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First- and second-generation immunometric PTH assays during treatment of hyperparathyroidism with cinacalcet HCl

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Background. First-generation immunometric assays for "intact" parathyroid hormone (iPTH) also measure large N-terminally truncated PTH fragments, whereas second-generation assays, such as the "bio-intact" PTH (biPTH) assay, measure only full-length biologically active PTH(1–84). This study compared iPTH and biPTH assays during cinacalcet treatment in subjects with secondary HPT receiving dialysis.

Methods. Four hundred and ten subjects were enrolled in a 26-week randomized, double-blind, placebo-controlled trial of oral cinacalcet (or placebo), 30 to 180 mg once daily, and efficacy was assessed using biPTH and iPTH assays.

Results. Compared with control treatment, cinacalcet improved the management of secondary HPT. Both biPTH and iPTH decreased by 38% ± 3% during weeks 13 to 26 in the cinacalcet group; biPTH increased by 23% ± 4% and iPTH increased by 9.5% ± 3% in the control group ($P < 0.001$). Fifty-six percent of cinacalcet subjects and 10% of control subjects had a ≥30% reduction in biPTH, and 61% and 11%, respectively, had a ≥30% reduction in iPTH. Significant correlations between biPTH and iPTH levels were observed throughout the study. Both assays correlated similarly with bone-specific alkaline phosphatase levels. The ratio of biPTH to iPTH was maintained at 56% ± 1% after treatment in both treatment groups. Increasing serum calcium levels were associated with a decreasing ratio of biPTH to (iPTH–biPTH).

Conclusion. These data show that PTH can be monitored with either iPTH or biPTH assays during therapy with cinacalcet, and that cinacalcet therapy does not exert a major influence on the ratio between PTH(1–84) and large, N-terminally truncated PTH fragments.

Key words: bio-intact PTH, cinacalcet HCl, dialysis, immunometric PTH assay, intact PTH, parathyroid hormone, PTH fragments, secondary hyperparathyroidism.

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Measurements of parathyroid hormone (PTH) are essential for the diagnosis of secondary hyperparathyroidism (HPT) and for monitoring the patient's response to therapy. In addition, measurement of PTH helps avoid oversuppression of PTH secretion that contributes to adynamic bone disease [1–5], an increased risk of fractures in adults, and further impairment of longitudinal growth in children [6–8]. Measurement of PTH using the first-generation immunometric assay has been the most commonly used method over the past decade to diagnose and treat secondary HPT.

First-generation immunometric assays for PTH employ 2 antibodies; the detection antibody is directed toward an epitope within the N-terminal region of PTH, while the capture antibody is directed toward an epitope within the C-terminal region of PTH [1, 9]. Initially, first-generation immunometric assays were thought to detect exclusively full-length PTH(1–84), thus, the term "intact" PTH (iPTH) assay. Subsequently, it was found that such assays also detect large N-terminally truncated fragments, which behave on high-performance liquid chromatography (HPLC) similarly to PTH(7–84) [10, 11]. These large PTH fragments have not yet been defined chemically, but in patients with renal failure, they appear to be present in higher concentrations than in normal subjects [10–12]. Animal studies suggest this may be because of a somewhat reduced clearance of PTH fragments by the kidney and/or increased secretion of fragments from the parathyroid gland [13, 14].

Second-generation immunometric PTH assays were developed by different laboratories using detection antibodies that specifically recognize the first 1 or 2 N-terminal amino acids of PTH, and capture antibodies that recognize an epitope within the C-terminal region. The resulting sandwich assays are thus likely to exclusively detect the full-length, biologically active PTH molecule [i.e.,

PTH(1–84)] [15–17], unless PTH fragments truncated at the C-terminus are also present. Although theoretically, such second-generation PTH immunometric assays [e.g., Bio-Intact PTH Assay (Nichols Institute, San Clemente, CA, USA) (biPTH) [9], Whole PTH Assay (Scantibodies, San Clemente, CA, USA) [16], and Bioactive Intact PTH Assay (Immutopics, San Clemente, CA, USA)] [17], should be superior to the first-generation "intact" PTH assay, most current data suggest that PTH levels measured with either assay system provide a similar prediction of bone turnover in patients with end-stage renal disease (ESRD) [17].

