Pharmacokinetics and Pharmacodynamics of Subcutaneous Recombinant Parathyroid Hormone (1–84) in Patients With Hypoparathyroidism: An Open-Label, Single-Dose, Phase I Study

Bart L. Clarke, MD1; Jolene Kay Berg, MD2,*; John Fox, PhD3; Jane A. Cyran, PhD3; and Hjalmar Lagast, MD3

1Division of Endocrinology, Diabetes, Metabolism, and Nutrition, Mayo Clinic, Rochester, Minnesota; 2PRACS Institute, San Antonio, Texas; and 3NPS Pharmaceuticals, Inc, Bedminster, New Jersey

ABSTRACT

Background: Impaired mineral homeostasis affecting calcium, phosphate, and magnesium is a result of parathyroid hormone (PTH) deficiency in hypoparathyroidism. The current standard of treatment with active vitamin D and oral calcium does not control levels of these major minerals. Recombinant full-length human PTH 1–84 (rhPTH[1–84]) is being developed for the treatment of hypoparathyroidism.

Objective: The goal of this study was to investigate the pharmacokinetics and pharmacodynamics of a single subcutaneous injection of rhPTH(1–84) in patients with hypoparathyroidism.

Methods: This was an open-label, dose-escalating study of single subcutaneous administration of 50 µg and then 100 µg of rhPTH(1–84). Enrolled patients (age range, 25–85 years) had ≥12 months of diagnosed hypoparathyroidism defined according to biochemical evidence of hypocalcemia with concomitant low-serum intact PTH and were taking doses ≥1000 mg/d of oral calcium and ≥0.25 µg/d of active vitamin D (oral calcitriol). The patient’s prescribed dose of calcitriol was taken the day preceding but not on the day of or during the 24 hours after rhPTH(1–84) administration. Each patient received a single 50-µg rhPTH(1–84) dose, had at least a 7-day washout interval, and then received a single 100-µg rhPTH(1–84) dose. The following parameters were assessed: plasma PTH; serum and urine total calcium, magnesium, phosphate, and creatinine; and urine cyclic adenosine monophosphate.

Results: After administration of rhPTH(1–84) 50 µg (n = 6) and 100 µg (n = 7), the approximate t½ was 2.5 to 3 hours. Plasma PTH levels increased rapidly, then declined gradually back to predose levels at ~12 hours. The median AUC was similar with calcitriol and rhPTH(1–84) for serum 1,25-dihydroxyvitamin D (calcitriol, 123–227 pg · h/mL; rhPTH[1–84], 101–276 pg · h/mL), calcium (calcitriol, 3.3–3.7 mg · h/dL; rhPTH[1–84], 3.3–7.6 mg · h/dL), and magnesium (calcitriol, 0.7–0.9 mg · h/dL; rhPTH[1–84], 1.3–2.8 mg · h/dL). In contrast, the median AUC for phosphate was strongly negative with rhPTH(1–84) (calcitriol, −1.0 to 0.8 mg · h/dL; rhPTH[1–84], −21.3 to −26.5 mg · h/dL). Compared with calcitriol, rhPTH(1–84) 50 µg reduced 24-hour calcium excretion and calcium-to-creatinine ratios by 12% and 23%, respectively, and rhPTH(1–84) 100 µg reduced them by 26% and 27%. There was little overall impact on urine magnesium levels. Compared with calcitriol, rhPTH(1–84) 50 µg increased urinary phosphate excretion and phosphate-to-creatinine ratios by 53% and 54%, respectively, and rhPTH(1–84) 100 µg increased them by 45% and 42%. Urine cyclic adenosine monophosphate-to-creatinine ratio increased with rhPTH(1–84) by 2.3-fold (50 µg) and 4.4-fold (100 µg) compared with calcitriol.

Conclusions: PTH replacement therapy with rhPTH(1–84) regulated mineral homeostasis of calcium, magnesium, phosphate, and vitamin D metabolism toward normal in these study patients with hypoparathyroidism. (Clin Ther. 2014;36:722–736) © 2014 The Authors. Published by Elsevier HS Journals, Inc. All rights reserved.

*Current address: DaVita Clinical Research, Minneapolis, Minnesota.
Key words: calcium, hypoparathyroidism, parathyroid hormone, pharmacokinetics, phosphate, rhPTH(1–84).

INTRODUCTION
Hypoparathyroidism is a rare, complex endocrine disorder of unbalanced mineral homeostasis resulting from absent or inappropriately low levels of parathyroid hormone (PTH). The hallmark clinical presentation is characterized by hypocalcemia, although other clinical characteristics often include hyperphosphatemia and may include hypomagnesemia. Current therapy for chronic hypoparathyroidism, aimed at controlling the symptoms of neuromuscular excitability (eg, paresthesias, cramps, tetany, seizures) due to hypocalcemia, consists of large doses of oral calcium and active forms of vitamin D; this therapy is designed to raise serum calcium levels by increasing intestinal calcium absorption. Patients may need thiazide diuretics to decrease hypercalciuria and dietary phosphate restriction or binders to control hyperphosphatemia. These suboptimal approaches present specific challenges for clinical care because they are a balance between partial symptom management and the ensuing long-term complications of soft tissue calcifications and kidney stones.

