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Characterization of the effect of chronic administration of a calcium-sensing receptor antagonist, ronacaleret, on renal calcium excretion and serum calcium in postmenopausal women

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ABSTRACT

Ronacaleret is an orally-active calcium-sensing receptor (CaSR) antagonist that has the potential for therapeutic utility in the stimulation of PTH release, notably as a bone anabolic agent comparable to recombinant human PTH(1–34) (rhPTH(1–34)). A recent study has shown that, despite the ability to increase circulating PTH levels in postmenopausal women in a dose-dependent manner, minimal effects of ronacaleret on bone mineral density have been observed. Therefore, the purpose of this study was to characterize the PTH profile as well as calcium metabolism parameters as a marker of PTH biological activity following the administration of ronacaleret or rhPTH(1–34). Administration of ronacaleret led to lower peak levels of PTH than were observed with rhPTH(1–34), however, greater total PTH exposure was observed. Further, chronic administration of either agent was associated with increases in urinary calcium excretion and serum calcium levels, with the magnitude of the changes following ronacaleret significantly greater than that for rhPTH(1–34). The greater magnitude of effects observed with ronacaleret is likely due to the greater total PTH exposure, and is potentially reflective of a state comparable to mild hyperparathyroidism. It is not clear whether the administration of all calcilytics would lead to a similar result, or is due to characteristics specific to ronacaleret.

Introduction

Calcium-sensing receptors (CaSRs) are the means by which specialized cells sense and respond to changes in extracellular calcium concentrations, thus maintaining calcium homeostasis [1–5]. In the parathyroid glands, CaSRs are pivotal in maintaining calcium homeostasis by coupling small decreases in serum calcium to increases in the secretion of parathyroid hormone (PTH) [6–8]. Ronacaleret is an orally-active, potent and selective calcium-sensing receptor (CaSR) antagonist (calcilytic) that was under development for the treatment of postmenopausal osteoporosis. Oral administration of ronacaleret at doses between 100 to 400 mg to postmenopausal women has been shown to result in dose-dependent, transient increases in circulating plasma PTH(1–84) levels. This increased circulating PTH was associated with elevated serum calcium levels, as well as increases in biomarkers of bone formation (P1NP, BSAP), effects which are consistent with PTH biological activity. By virtue of the ability to increase the endogenous secretion of PTH from the parathyroid glands, ronacaleret was thought to represent a novel bone-forming agent for the treatment of patients with osteoporosis. However, a recent investigation of the effect of ronacaleret on bone mineral density (BMD) in postmenopausal women with low BMD showed that daily administration for 12 months led to small decreases in total hip, femoral neck, and trochanter BMD, with a small increase in lumbar spine BMD [9].

rhPTH(1–34) (teriparatide; Forteo®) is a marketed agent indicated for the treatment of postmenopausal women with osteoporosis who are at high risk of fracture, given as a single 20 μg/day subcutaneous injection. The commercially-available formulation contains human parathyroid hormone (1–34), of recombinant DNA origin [rhPTH(1–34)], and has the identical sequence to the 34 N-terminal amino acids of the 84-amino acid human parathyroid hormone [PTH(1–84)]. The biological effects of rhPTH(1–34) include increases in serum calcium, increases in serum $1,25(OH)_2D$, and slight increases in mean 24-hour urinary calcium excretion [10]. rhPTH(1–34), administered at the indicated dose, led to increases in lumbar spine, total hip, femoral neck and trochanter BMD in the investigation of ronacaleret described above [9].

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Administration of ronacaleret increases endogenous PTH secretion to levels comparable to rhPTH(1–34) administration, however the therapeutic response to the chronic administration of ronacaleret in a postmenopausal population with low BMD was not comparable to what was observed with rhPTH(1–34) [9,11]. As other CaSR antagonists are in development for the treatment of osteoporosis, it is important to gain some understanding of the mechanism for the apparent minimal therapeutic effect of ronacaleret. Therefore, the purpose of this study was to characterize the effects of rhPTH(1–34) and ronacaleret on renal calcium excretion and serum calcium levels in postmenopausal women as a measure of PTH biological activity.

Material and methods

Study design and ethics

This was an exploratory, open-label, randomized, parallel group study, involving 5 U.S. centers conducted in compliance with Good Clinical Practices, GlaxoSmithKline Standard Operating Procedures and the guiding principles of the Declaration of Helsinki. This study was registered at clinicaltrials.gov (identifier: NCT00532077). All subjects provided written informed consent. Ethical approval for the study was received from the Independent Investigational Review Board, Inc., Plantation, Florida.

The primary study endpoint was urinary calcium excretion. Secondary study endpoints were levels of albumin-adjusted serum calcium, serum phosphate, urinary phosphate excretion, and plasma levels of ronacaleret, PTH(1–84) and rhPTH(1–34).

