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Pharmacokinetics in rats of a long-acting human parathyroid hormone-collagen binding domain (PTH-CBD) 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 to 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 6-fold higher due to a systemic clearance of approximately 20% relative to PTH(1-34); however, these kinetics were consistent with >95% of a dose being eliminated from serum within 24 hours. Results obtained support continued investigation of PTH-CBD as a bone targeted anabolic agent for the treatment of post-menopausal osteoporosis.

Keywords

collagen binding; osteoporosis; peptides; peptide delivery; population pharmacokinetics; preclinical pharmacokinetics; PTH(1-34); teriparatide

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Chemical Compounds studies in this article

Human parathyroid hormone, PTH(1-34) (PubChem CID: 16129682)

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 which incorporates into hydroxylapatite crystals in bone, resulting in targeted delivery and prolonged duration of action, up to one year with a single IV dose (1). However, because they function as antiresorptive rather than anabolic agents, their efficacy is limited. Parathyroid hormone is an anabolic bone agent that is more effective at treating osteoporosis than bisphosphonates (2); however, daily subcutaneous injections are required. To improve delivery and retention of parathyroid hormone to bone, we developed a hybrid peptide of the active domain of parathyroid hormone, PTH(1-33), and a collagen binding domain 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 ovariectomized rats showed a statistically significant increase of 10.4% in bone mineral density 6 months after a single subcutaneous injection compared to vehicle controls (4). This increase was similar to that observed following daily administration of recombinant human parathyroid hormone, 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 BMD observed following a single dose of PTH-CBD were not accompanied by increases in serum calcium levels.

While PTH-CBD was designed as an intrinsically bone-targeted anabolic agent, it remains to be determined if the sustained effects are the result of this tissue targeting, or if 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 to 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 > 95% of PTH-CBD was eliminated from serum within 24 hours 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.

Experimental

Materials

PTH(1-34) was purchased from Sigma-Aldrich Company. PTH-CBD is a peptide construct consisting of PTH(1-33) that is linked at the C-terminal end to the collagen binding domain (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 preparation of PTH-CBD, including its biosynthesis in *E. coli* and purification, have been described (7). Tris HCl and CaCl₂ were also obtained from Sigma-Aldrich and were used for the preparation of collagen binding buffer (CBB), which was 50 mM Tris HCl, pH 7.5 and 5 mM CaCl₂.

Methods

Three month-old female Sprague Dawley rats (200–240 grams) were obtained from Charles River (Wilmington, MA) and were acclimated for two 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 nmoles/kg PTH(1-34) while another four animals were injected with an 18.1 nmoles/kg dose of PTH-CBD by this route. Blood samples were collected out to 6 hours. Initial PK analysis of these animals determined that extrapolated areas of PTH-CBD exposure were > 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 hours. On a separate date, four animals received a 2.4 nmoles/kg intravenous bolus dose of PTH(1-34) while another four received a 2.3 nmoles/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 minutes post-administration, and at 60, 180, 720, 1440, 2160 and 2880 minutes 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 minutes post-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 14,000 rpm for 10 minutes under refrigerated conditions to recover serum. Serum samples were stored at –80°C until time of analysis.

Concentrations of immunoreactive hPTH (1-34) were measured using enzyme-linked immunosorbent assay (ELISA) technique (Immutopics, Inc., San Clemente, CA). Comparison of standard curves of PTH(1-34) vs. 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 rPTH(1-84) was measured using the rat specific ELISA assay also available from Immutopics. The manufacturer has verified that PTH(1-34) is not measured in this assay (Rat Intact PTH ELISA Kit product literature, Immutopics). Serum calcium concentrations were measured using the QuantiChrom™ Assay Kit (BioAssay Systems, Hayward, CA).

Phoenix with Non-Linear Mixed Effects (NLME), version 1.2 (Pharsight, Mountain View, CA) 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 1- vs. 2-compartment model was evaluated for the intravenous route. Based on goodness-of-fit of measured concentrations vs. estimated concentrations, precision of the estimates and Akaike Information Criterion (AIC), a 1-compartment model was selected for PTH(1-34) and a 2-compartment model for PTH-CBD. For subcutaneous administration, absorption of both peptides was modeled according to a 1st 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 inter-subject variability using First-Order Conditional Estimation resulted in models with higher AIC and inability to reliably estimate inter-subject 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 observed following a single subcutaneous dose in approximately equivalent amounts on a mole basis (19.4 nmoles/kg for PTH(1-34) and 18.1 nmoles/kg for PTH-CBD) and sampling out to 360 minutes post-administration are shown in Figure 1. PTH(1-34) peak concentrations were observed between 20 and 60 minutes and returned to background levels by 6 hours. 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 minutes. 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 3 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.