Hypoparathyroidism is the last remaining classic hormone deficiency endocrine disorder for which replacement therapy using the native hormone has not been approved. Initial clinical studies investigated whether a synthetic truncated PTH(1–34) would be an effective treatment. Results showed that subcutaneous (SC) injections BID were required to maintain normal serum calcium levels while reducing fluctuations in calcium levels and hypercalciuria. Further improvements in the serum and urine calcium fluctuations were observed when PTH(1–34) was delivered via a modified insulin pump. The effect of treatment using full-length recombinant human PTH (rhPTH(1–84)), which is identical to endogenous human PTH, has been investigated. Several Phase I/II, single-center, open-label studies demonstrated biologic activity of rhPTH(1–84) administered as SC injections daily or on alternate days in patients with hypoparathyroidism. A large, randomized, placebo-controlled, Phase III registration trial (REPLACE [Use of NPSP558 in the Treatment of Hypoparathyroidism]; NCT00732615) was recently completed in 134 patients with hypoparathyroidism who received SC injections of 50 μg/d of rhPTH(1–84), with permitted dose escalation up to 75 μg/d and then 100 μg/d. Results showed that 53% of patients in the rhPTH(1–84) group and 2% of patients in the placebo group met the primary end point of reducing calcium and active vitamin D doses by ≥50% while maintaining serum calcium levels above baseline.

The current study in treatment-naive patients with hypoparathyroidism incorporated the highest and lowest rhPTH(1–84) dose levels included in the registration study. It was intended to provide supportive pharmacokinetic (PK) and pharmacodynamic (PD) data in serum and urine to better interpret findings from other studies investigating the use of rhPTH(1–84) as a therapy for hypoparathyroidism.

PATIENTS AND METHODS
Design
This was an open-label, dose-escalating, single-dose, 2-center study. It was conducted in accordance with the Good Clinical Practice Guidelines and the Declaration of Helsinki. The investigational sites’ institutional review boards approved the protocol before study initiation. Written informed consent was obtained from patients before study participation.

The maximum enrollment was 8 male or female (postmenopausal or nonpregnant) patients with hypoparathyroidism, aged 25 to 85 years, at the Mayo Clinic (Rochester, Minnesota) and Cetero Research (San Antonio, Texas). The main inclusion criteria were hypoparathyroidism for ≥12 months defined by hypocalcemia with concomitant serum PTH concentrations below the lower limit of the laboratory normal range, doses ≥1000 mg/d of oral calcium and ≥0.25 μg/d of active vitamin D (oral calcitriol), serum magnesium levels within the normal laboratory range, serum 25-hydroxyvitamin D (25(OH)D) levels ≤1.5-fold of the laboratory upper limit of normal (ULN), serum creatinine levels <1.5 mg/dL, and creatinine clearance >60 mL/min. The main exclusion criteria were hypoparathyroidism resulting from an activating mutation in the calcium-sensing receptor gene, pseudohypoparathyroidism, any non-hypoparathyroidism disease that might affect calcium metabolism or calcium–phosphate homeostasis, regular parenteral calcium infusions, history of hypercalcemia and disturbances in calcium and/or phosphate metabolism,
use of diuretics within 14 days of study drug administration, calcitriol or cinacalcet hydrochloride use within 3 months, and previous treatment with PTH(1–84), N-terminal PTH, or PTH-related peptides or analogues within 3 months.

rhPTH(1–84) (NPS Pharmaceuticals, Inc., Bedminster, New Jersey) was provided as a lyophilized powder in a dual-chamber, 1-mL injection pen that contained solution for reconstitution. Two strengths were provided, enabling 50 or 100 μg to be reconstituted in 71.4 μL and directly administered as an SC injection in the thigh by trained personnel. Each patient received a single 50-μg rhPTH(1–84) dose during treatment period 1, had at least a 7-day washout interval, and then received a single 100-μg rhPTH(1–84) dose during treatment period 2. Calcium intake (dietary and supplemental) was to be held constant throughout the study and was monitored on the day before and up to the final blood draw after rhPTH(1–84) administration. Calcitriol was taken at the patient’s prescribed dose at time 0 on day −1 but was not taken on day 1 or during the 24 hours after rhPTH(1–84) administration in each period. The changes on day −1 represent the responses to each patient’s habitual regimen of oral calcium and calcitriol supplements. Both calcitriol (day −1) and rhPTH (1–84) (day 1) were administered after an overnight minimal 8-hour fast in both treatment periods. Fasting continued ~2 hours after calcitriol and rhPTH(1–84) administration, at which time a standardized meal was served. The protocol from day −1 was followed as closely as possible on day 1 of each treatment period.

### Assessments

Plasma PTH(1–84) levels were measured in blood samples obtained during the 24 hours before and after rhPTH(1–84) administration in each period by using a whole PTH(1–84) immunoradiometric assay (Scantibodies Laboratory, Inc, Santee, California). The assay has a lower limit of quantification of 10 pg/mL, quantifies only full-length PTH(1–84), and does not distinguish between rhPTH(1–84) and endogenous PTH. Baseline for each treatment period was the average of 3 plasma samples collected during the day immediately before study drug administration (day −1). The PK of PTH(1–84) were calculated in validated version 5.2 of WinNonlin Enterprise (Pharsight Corporation, Cary, North Carolina) by using noncompartmental methods on individual concentration–time data of rhPTH(1–84).