Subjects

Healthy postmenopausal women 40 to 65 years of age, inclusive, with a body weight $\geq$50 kg and a BMI within the range of 19 kg/m^2–32 kg/m^2 were eligible for the study. Subjects with significant renal, gastrointestinal or cardiovascular disease or those at increased risk of osteosarcoma were excluded. Other key inclusion criteria included normal serum levels of albumin-adjusted calcium, total calcium, PTH and urinary calcium. Subjects with Vitamin D deficiency, defined by serum 25-hydroxy vitamin D <20 ng/mL (equivalent to 50 nmol/L) at screening could be repleted per local standard of care and re-screened for serum 25-hydroxy vitamin D once within 2 weeks of initial screening. Subjects were excluded if there was evidence of significant renal disease as defined by estimated glomerular filtration rate (eGFR) <60 mL/min as determined from serum creatinine (SCr) and demographic data using the Modification of Diet in Renal Disease (MDRD) formula [12].

Treatment

Subjects were randomized in a ratio of 1:1:1 to receive oral ronacaleret 100 mg or 400 mg once daily in the morning for 28 days, or subcutaneous (sc) rhPTH(1–34) (teriparatide; Forteo®) 20 μg once daily in the morning for 28 days. Daily oral calcium (500–660 mg) and vitamin D (400 IU) supplements were taken in the evenings from Day −14 through Day 30.

Assessments

Subjects were admitted to the clinical research unit (CRU) on Days −2, 13 and 27 and stayed until the mornings of Days 2, 15 and 31, respectively; subjects reported to the CRU each morning for dosing on all other dosing days.

Blood samples were taken via an intravenous cannula on Days 1, 14 and 28 for the measurement of serum calcium and phosphate up to 18 h post-dose; serum calcium and phosphate were also measured on Days −1, 29 and 30. Urine samples were collected on Days 1, 14 and 28 for the measurement of calcium and phosphate up to 24 h post-dose; urine calcium and phosphate were also measured on Days −1, 29 and 30. Plasma concentrations of PTH(1–84) were determined by Pacific Biometrics, Inc. (Seattle, WA, USA) using a validated immunoradiometric assay specific for whole PTH(1–84) (Scantibodies Whole PTH [wPTH] assay), with a lower limit of quantitation of 1.91 pg/mL. Plasma PTH(1–84) levels were measured pre-dose and at various times up to 24 h post-dose on Days 1, 14 and 28 with molar concentrations calculated using a molecular weight of 9426 g/mol.

Blood samples for pharmacokinetic analyses were obtained via an intravenous cannula on Days 1, 14 and 28 and collected pre-dose and at various times up to 6 h post-dose for the measurement of rhPTH(1–34) and at various times over 24 h post-dose for the measurement of ronacaleret. Human plasma samples were analyzed for rhPTH(1–34) by a commercially available two-site enzyme-linked immunosorbent assay (High Sensitivity Human PTH(1–34) ELISA Kit; Immunotopics Inc., San Clemente, CA, USA) with a lower limit of quantitation of 0.9 pg/mL (using the WHO International Standard Parathyroid Hormone 1–34, Recombinant, Human) and less than 3% cross-reactivity to PTH(1–84). Molar concentrations were calculated using a molecular weight of 4115 g/mol. Blood levels of ronacaleret were determined using a validated analytical method based on protein precipitation, followed by HPLC/MS/MS analysis. The lower limit of quantification (LLQ) for ronacaleret was 10 ng/mL, using a 50 μL aliquot of hemolyzed human blood with a higher limit of quantification (HLQ) of 10,000 ng/mL (GlaxoSmithKline Internal Report, on file). The pharmacokinetic parameters estimated from the analyses were AUC(0–∞) [area under the concentration versus time curve from time zero to infinity], AUC(0–t) [area under the concentration versus time curve from time zero to the last quantifiable concentration], Cmax [maximum observed plasma concentration], t1/2 [apparent half-life], and tmax [time of maximum observed plasma concentration].

Statistical analysis

Descriptive statistics were calculated as the arithmetic means ± standard deviation (SD) except for the reported pharmacokinetic parameters, which were either the geometric means ± SD (Cmax and AUC) or median (for tmax). Between treatment effects were assessed for statistical significance by single-factor ANOVA and/or *t*-test, as appropriate, with $p < 0.05$ considered statistically significant. Statistical significance of treatment effects within subjects was assessed through calculation of the change from baseline for each subject at each time point, with the mean and 95% confidence interval determined for each treatment group. Values were considered statistically significant if the change from baseline ± the 95% confidence interval did not contain zero.

Results

Subject disposition and baseline characteristics

All enrolled subjects were healthy, postmenopausal women, as defined by specific inclusion/exclusion criteria, aged 44–65 years, with a body weight between 51.2 and 83.5 kg and a body mass index (BMI) within the range 20–32 kg/m^2. A total of 52 eligible subjects were randomly assigned to one of the three treatment groups (rhPTH(1–34), n = 18; ronacaleret 100 mg, n = 15; ronacaleret 400 mg, n = 19) with 50 subjects completing the study as planned; 2 subjects were withdrawn from the 400 mg ronacaleret treatment group prior to study completion due to adverse events unrelated to treatment. Baseline subject characteristics were well balanced across the treatment groups (Tables 1 and 2).