>17.8 ± 7.3François</td>
<td>17.9 ± 5.6François</td>
</tr>
<tr>
<td>Alkaline phosphatase (25–97 U/L)</td>
<td>52.6 ± 21.9</td>
<td>81.6 ± 17.1François</td>
<td>72.4 ± 17ᵈ Multiply</td>
<td>90.7 ± 40.6ᵃ Multiply</td>
</tr>
<tr>
<td>25(OH)D (35–105 nmol/L)</td>
<td>43.4 ± 14.0</td>
<td>41.9 ± 18.7</td>
<td>28.8 ± 17.9</td>
<td>63.2 ± 24.8ᵇ</td>
</tr>
<tr>
<td>1,25(OH)D₂ (38–133 pmol/L)</td>
<td>69.4 ± 24.2</td>
<td>60.3 ± 14.7</td>
<td>25.2 ± 10ᵉ François</td>
<td>38.2 ± 9.1ᵇ,ᵇ</td>
</tr>
<tr>
<td>I-PTH (1–6.8 pmol/L)</td>
<td>2.28 ± 0.63</td>
<td>4.04 ± 2.0ᵃ Multiply</td>
<td>7.37 ± 4.4ᵃ Multiply</td>
<td>11.5 ± 5.4ᵃ Multiply</td>
</tr>
<tr>
<td>C-PTH (2.6–10.6 pmol/L)</td>
<td>5.25 ± 1.11</td>
<td>11.7 ± 4.9ᵃ Multiply</td>
<td>19.8 ± 7.1ᵃ Multiply</td>
<td>45.8 ± 20ᵃFrançois</td>
</tr>
</tbody>
</table>

ᵃ Results are mean ± SD. Reference ranges for analytes in parentheses.
ᵇ–ʲ Compared with healthy subjects:ᵇ P < 0.05; ᵍ P < 0.001; ᵉ P < 0.01.
ᵇ–ʲ Compared with patients with GFR between 60 and 100 mL·min⁻¹·1.73 m⁻²:ᵇ P < 0.05; ᵉ P < 0.001; ᵉ P < 0.01.
ᵇ–ʲ Compared with patients with GFR between 30 and 60 mL·min⁻¹·1.73 m⁻²:ᵇ P < 0.001; ᵉ P < 0.01.
ᵇ–ʲ Compared with patients with GFR between 60 and 100 mL·min⁻¹·1.73 m⁻²:ᵇ P < 0.05; ᵉ P < 0.001; ᵉ P < 0.01.
and accumulates in hemodialyzed patients to account for 50% of I-PTH immunoreactivity compared with 20% in healthy individuals (2). We studied five groups of individuals with GFR values ranging from normal to almost nonexistent. This enabled us to study I-PTH composition as a function of the GFR.

I- and C-PTH were increased in patients with a GFR between 30 and 100 mL·min⁻¹·1.73 m²⁻² even if mean Ca²⁺, phosphate, 25(OH)D, and 1,25(OH)₂D values were in the reference ranges. I- and C-PTH concentrations were higher in patients with a GFR <30 mL·min⁻¹·1.73 m²⁻² and in hemodialyzed patients, but in these groups, the mean Ca²⁺ and 1,25(OH)₂D concentrations were low and the phosphate concentration was increased. The GFR and Ca²⁺, 1,25(OH)₂D, I-PTH, and C-PTH were all correlated when all patients with progressive renal failure were considered. Only the correlation between the GFR and I- and C-PTH remained significant when patients with a GFR <30 mL·min⁻¹·1.73 m²⁻² were excluded. The main correlations between I- and C-PTH concentrations in progressive renal failure were with Ca²⁺, CrCl, 25(OH)D, and 1,25(OH)₂D concentrations by simple regression. Only the correlations with Ca²⁺ and CrCl remained significant by multivariate analysis when all patients with progressive renal failure were considered, and only the correlations with 25(OH)D and CrCl remained significant when patients with a GFR <30 mL·min⁻¹·1.73 m²⁻² were excluded. Our study was performed at the end of winter, and this may explain why 35% of our patients with progressive renal failure had 25(OH)D concentrations below the lower limit of normal and why 25(OH)D was associated with I-PTH concentrations. It remains that the biochemical findings in our patients were similar to those described by others in similar patients (27–31), with minor differences from one study to the other. In particular, the concentration of I-PTH relative to the GFR of the patients is surprisingly similar (28–30) when the same or similar I-PTH assays are used.

The composition of I-PTH as a function of the GFR was our main focus. There were minor changes in composition before a GFR <30 mL·min⁻¹·1.73 m²⁻² was reached. At this stage, PTH(1-84) had decreased from 79% in healthy individuals to 68% in patients with progressive renal failure, and non-(1-84) had increased from 21% in healthy
individuals to 32% in patients with progressive renal failure. The greatest changes were seen in hemodialyzed patients, where PTH(1-84) and non-(1-84) PTH each accounted for one-half of the I-PTH. This means that the accumulation of non-(1-84) PTH becomes a more significant phenomenon in the last stage of progressive renal failure evolution. For an 8-fold increase in total I-PTH between healthy individuals and hemodialyzed patients,
there is a 5-fold increase in PTH(1-84) but an 18-fold increase in non-(1-84) PTH. This 3.6-fold difference between PTH(1-84) and non-(1-84) PTH may well represent decreased renal clearance of this C-terminal fragment. It is interesting to note that the difference in C-PTH between healthy individuals and hemodialyzed patients is also 18-fold; this assay reacts predominantly with C-terminal fragments, reinforcing the decreased clearance hypothesis.

Overall, our study demonstrated that the GFR influences the composition of I-PTH in progressive renal failure, with non-(1-84) PTH representing a greater proportion of total I-PTH immunoreactivity with each decrease. When renal function is abolished, I-PTH concentrations 1.6-fold higher are required to achieve PTH(1-84) concentration similar to those in healthy subjects. The role of non-(1-84) PTH in the PTH resistance of renal failure remains to be elucidated because theoretically, this large C-terminal fragment could bind to both the classical PTH/PTHrP (32–34) and C-PTH (35) receptors and therefore interfere with PTH(1-84) biological effects. Recent results obtained in vivo in rats suggest that the synthetic fragment PTH(7-84), possibly related to non-(1-84) PTH, is a potent in vivo antagonist of PTH(1-84) (36). The recent introduction of a “whole” PTH assay free of interference from non-(1-84) PTH should be very helpful in clarifying these issues (37, 38).

References