Serum total calcium, magnesium, phosphate, and creatinine were measured by using standard tests from blood samples obtained during the 24 hours before (day −1) and after (day 1) the administration of rhPTH(1–84) in each period (the patient’s prescribed doses of calcitriol were taken at time 0 on day −1 and rhPTH[1–84] at time 0 on day 1). Serum 25(OH)D and 1,25-dihydroxyvitamin D (1,25(OH)2D) levels were determined by using LC-MS/MS. The PD parameters were calculated in WinNonlin by using noncompartmental methods on time points relative to the time of calcitriol (day −1) and study drug (day 1) administration in both treatment periods. The serum total calcium values were not albumin adjusted; measurements of serum albumin levels indicated no substantial changes in serum albumin concentrations during the treatment and monitoring periods. To assess the renal responses, urine samples were collected at 0 to 3, 3 to 6, 6 to 10, 10 to 16, and 16 to 24 hours after calcitriol (day −1) and rhPTH(1–84) (day 1) administration in each period, and the fractional excretion of calcium (FE\textsubscript{Ca}), magnesium (FE\textsubscript{Mg}), and phosphate (FE\textsubscript{p}) were calculated, as were the excretion of creatinine and cyclic adenosine monophosphate (cAMP).

FE\textsubscript{Ca}, FE\textsubscript{Mg}, and FE\textsubscript{p} were calculated by using the following formula: [urine-specific mineral (mg/dL) × serum creatinine (mg/dL) × 100]/[serum-specific mineral (mg/dL) × urine creatinine (mg/dL)].

The safety profile was monitored throughout the study in all patients who entered the treatment phase.

### Statistics

All parameters were summarized with descriptive statistics because of the small size of the study population.

### RESULTS

#### Patients

PK and PD analysis for rhPTH(1–84) was performed in 7 enrolled patients with hypoparathyroidism; 1 additional patient was discontinued before receiving study drug. Six patients received rhPTH(1–84) 50 μg in period 1, and 7 patients received rhPTH (1–84) 100 μg in period 2. The patient excluded from the 50-μg rhPTH(1–84) analysis in period 1 had a failed dose due to an error in use of the injection pen. Table 1 summarizes patient demographic characteristics and biochemistry findings at baseline. Patient
demographics and disease characteristics at baseline were consistent with the general characteristics of hypoparathyroidism. Serum total 25(OH)D and creatinine levels were within the normal range. Serum total calcium, magnesium, and 1,25(OH)₂D levels were near the lower limit of normal, and serum phosphate levels were at the ULN. The individual baseline daily calcium and calcitriol doses for each patient were held constant throughout the study (the range for all patients is noted in Table I).

**Pharmacokinetics**

Baseline plasma PTH levels were below the limit of quantification (10 pg/mL) throughout day –1 in both treatment periods, with the exception of 1 measureable concentration (11 pg/mL) in 1 patient in period 2. After the 50- and 100-µg rhPTH(1–84) injections, a double PTH peak was observed, with an initial peak occurring at a median of 10 to 15 minutes and a second peak at 1 to 3 hours (Figure 1). PTH levels became undetectable by 12 or 24 hours. Table II summarizes the baseline-adjusted PK parameters. The t½ was 2.5 to 3 hours with both doses.

**Pharmacodynamics**

Before calcitriol administration on day –1 of each treatment period, serum levels of 1,25(OH)₂D, total calcium, magnesium, phosphate, and creatinine were comparable (Table I).

**Serum 1,25(OH)₂D**

Serum 1,25(OH)₂D increased promptly and peaked at a median of 3 to 5 hours after calcitriol administration and then generally decreased and reached baseline levels by 24 hours (Figure 2). After rhPTH(1–84) administration, median serum 1,25(OH)₂D increased more slowly over 8 to 16 hours before decreasing; levels remained above baseline at 24 hours. PD analysis of the baseline-adjusted serum 1,25(OH)₂D profiles is shown in Table III. The median maximum effect (Eₘₐₓ) after calcitriol or rhPTH(1–84) administration was similar. The difference between the AUC that was above and below the baseline value (AUCₙₑᵗ) was 27% higher with the 50-µg dose versus the 100-µg dose.