Serum calcium and endogenous rat PTH (rPTH(1-84)) were measured in the same rats used in the subcutaneous pharmacokinetic evaluations of the two peptides out to 360 minutes post-administration. Concentrations in individual animals and average concentration – time profiles for both analytes are summarized in Figure 2 (calcium) and Figure 3 (rPTH(1-84)). Serum calcium was also measured out to 48 hours in a separate cohort of four animals following the same 18.1 nmoles/kg subcutaneous dose of PTH-CBD. These results are also shown in Figure 2. In general, the calcium concentration vs. time profiles revealed a pattern observed by others following the same subcutaneous dose of PTH(1-34) (8), that is,

evidence of an early hypocalcemic effect in the first 30 minutes following administration that then returned to baseline levels. There was also no difference in these profiles between PTH(1-34) and PTH-CBD, even though PTH-CBD exposure was prolonged relative to PTH(1-34).

When administered subcutaneously, PTH(1-34) disposition in both rats and humans is characterized by absorption-rate limited kinetics (8–9). Based on this behavior and the higher molecular weight of PTH-CBD (17694 daltons vs. 4118 daltons for PTH(1-34)), expectation of slower absorption as the cause of the longer terminal half-life observed for PTH-CBD was reasonable. To evaluate this hypothesis, both peptides were administered intravenously by a bolus dose that was similar on a mole basis (2.4 and 2.3 nmoles/kg, respectively, for PTH(1-34) and PTH-CBD). Observed serum concentrations for this route are shown in Figure 1 alongside concentrations observed following subcutaneous dosing. For a given peptide, the different subcutaneous vs. intravenous doses resulted in a similar range of serum concentrations. As shown in Figure 2, mean serum calcium concentrations observed following intravenous administration of either peptide tended to decline within the first 30 minutes and then returned to baseline by one hour. These serum calcium profiles were similar to those observed following subcutaneous administration.

Simultaneous population modeling of both routes of PTH(1-34) administration were best described by a one-compartment model with first-order absorption and confirmed the absorption rate-limited pharmacokinetics of PTH(1-34) observed in rats (8) and humans (9) also using a compartmental modeling approach. The estimated absorption half-life was 41 minutes (coefficient of variation of 6.9%) versus 26 minutes (5.8%) for elimination. Structural model parameters were estimated with good precision (Table 2). Figure 4 summarizes the relationship between observed, and individual- and population-predicted PTH(1-34) serum concentrations. A visual predictive check of 1000 simulations of the PTH(1-34) time course that was stratified by route of administration and based on the final population model estimates is depicted in Figure 5. More than 90% of the observed concentrations were within the 90% confidence intervals of the predicted concentrations.

Results of simultaneous population modeling of PTH-CBD concentrations observed following subcutaneous and intravenous administration are shown alongside the PTH(1-34) results in Table 2. Unlike PTH(1-34), PTH-CBD pharmacokinetics were best described by a 2-compartment model. As with PTH(1-34), structural parameters were estimated with good precision (%SEE 20% or less). The relationship between observed and predicted concentrations is shown in Figure 4. A visual predictive check resulted in more than 90% of the observed concentrations being within the 90% confidence intervals of the predicted concentrations (Figure 5).

Absorption rate and absolute bioavailability from the subcutaneous route were similar for the two peptides (Table 2). Therefore, in contrast to PTH(1-34) being characterized by absorption rate-limited kinetics, the terminal half-life of PTH-CBD following subcutaneous administration represents elimination. Systemic clearance of PTH-CBD was approximately 20% of PTH(1-34) clearance, indicating slower elimination of this modified form of PTH(1-34) from the systemic circulation.

Discussion

Observed concentrations and PK analyses resulting from intravenous administration of PTH(1-34) agree well with those reported by Jones et al. (10) who evaluated the same 10 µg/kg (2.4 nmoles/kg) bolus dose that we used. Their estimate of clearance (98 mL/min/kg) agrees well with ours (83 mL/min/kg using an average body weight of 0.3 kg). In that study, PTH(1-34) demonstrated dose-dependent pharmacokinetics due to saturable elimination. PTH(1-34) exposures we observed following an eight-fold higher subcutaneous dose were similar to the intravenous dose exposures, which supports the assertion that clearance was similar for the two routes and our estimate of subcutaneous bioavailability of 47%. The fact that we could estimate clearance precisely (Table 2) from simultaneous population analyses of the two routes also supports the assertion of a common clearance.