Oral calcimimetic agents are small molecules that directly and rapidly lower PTH secretion by binding directly to the calcium-sensing receptor on chief cells in the parathyroid gland and increasing its sensitivity to extracellular ionized calcium [18]. Cinacalcet HCl (Sensipar®, Mimpara®, hereafter cinacalcet; Amgen, Inc., Thousand Oaks, CA, USA), the first calcimimetic agent to be evaluated in clinical trials for the management of secondary HPT [19–21], has recently been approved in the United States, Canada, and Europe for the treatment of secondary HPT in dialysis patients. The PTH values used in those studies to manage dose adjustments in cinacalcet therapy and to evaluate the efficacy of cinacalcet were measured using a first-generation PTH immunometric assay (iPTH Assay; Nichols Institute). The present analysis was conducted to determine whether second-generation PTH assays can be used to evaluate response to cinacalcet therapy with similar accuracy as first-generation assays, and to estimate the conversion factor from iPTH to biPTH levels during cinacalcet therapy. In addition, we sought to determine if demographic and laboratory parameters influence the ratio of biPTH to (iPTH–biPTH).

METHODS

Subjects

This prospective, multicenter, randomized, placebo-controlled trial was conducted at 65 centers in the United States and Canada. The primary inclusion criteria were age ≥18 years, thrice-weekly hemodialysis for at least 3 months, mean plasma iPTH level ≥300 pg/mL despite ongoing treatment with diet, vitamin D therapy, and/or phosphate-binding therapy, and mean serum calcium level ≥8.4 mg/dL. Enrollment of subjects with plasma iPTH >800 pg/mL at baseline was limited to 20% of the population. All subjects provided informed written consent to participate, and institutional review boards approved the study design.

Study design

Eligible subjects were randomly (computer-generated randomization) assigned in a 1:1 ratio to double-blind treatment with cinacalcet or matching placebo taken orally once daily; randomization was stratified by baseline iPTH and Ca × P level. Blood for biochemical measurements was drawn weekly from weeks 1 to 12 and then biweekly from weeks 13 to 26. Plasma PTH concentrations were measured by a first-generation assay, the Intact PTH Assay (iPTH) (Nichols Institute), which detects PTH(1–84) as well as PTH(7–84) [9, 15–17], and by a second-generation Bio-Intact PTH (biPTH) Assay (Nichols Institute), which detects PTH(1–84) [9]. The biPTH to iPTH ratio was determined by dividing the value obtained from the Bio-Intact PTH Assay by the value obtained from the Intact PTH Assay. The biPTH to (iPTH–biPTH) ratio was determined by dividing the value obtained from the Bio-Intact PTH Assay by the value obtained from the Intact PTH Assay minus the value obtained from the Bio-Intact PTH Assay. Serum bone-specific alkaline phosphatase (BALP) levels were determined before treatment and at weeks 12 and 26.

During the first 12 weeks, cinacalcet (or placebo) doses were titrated sequentially every 3 weeks from a starting dose of 30 mg to doses of 60, 90, 120, and 180 mg if plasma iPTH was >200 pg/mL and serum calcium was ≥7.8 mg/dL. The dose could be modified further as needed every 4 weeks during weeks 13 to 26. Dose reductions were permitted for symptomatic hypocalcemia, serum calcium <7.5 mg/dL, plasma iPTH <100 pg/mL on 3 consecutive study visits, or a dose-related adverse event.

Statistical analysis

Baseline values were obtained during the screening period. Mean values for iPTH and biPTH, calcium, phosphorus, and Ca × P were determined from all available results from weeks 13 to 26 (up to 7 values per subject). Evaluations included the proportion of subjects with an average iPTH ≤250 pg/mL and biPTH ≤140 pg/mL from weeks 13 to 26, and the proportion of subjects with an average iPTH and biPTH level from weeks 13 to 26 that was reduced by ≥30% from baseline. Subjects who withdrew before week 13 were included in the analysis and considered not to have met the study end points. The proportions of subjects who met the therapeutic end points were compared between groups by a Cochran-Mantel-Haenszel test, stratified by iPTH and Ca × P level at baseline.

Mean percentage changes in biPTH and iPTH from baseline were determined at each visit. The mean percentage changes from baseline during weeks 13 to 26 were compared between groups by a generalized Cochran-Mantel-Haenszel test using rank, stratified by iPTH and Ca × P values at baseline. The mean of the last 2 on-study values was carried forward for subjects who withdrew before week 13.

Correlation and linear regression analyses of plasma biPTH and iPTH values were performed for randomized subjects in each treatment group at baseline, as well as for