**Calcium**

The pattern of change in serum total calcium levels was similar after both calcitriol and rhPTH(1–84):

![Table I. Patient demographic and baseline characteristics. Unless otherwise indicated, values are given as median (range).](image)
Peak levels occurred after 8 to 12 hours with calcitriol and after 12 hours with 50 and 100 mg of rhPTH(1–84) (Figure 3). Predose levels were not restored by 24 hours with either treatment. PD analysis (Table III) showed that the median E_{\text{max}} and AUC_{\text{net}} for serum total calcium after injection of rhPTH(1–84) were comparable to those obtained for calcitriol administration in the same treatment period. Unlike the serum responses, there were marked treatment differences in urine calcium excretion. After administration of oral calcitriol, there was an increase in FE_{\text{Ca}} followed by a plateau. In contrast, with rhPTH (1–84), there was an immediate and substantial decrease in FE_{\text{Ca}} to a nadir in the 3- to 6-hour urine sample. Total 24-hour urinary calcium excretion and calcium-to-creatinine ratios were similar on each day of calcitriol treatment (Table IV). Compared with calcitriol, the 50-\(\mu\)g rhPTH(1–84) dose reduced 24-hour calcium excretion and calcium-to-creatinine ratios by 12% and 23%, respectively. The 100-\(\mu\)g rhPTH(1–84) dose reduced 24-hour calcium excretion and calcium-to-creatinine ratios by 26% and 27%.

**Magnesium**

Peak levels occurred with calcitriol after 5 to 12 hours (period 1) and 10 to 24 hours (period 2), and with 50 \(\mu\)g of rhPTH(1–84) after 8 to 12 hours and with 100 \(\mu\)g of rhPTH(1–84) after 8 to 16 hours (Figure 4). Unlike the calcium response, serum magnesium levels returned to baseline or lower by 24 hours after rhPTH(1–84) administration. PD analysis

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**Table II. Baseline-adjusted pharmacokinetic parameters for parathyroid hormone (1–84). Values are given as median (range).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>rhPTH(1–84) 50 (\mu)g (n = 6)</th>
<th>rhPTH(1–84) 100 (\mu)g (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{0–last} pg \cdot h/mL</td>
<td>554 (449–752)</td>
<td>968 (647–1087)</td>
</tr>
<tr>
<td>AUC_{0–\infty} pg \cdot h/mL</td>
<td>618 (520–822)</td>
<td>1117 (722–1141)</td>
</tr>
<tr>
<td>C_{\text{max}} pg/mL</td>
<td>175 (92–241)</td>
<td>189 (139–463)</td>
</tr>
<tr>
<td>T_{\text{max}} h</td>
<td>0.25 (0.17–2.00)</td>
<td>0.17 (0.08–1.50)</td>
</tr>
<tr>
<td>(\tau_{\frac{1}{2}}) h</td>
<td>2.40 (2.00–4.70)</td>
<td>2.96 (1.80–3.74)</td>
</tr>
<tr>
<td>CL/F, L/h</td>
<td>82 (61–96)</td>
<td>90 (88–139)</td>
</tr>
<tr>
<td>V_{ss}/F, L</td>
<td>267 (221–585)</td>
<td>443 (253–722)</td>
</tr>
</tbody>
</table>

rhPTH(1–84) = recombinant human parathyroid hormone 1–84; \(V_{ss}/F\) = apparent volume of distribution at steady-state.
showed that, as for serum total calcium, the median E\text{max} and \text{AUC}_{\text{net}} for serum magnesium after injection of rhPTH(1–84) were similar to those obtained for calcitriol administration in the same treatment period, although the highest values occurred with 100 µg of rhPTH(1–84) (Table III). \text{FEMg} followed a pattern similar to that observed for \text{FECa} (Figure 3), with an initial increase in \text{FEMg} after oral calcitriol and a rapid and marked decrease after rhPTH(1–84) administration. Total 24-hour urinary magnesium excretion and magnesium-to-creatinine ratios were similar after each calcitriol administration (Table IV). In contrast to its effects on calcium excretion, rhPTH(1–84) had little impact on overall urine magnesium, primarily because excretion increased above predose levels in the last 8 hours of each study day (Figure 4).

**Phosphate**

Calcitriol resulted in an initial small decrease in serum phosphate levels before reaching an above-ULN peak (12 hours), and finally a return to predose levels (16 hours), in both periods (Figure 4). In contrast, after rhPTH(1–84) administration, serum phosphate levels decreased rapidly (−1.6 mg/dL at 5 hours) to a level that was in the lower portion of the normal range, and thereafter progressively increased. However, baseline levels were not restored within 24 hours with either dose and remained at least 0.5 mg/dL below the ULN. PD analysis found that the magnitude of the initial decrease and the subsequent increase in serum phosphate after calcitriol administration were similar and resulted in an \text{AUC}_{\text{net}} that showed little overall change (Table III). In marked contrast, the median \text{E}\text{max} for serum phosphate after rhPTH(1–84) administration was negative because all postinjection values were below baseline findings. The minimum effect for serum phosphate after rhPTH(1–84) administration was −1.5 mg/dL with both doses, and the \text{AUC}_{\text{net}} values were also strongly negative. The pattern of \text{FEP} was similar after oral calcitriol administration in both treatment periods, with only a small increase to a level that was generally maintained (Figure 4). In contrast, after rhPTH(1–84) administration, \text{FEP} increased 2.6- and 3.3-fold with 50 and 100 µg of rhPTH(1–84),
respectively, after 3 to 6 hours compared with calcitriol levels from 16 to 24 hours on day C0. Thereafter, FEP after rhPTH(1–84) administration decreased progressively and returned to predose levels (16–24 hours). Total 24-hour urinary phosphate excretion was increased by 53% and 45% with 50 and 100 μg of rhPTH(1–84), respectively, compared with each preceding calcitriol day (Table IV). The 24-hour urinary phosphate-to-creatinine ratios were also increased by 54% and 42%.

