Pharmacokinetics in Rats of a Long-Acting Human Parathyroid Hormone–Collagen Binding Domain Peptide Construct

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ABSTRACT: The pharmacokinetics of a hybrid peptide consisting of the N-terminal biologically active region of human parathyroid hormone (PTH) linked to a collagen-binding domain (CBD) were evaluated in female Sprague–Dawley rats. The peptide, PTH–CBD, consists of the first 33 amino acids of PTH linked as an extension of the amino acid chain to the CBD peptide derived from ColH collagenase of *Clostridium histolyticum*. Serum concentrations arising from single dose administration by the subcutaneous and intravenous routes were compared with those measured following route-specific mole equivalent doses of PTH(1–34). Population-based modeling demonstrated similar systemic absorption kinetics and bioavailability for both peptides. Exposure to PTH–CBD was sixfold higher because of a systemic clearance of approximately 20% relative to PTH(1–34); however, these kinetics were consistent with more than 95% of a dose being eliminated from serum within 24 h. Results obtained support continued investigation of PTH–CBD as a bone-targeted anabolic agent for the treatment of postmenopausal osteoporosis. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:768–775, 2014

Keywords: collagen binding; osteoporosis; peptides; peptide delivery; pharmacokinetic/pharmacodynamic models; population pharmacokinetics; preclinical pharmacokinetics; PTH(1–34); teriparatide

INTRODUCTION

Osteoporosis is a disease characterized by low bone mass and deterioration of bone tissue, both of which lead to bone fragility and increased risk of hip and spine fracture. The disease is a major public health problem, particularly in elderly women, affecting approximately 10 million Americans. Currently, pharmacotherapy of osteoporosis is managed primarily by bisphosphonates, which work by inhibiting osteoclast action, thus reducing bone resorption. Bisphosphonates are so named because they are based on a pyrophosphate backbone that incorporates into hydroxylapatetite crystals in bone, resulting in targeted delivery and prolonged duration of action, up to 1 year with a single intravenous dose.[1] However, because they function as antiresorptive rather than anabolic agents, their efficacy is limited. Parathyroid hormone (PTH) is an anabolic bone agent that is more effective at treating osteoporosis than bisphosphonates[2]; however, daily subcutaneous injections are required. To improve the delivery and retention of PTH to bone, we developed a hybrid peptide of the active domain of parathyroid hormone, PTH(1–33), and a collagen-binding domain (CBD) derived from ColH1 collagenase of *Clostridium histolyticum*.[3] The compound was designed to combine the bone-targeting effects of the bisphosphonates with the superior efficacy of an anabolic agent, resulting in an overall superior therapy for osteoporosis. Testing of this compound, PTH–CBD, in ovarectomized rats showed a statistically significant increase of 10.4% in bone mineral density 6 months after a single subcutaneous injection compared with vehicle controls.[4] This increase was similar to that observed following daily administration of recombinant PTH, amino acids 1–34, teriparatide [PTH(1–34)] to rats for 2 weeks. Similar comparative results for the two peptides were observed in mice.[5] In both species, these long-term increases in bone mineral density observed following a single dose of PTH–CBD were not accompanied by increases in serum calcium levels.

Although PTH–CBD was designed as an intrinsically bone-targeted anabolic agent, it remains to be determined whether the sustained effects are the result of this tissue targeting, or whether the collagen-binding activity provides a reservoir for sustaining serum levels over a longer period of time. In this study, we describe the single dose pharmacokinetics of PTH–CBD, as compared with PTH(1–34), to test the hypothesis that a single subcutaneous dose of PTH–CBD provides a depot, either through delayed absorption or through prolonged release from a collagen-bound reservoir, resulting in long-term elevated serum levels. Our finding of similar absorption kinetics to PTH(1–34) and that more than 95% of PTH–CBD was eliminated from serum within 24 h after a single subcutaneous dose suggests that the prolonged effect on bone growth does not appear to be a consequence of prolonged PTH–CBD release into the systemic circulation from a depot. Rather, the pharmacokinetics observed support continued investigation of the hypothesis that PTH–CBD acts as a bone-targeted anabolic agent.

Abbreviations used: AIC, akaike information criterion; CBD, collagen-binding domain; PTH, human parathyroid hormone; PTH(1–34), recombinant human parathyroid hormone, amino acids 1–34, teriparatide; rPTH(1–84), endogenous rat parathyroid hormone; VPC, visual predictive check.

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EXPERIMENTAL

Materials

Human parathyroid hormone(1–34) was purchased from Sigma–Aldrich Company (St. Louis, Missouri). PTH–CBD is a peptide construct consisting of PTH(1–33) that is linked at the C-terminal end to the CBD of ColH collagenase (amino acids 861–981) from *Clostridium histolyticum*. The CBD peptide has been shown to be biologically inert and binds to the triple helical region of collagen with micromolar affinity.[6] Details regarding the preparation of PTH–CBD, including its biosynthesis in *E. coli* and purification, have been described.[7] Tris–HCl and $CaCl_2$ were also obtained from Sigma–Aldrich and were used for the preparation of collagen-binding buffer, which was 50 mM Tris–HCl, pH 7.5, and 5 mM $CaCl_2$.