### Calcium × Phosphate Product

At 12 hours after rhPTH(1–84) administration (Figure 5), the time of peak serum calcium levels and low serum phosphate levels, the calcium × phosphate product was lower with both doses of rhPTH(1–84) (35–36 mg²/dL²) than at baseline (41–42 mg²/dL²). In contrast, after calcitriol administration, the calcium × phosphate product was higher (44–46 mg²/dL²) than baseline (39 mg²/dL²). Furthermore, a product of 55 mg²/dL² was recorded in both periods in 1 patient and in period 2 in a second patient at 12 hours after calcitriol administration.

### Urine cAMP

In the 24 hours after calcitriol administration in both periods, cAMP excretion was relatively stable (Figure 6). In contrast, there was a substantial, dose-related increase

### Table III. Baseline-adjusted serum pharmacodynamic parameters in each treatment period. Values are given as median (range).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period 1</th>
<th>Period 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calcitriol (n = 7)</td>
<td>rhPTH(1-84) 50 μg (n = 6)</td>
</tr>
<tr>
<td>1,25(OH)₂D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emax, pg/mL</td>
<td>21.0 (8.0 to 26.0)</td>
<td>24.0 (7.0 to 52.0)</td>
</tr>
<tr>
<td>AUCabove, pg · h/mL</td>
<td>149 (71.5 to 352)</td>
<td>276 (14.0 to 458)</td>
</tr>
<tr>
<td>AUCbelow, pg · h/mL</td>
<td>2.0 (0 to 29.5)</td>
<td>1.1 (0 to 75.5)</td>
</tr>
<tr>
<td>AUCnet, pg · h/mL</td>
<td>123 (69.5 to 352)</td>
<td>276 (-61.5 to 458)</td>
</tr>
<tr>
<td>Total calcium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emax, mg/dL</td>
<td>0.7 (0.1 to 0.9)</td>
<td>0.7 (0.2 to 1.2)</td>
</tr>
<tr>
<td>AUCabove, mg · h/dL</td>
<td>4.7 (1.2 to 15.8)</td>
<td>4.9 (1.0 to 12.4)</td>
</tr>
<tr>
<td>AUCbelow, mg · h/dL</td>
<td>1.3 (0 to 7.2)</td>
<td>2.0 (0 to 6.9)</td>
</tr>
<tr>
<td>AUCnet, mg · h/dL</td>
<td>3.3 (-5.6 to 15.8)</td>
<td>3.3 (-5.4 to 12.4)</td>
</tr>
<tr>
<td>Magnesium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emax, mg/dL</td>
<td>0.1 (0 to 0.3)</td>
<td>0.2 (0 to 0.2)</td>
</tr>
<tr>
<td>AUCabove, mg · h/dL</td>
<td>1.0 (0 to 3.7)</td>
<td>1.5 (0 to 2.9)</td>
</tr>
<tr>
<td>AUCbelow, mg · h/dL</td>
<td>0.3 (0 to 1.5)</td>
<td>0.2 (0 to 1.6)</td>
</tr>
<tr>
<td>AUCnet, mg · h/dL</td>
<td>0.7 (-1.5 to 3.7)</td>
<td>1.3 (-1.6 to 2.9)</td>
</tr>
<tr>
<td>Phosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emax, mg/dL</td>
<td>0.4 (0.1 to 1.3)</td>
<td>-0.3 (-0.8 to 0.1)</td>
</tr>
<tr>
<td>Emin, mg/dL</td>
<td>-0.7 (-1.0 to 0.1)</td>
<td>-1.6 (-2.0 to -0.6)</td>
</tr>
<tr>
<td>AUCabove, mg · h/dL</td>
<td>1.6 (0.1 to 16.8)</td>
<td>0 (0 to 0.1)</td>
</tr>
<tr>
<td>AUCbelow, mg · h/dL</td>
<td>4.7 (0 to 8.5)</td>
<td>21.3 (7.3 to 35.1)</td>
</tr>
<tr>
<td>AUCnet, mg · h/dL</td>
<td>-1.0 (-8.4 to 16.8)</td>
<td>-21.3 (-35.1 to -7.3)</td>
</tr>
</tbody>
</table>

rhPTH(1-84) = recombinant human parathyroid hormone 1-84; 1,25(OH)₂D = 1,25-dihydroxyvitamin D; Emax = median maximum effect; Emin = median minimum effect; AUCabove = AUC that is above the baseline value; AUCbelow = AUC that is below the baseline value; AUCnet = difference between AUCabove and AUCbelow.
in the cAMP-to-creatinine ratio in the first urine sample (0–3 hours) collected after rhPTH(1–84) injection. The urine cAMP-to-creatinine ratio increased by 2.3- and 4.4-fold with the 50- and 100-mg doses of rhPTH(1–84), respectively, compared with the sample collected at 16 to 24 hours after calcitriol administration.

Creatinine

In the 24 hours after calcitriol administration in both periods, median serum creatinine levels remained constant (0.9 [range, 0.5–1.5] to 1.1 [range, 0.6–1.5] mg/dL). In contrast, in the 12 hours after rhPTH(1–84) administration, creatinine levels decreased progressively from 0.9 (range, 0.7–1.6) mg/dL to 0.8 (range, 0.6–1.5) mg/dL (50-µg dose) and from 1.0 (range, 0.7–1.5) mg/dL to 0.9 (range, 0.5–1.3) mg/dL (100-µg dose), before returning close to baseline levels at 16 to 24 hours. This decrease correlated with increased glomerular filtration rate; median (range) creatinine clearance findings in the 3- to 6-hour samples were 125 (75–155) mL/min (50-µg dose) and 108 (95–158) mL/min (100-µg dose), compared with 91 (63–120) mL/min (calcitriol, period 1) and 92 (55–169) mL/min (calcitriol, period 2).

Safety Profile

rhPTH(1–84) was well tolerated; no serious adverse events (AEs) occurred, and no patient discontinued treatment because of a treatment-emergent AE. Five of the 7 study patients reported treatment-emergent AEs; all were deemed unrelated to study drug by the investigators. A total of 7 of 8 AEs were mild in severity (nausea, vessel puncture site hematoma, muscle strain, dysmenorrhea, tachypnea, and 2 incidents of vaginal hemorrhage in the patient who

Figure 3. Changes in baseline-adjusted serum (left panels) and urinary fractional excretion (FE\textsubscript{Ca}; right panels) of total calcium levels after oral administration of calcitriol (n = 7) or subcutaneous injection of 50 µg (n = 6) or 100 µg (n = 7) of recombinant human parathyroid hormone 1–84 (rhPTH[1–84]) in patients with hypoparathyroidism. The arrows indicate the time of treatment administration. Note that the serum baseline resets to zero (horizontal dotted line) after the 24-hour calcitriol time point. Line graphs (left panels; triangles = calcitriol, circles = rhPTH[1–84]) of median with minimum and maximum values and the 25th and 75th percentiles are shown by dashed lines or box and whisker plots (right panels) of median (line within box), minimum and maximum values (ends of the lower and upper whiskers, respectively), and 25th and 75th percentiles (top and bottom of the box).
also reported dysmenorrhea), and 1 of 8 was moderate in severity (gout); however, this patient did not receive study drug in period 1 because of an error in using the injection pen.

DISCUSSION

This study characterized the PK and the acute serum and urine PD responses in the 24-hour period after SC administration of 50- and 100-µg doses of rhPTH(1–84) in patients with hypoparathyroidism. These doses spanned those evaluated in the 24-week, Phase III REPLACE registration study. Plasma PTH levels increased rapidly, resulting in a double-peak concentration profile that was followed by a slow decline back to predose levels at ~12 hours. The mechanism responsible for the profile and extended duration of exposure to PTH is unknown. Mean exposure parameters with the 100-µg dose were lower in the present study than observed previously in an unpublished Phase Ib trial (Cmax, 233 vs 303 pg/mL; AUC, 924 vs 1349 pg · h/mL), perhaps because the patients in this study were heavier (median weight, 78 vs 69 kg) (data on file, NPS Pharmaceuticals, Inc., Bedminster, NJ; 2014).

Serum 1,25(OH)₂D₃, total calcium, magnesium, and phosphate levels were assessed because they are adversely affected in hypoparathyroidism. Analysis of urine samples across 24 hours for calcium, magnesium, phosphate, and cAMP provided additional information to better understand the mechanisms involved in the serum PD responses. A comparison was made with PD responses after administration of oral calcium and the active vitamin D metabolite calcitriol during the 24-hour period preceding each day of rhPTH(1–84) because these are normal or habitual treatments that patients use to control symptoms.

In this study, a comparison of the changes in serum 1,25(OH)₂D₃ levels resulting from calcitriol versus PTH-stimulated renal 1,25(OH)₂D₃ synthesis was possible because no calcitriol was taken when rhPTH(1–84) was administered. The 50-µg rhPTH(1–84) dose resulted in a larger 1,25(OH)₂D₃ response than the 100-µg dose, although both peaked after 8 hours. Renal 1-hydroxylase is regulated not only by PTH, but its activity is also directly inhibited by calcium. The higher calcium response to the 100-µg