Methods

Three-month-old female Sprague–Dawley rats (200–240 g) were obtained from Charles River (Wilmington, Massachusetts) and were acclimated for 2 weeks. Institutional animal care approval was obtained from the Children's hospital at Montefiore/Albert Einstein College of Medicine, Bronx, New York. Four animals were injected with a single subcutaneous dose of 19.4 nmol/kg PTH(1–34), whereas another four animals were injected with an 18.1 nmol/kg dose of PTH–CBD by this route. Blood samples were collected out to 6 h. Initial pharmacokinetics (PK) analysis of these animals determined that extrapolated areas of PTH–CBD exposure were more than 20%; thus, an additional study was conducted at a later date in four animals receiving the same PTH–CBD dose with blood collection out to 48 h. On a separate date, four animals received a 2.4-nmol/kg intravenous bolus dose of PTH(1–34), whereas another four received a 2.3-nmol/kg dose of PTH–CBD by this route.

For the subcutaneous route of administration, blood samples were collected from the tail vein at 0, 2, 5, 10, 20, 30, 60, 180, and 360 min after administration, and at 60, 180, 720, 1440, 2160, and 2880 min in the second group of rats receiving PTH–CBD. Blood samples were collected from the tail vein at 0, 5, 10, 15, 30, 60, 90, 120, 180, and 360 min after administration for both peptides following intravenous administration. In all studies, blood samples were placed into microtubes and allowed to clot at ambient temperature. Subsequently, these were centrifuged at 18,000 x g for 10 min under refrigerated conditions to recover serum. Serum samples were stored at $-80^{\circ}C$ until the time of analysis.

Concentrations of immunoreactive hPTH (1–34) were measured using enzyme-linked immunosorbent assay (ELISA) technique (Immutopics, Inc., San Clemente, California). Comparison of standard curves of PTH(1–34) versus PTH–CBD indicated a cross reactivity of the assay with PTH–CBD of 17%. The assay limit of quantitation was 5 pM for PTH(1–34) and 100 pM for PTH–CBD. Within-day accuracy ranged from 70% to 130% and precision ranged from 2.0% to 13.6%. Concentrations below the limit of quantitation were not used in pharmacokinetic analyses.

Endogenous rat parathyroid hormone(1–84) was measured using the rat-specific ELISA assay also available from Immutopics, Inc. The manufacturer has verified that PTH(1–34) is not measured in this assay (Rat Intact PTH ELISA Kit product literature, Immutopics, Inc.). Serum calcium concentrations were measured using the QuantiChrom™ Assay Kit (BioAssay Systems, Hayward, California).

Phoenix with nonlinear mixed effects, version 1.2 (Pharsight, Mountain View, California) was used for pharmacokinetic analysis of PTH(1–34) and PTH–CBD serum concentration–time profiles. Concentration data were log-transformed prior to model fitting. A sequential approach was used to construct the final population model for each peptide. This consisted of optimizing a model for each route of administration followed by simultaneous modeling of the two routes. This approach supported assurance of the accuracy of the parameter estimates in the final model. For both peptides, a one- versus two-compartment model was evaluated for the intravenous route. On the basis of goodness-of-fit of measured concentrations versus estimated concentrations, precision of the estimates and akaike information criterion (AIC), a one-compartment model was selected for PTH(1–34) and a two-compartment model for PTH–CBD. For subcutaneous administration, absorption of both peptides was modeled according to a first-order process, which has been employed for PTH(1–34) in both rats[8] and humans.[9] A zero-order process was investigated for both peptides; however, this resulted in a poorer fit (higher AIC values). Analysis of the combined routes of administration for each peptide was based on a naïve-pooled approach. Attempts to measure intersubject variability using first-order conditional estimation resulted in models with higher AIC and inability to reliably estimate intersubject variability for the structural parameters. A log-additive error model was used to estimate random error arising from errors in dosing, concentration measurement, and model misspecification. Population parameter estimates are reported along with their percent standard error of the estimate (%SEE). A visual predictive check (VPC) was conducted on the final model for each peptide to confirm that final structural estimates provided an adequate description of the observed concentration data. The VPC consisted of stratifying by route of administration (both peptides) and administration day (for subcutaneous PTH–CBD only, which was evaluated on two separate occasions with four rats on each occasion). One thousand simulations were conducted for each peptide.

RESULTS

Mean serum concentrations of the two peptides were observed following a single subcutaneous dose in approximately equivalent amounts on a mole basis [19.4 nmol/kg for PTH(1–34) and 18.1 nmol/kg for PTH–CBD] and sampling out to 360 min after administration are shown in Figure 1. PTH(1–34) peak concentrations were observed between 20 and 60 min and returned to background levels by 6 h. This disposition time course agrees well with that observed previously using the same PTH(1–34) subcutaneous dose.[8] PTH–CBD peak concentrations were also observed between 20 and 60 min. Summary exposure parameters for the two peptides administered by this route are listed in Table 1. PTH–CBD dose-normalized exposure (area under the curve, AUC) was 5.6-fold higher and the terminal half-life for PTH–CBD was approximately three times longer than that observed for PTH(1–34). Both the higher C_{max} and longer terminal half-life contributed to the markedly higher AUC for PTH–CBD.