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period 1</th>
<th>Period 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calcitriol</td>
<td>rhPTH(1–84) 50 µg</td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount, mg</td>
<td>394 (130–495)</td>
<td>347 (86–549)</td>
</tr>
<tr>
<td>Ratio, mg/mg</td>
<td>0.34 (0.08–0.40)</td>
<td>0.27 (0.04–0.44)</td>
</tr>
<tr>
<td>Magnesium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount, mg</td>
<td>104 (58–205)</td>
<td>103 (52–223)</td>
</tr>
<tr>
<td>Ratio, mg/mg</td>
<td>0.08 (0.03–0.17)</td>
<td>0.08 (0.03–0.16)</td>
</tr>
<tr>
<td>Phosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount, mg</td>
<td>591 (247–1014)</td>
<td>904 (669–1278)</td>
</tr>
<tr>
<td>Ratio, mg/mg</td>
<td>0.46 (0.21–0.72)</td>
<td>0.71 (0.49–0.94)</td>
</tr>
<tr>
<td>Creatinine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ratio = amount excreted normalized by creatinine.
Figure 4. Changes in baseline-adjusted serum (left panels) and urinary fractional excretion (FE; right panels) of magnesium and phosphate levels after oral administration of calcitriol (n = 7) or subcutaneous injection of 50 µg (n = 6) or 100 µg (n = 7) of recombinant human parathyroid hormone 1–84 (rhPTH[1–84]) in patients with hypoparathyroidism. The arrows indicate the time of treatment administration. Note that the serum baseline resets to zero (horizontal dotted line) after the 24-hour calcitriol time point. Line graphs (left panels; triangles = calcitriol, circles = rhPTH[1–84]) of median with minimum and maximum values, and the 25th and 75th percentiles are shown by dashed lines or box and whisker plots (right panels) of median (line within box), minimum and maximum values (ends of the lower and upper whiskers, respectively), and 25th and 75th percentiles (top and bottom of the box).
dose may provide a greater inhibition of enzyme activity and, therefore, a smaller 1,25(OH)2D response. Thus, rhPTH(1–84) produces a serum 1,25(OH)2D response that is essentially equivalent to that observed with calcitriol but with a delayed and prolonged time course; peak levels occur at 8 to 9 hours after rhPTH(1–84) administration compared with 3 hours after calcitriol administration. The stimulation of endogenous 1,25(OH)2D formation provides an underlying mechanism to reduce the administration of active forms of vitamin D in long-term treatment of hypoparathyroidism with rhPTH(1–84).12–14,19

The greatest effects on serum calcium and magnesium were observed with 100 µg of rhPTH(1–84). The increase in serum calcium levels in this study was comparable to the response of SC rhPTH(1–84) 100 µg in normal postmenopausal women,16 with maximum levels achieved near 12 hours. PTH has major effects on phosphate levels, primarily by increasing urinary excretion, and its absence in hypoparathyroidism results in hyperphosphatemia. Both doses of rhPTH(1–84) had profound effects on serum phosphate, decreasing mean levels from the upper limit to the lower quartile of the normal range within 5 hours. The prolonged decrease in serum

Figure 5. Serum calcium x phosphate (Ca x P) product levels after oral administration of calcitriol (n = 7) or subcutaneous injection of 50 µg (n = 6) or 100 µg (n = 7) of recombinant human parathyroid hormone 1–84 (rhPTH[1–84]) in patients with hypoparathyroidism. The arrows indicate the time of treatment administration. Values are presented at each time point for individual patients (lines with symbols) and as the median for the study population (thick solid line). The horizontal dotted line indicates a Ca x P product level of 55 mg2/dL2.
phosphate after a single injection of rhPTH(1–84) is consistent with the progressive decrease in phosphate levels observed in long-term studies in patients with hypoparathyroidism.\textsuperscript{12,13,15,20} In contrast, the changes in serum phosphate after calcitriol administration were considerably smaller in magnitude than were observed with rhPTH(1–84) and were at or above baseline levels for most of the 24 hours after dosing. PD analysis confirmed that the AUC\textsubscript{net} for serum phosphate after calcitriol use was small but was strikingly negative with rhPTH(1–84). The decrease in serum phosphate after rhPTH(1–84) administration resulted in a corresponding decrease in the serum calcium × phosphate product. At 12 hours after rhPTH(1–84) administration (the time of peak serum calcium levels and normalized serum phosphate levels), the median calcium × phosphate product was 2 to 6 mg\textsuperscript{2}/dL\textsuperscript{2} lower than at baseline. In contrast, 12 hours after calcitriol administration, the calcium × phosphate product was 5 to 6 mg\textsuperscript{2}/dL\textsuperscript{2} higher than at baseline. We were surprised to find in 2 patients who were taking average daily oral calcium and calcitriol doses that the calcium × phosphate product reached a level that is associated with an increased risk of soft
tissue calcification (≥55 mg/dL). These instances occurred at 12 to 16 hours after calcitriol administration, when peak calcium and phosphate concentrations are rarely measured because most blood sampling is done in the morning.

Increased clearance of creatinine resulted in decreased serum creatinine levels after rhPTH(1–84) administration. The mechanism responsible for this increased clearance is unclear but may be related to the vasodilatory properties of PTH resulting in increased renal blood flow. cAMP is an important second messenger produced after activation of the PTH-1 receptor, and its secretion by renal tubules is increased after either exogenous administration of PTH or stimulation of endogenous PTH secretion. cAMP excretion increased and was maximal at 0 to 3 hours after rhPTH(1–84) administration. Thereafter, cAMP excretion declined in parallel with the decrease in plasma PTH levels. As expected, calcitriol had no effect on cAMP excretion. Measurements of urinary excretion patterns of calcium, magnesium, and phosphate in this study provided insights into the mechanism underlying the changes in the serum levels of these markers of PTH(1–84) activity. At baseline, FECa and total 24-hour urine calcium excretion (~3% and 380 mg, respectively) were relatively high, an expected consequence of hypoparathyroidism. FECa increased shortly after calcitriol administration, perhaps caused in part by concomitant calcium supplementation; however, there was little overall effect on calcium excretion. In contrast, both doses of rhPTH(1–84) decreased FECa by almost 70% within 6 hours before excretion slowly increased again to predose levels. Total 24-hour calcium excretion was decreased by 13% and 23%, respectively, with 50 and 100 μg of rhPTH(1–84).

Increased renal reabsorption of calcium plays an important role in the serum calcium response to rhPTH(1–84). Results from the PaTH study (Parathyroid Hormone and Alendronate for Osteoporosis Trial) provided insights into the relative contribution of kidney and bone to the increase in serum calcium after administration of rhPTH(1–84). Increased urinary calcium excretion is an important factor in the nephrolithiasis commonly observed in hypoparathyroidism. The ability of rhPTH(1–84) to increase serum calcium levels and, in contrast to calcitriol, also substantially decrease urine calcium excretion is an important observation for the potential use of rhPTH(1–84) in patients with hypoparathyroidism. Increased calcium retention by the body provides an additional mechanism supporting the reduction in oral calcium doses in the long-term treatment of hypoparathyroidism with rhPTH(1–84).

The urinary excretion of magnesium in the present study showed temporal patterns of changes that mirrored those of calcium. Although the initial decrease of ~65% in FEMg was similar to that of FECa, the duration of the effect was shorter and FEMg actually increased above the level observed in the previous day’s 16- to 24-hour urine collection. This resulted in 24-hour urinary magnesium excretion after rhPTH(1–84) being little different from calcitriol, and this finding may play a role in the smaller serum magnesium response compared with calcium. Finally, quantification of urinary phosphate excretion provided a clear explanation for the dramatic differences between the effects of rhPTH(1–84) and calcitriol on serum phosphate. FEP was relatively low on each day –1, an expected consequence of hypoparathyroidism. Short-term FEP increases after calcitriol are more likely to be a consequence of food intake rather than a renal response. In contrast, short-term rhPTH(1–84) increased FEP by >3-fold before excretion returned slowly to predose levels. Compared with excretion on each day –1, the total 24-hour urine phosphate level was increased by 45% to 53% after rhPTH(1–84) administration.

Although 50- and 100-μg doses of rhPTH(1–84) were used, the study has limitations that affect the ability to make conclusive statements based on a comparative analysis; the study design was a non-randomized, single-dose administration with a 7-day washout interval in a small population size. Despite limitations of a relatively small number of patients, single-dose administration, and descriptive statistics, this study provides valuable PK and PD data after the SC injection of rhPTH(1–84) in patients with hypoparathyroidism. rhPTH(1–84) was well tolerated, with no serious AEs and no investigator-assessed study drug–related AEs. Although baseline plasma PTH levels were approached between 12 and 24 hours, the prolonged effects of rhPTH(1–84) administration on serum calcium, phosphate, and 1,25(OH)2D levels provide an explanation of the proven efficacy of once-daily rhPTH(1–84) in clinical trials in patients with an orphan disease such as hypoparathyroidism. Moreover, the decreased
serum calcium × phosphate product and urine calcium excretion after rhPTH(1–84) administration has the potential to decrease the trend toward soft tissue calcification, particularly in the kidneys, which is an important cause of the chronic morbidity associated with hypoparathyroidism. Thus, in contrast to standard therapy with calcitriol, PTH replacement therapy with rhPTH(1–84) regulates mineral homeostasis of calcium, magnesium, phosphate, and vitamin D metabolism toward normal in patients with hypoparathyroidism.

CONCLUSIONS

PTH replacement therapy with rhPTH(1–84) regulated mineral homeostasis of calcium, magnesium, phosphate, and vitamin D metabolism toward normal in these study patients with hypoparathyroidism.

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The publication contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

Drs. Clarke and Berg were the study investigators. Drs. Clarke, Berg, and Fox contributed to the study design. All of the authors were given access to the clinical study report and were responsible for data interpretation contained within the manuscript and for the preparation, review, and final approval of the manuscript. NPS Pharmaceuticals, Inc, was responsible for the design and conduct of the study. Collection, management, and analysis of the data were performed by NPS Pharmaceuticals, Inc, who has reviewed the manuscript for verification of the data.

CONFLICTS OF INTEREST

Dr. Clarke has received institutional research grants from and served as an advisory group member for NPS Pharmaceuticals, Inc. Dr. Berg was an employee of the PRACS Institute, which contracted with NPS Pharmaceuticals, Inc, to perform the study. Drs. Fox and Cyran are consultants for NPS Pharmaceuticals, Inc. Dr. Lagast is an employee of NPS Pharmaceuticals, Inc. This study was funded by NPS Pharmaceuticals, Inc.

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Address correspondence to: Bart L. Clarke, MD, Division of Endocrinology, Diabetes, Metabolism, and Nutrition, Mayo Clinic, W18-A, 200 1st Street SW, Rochester, MN 55905. E-mail: clarke.bart@mayo.edu