Administration of different doses of PTH-CBD by the two routes also resulted in similar exposures to the hybrid peptide (Figure 1) and supported a subcutaneous bioavailability estimate that was similar to PTH(1-34) (Table 2). The rate of PTH-CBD absorption was also similar to that of PTH(1-34); thus, despite its larger mass (17694 daltons vs. 4118 daltons), PTH-CBD displayed similar absorption properties. However, the systemic kinetics of the hybrid peptide were described by a 2-compartment model with an elimination clearance that was lower than PTH(1-34) systemic clearance (Table 2). Attempts to estimate distributional clearances independently (that is, clearance from the central compartment to the peripheral compartment vs. clearance in the opposite direction) resulted in poor precision of all structural parameters and reduced model performance (higher AIC). Thus, the possibility that PTH-CBD would have a measurable lower distributional clearance from the periphery back to the central compartment due perhaps to retention via widespread binding to collagen in the periphery was not supported by model analyses. One possible reason for this is that the collagen binding mechanism is operative in both compartments and, possibly, was also responsible or contributed to the hybrid peptide having lower systemic clearance than PTH(1-34). Notably, analysis of PTH-CBD concentrations was based on the ELISA assay optimized for PTH(1-34), which utilizes two antibodies, one targeted to the N-terminal region and the other to the C-terminal region of the peptide. Development of a similar approach that targets an epitope on the N-terminal of the PTH portion of the hybrid peptide and another that targets an epitope on the C-terminal region of the CBD portion is ongoing. While the Immutopics assay for PTH(1-34) was able to detect PTH-CBD, albeit with reduced sensitivity due to a cross reactivity between 15 – 20%, it's possible that hydrolytic products of PTH-CBD, particularly C-terminal truncated peptides from the CBD end, akin to early assays for human PTH(1-84) (11), may be artificially inflating concentrations and altering PTH-CBD PK parameter estimates. However, if this is the case, use of a more selective assay would likely result in faster elimination of PTH-CBD and would not be expected to alter possible explanations (discussed below) for the anabolic effect observed at 6 months from a single dose.

The terminal half-life of PTH-CBD estimated from simultaneous modeling of the two administration routes was 270 ± 67.6 minutes (population estimate $\pm$ standard error of the estimate). Based on this estimate, it would take approximately 24 hours for 95% of a dose of PTH-CBD to be eliminated from the serum. The relationship between the pharmacokinetics

of exogenous PTH(1-34) and its pharmacodynamic effects in bone have been extensively investigated in rats (8,12). Results in those two studies were consistent in demonstrating an anabolic effect in bone when PTH(1-34) was administered as a single bolus dose for several days. In the latter study and consistent with its rapid elimination, PTH(1-34) levels returned to background within 3 hours of administration. Conversely, when the same daily dose of PTH(1-34) was administered intermittently over 6 hours, such that levels did not return to background until 8 hours after the first dose, a catabolic effect was observed after 18 days of this dose regimen. These results indicate that prolonged exposure to PTH(1-34) on a daily basis for 2 – 3 weeks is undesirable. Although a single dose of PTH-CBD would be expected to result in levels exceeding background for approximately 24 hours, an anabolic rather than catabolic effect was observed after one dose in 6 months (4). Furthermore, the anabolic effect was progressive over this time. Taken together, the pharmacokinetic profile of PTH-CBD relative to its bone building effect supports the hypothesis of a repository of the active peptide in bone that achieves net bone growth over several months. A reasonable explanation is that this repository exists in bone as a consequence of retention through collagen binding and which enables the PTH portion of the peptide to interact with PTH/PTHrP receptors on osteoblasts to achieve an anabolic effect. Of note, the dimeric peptide has been shown to be equipotent to PTH(1-34) (EC_{50} 's of approximately 10 nM) based on cAMP accumulation in PTH/PTHrP transfected HKrk-B7 cells (13) and is substantially greater than PTH-CBD concentrations observed at 720 minutes (average of 165 pM). This interpretation of selective retention in bone is also supported by the absence of hypercalcemia, which would be expected if there was prolonged exposure to PTH/PTHrP receptors in the kidney (14). Alternatively, it's possible that PTH-CBD circulates in a more highly bound form than PTH(1-34) such that, at least within the first few hours after administration, similar concentrations of the free form of the two peptides are available for interaction with receptors in the kidney. Clearly, more mechanistic studies are needed to support the interpretation of selective retention in bone that results in an anabolic effect without coincident hypercalcemia.

An approach to reduce the systemic clearance of PTH(1-34) by fusing it to the Fc fragment of human IgG1 (15) met with partial success. With that approach an anabolic effect was observed in ovariectomized rats following once-weekly administration; however, there was a concomitant hypercalcemic response. The latter response was attributed to prolonged renal exposure to the hybrid peptide that enhanced calcium reabsorption in the proximal tubules in a manner similar to prolonged intravenous infusion of PTH(1-34) (16). The mean residence time (MRT) of the PTH-Fc peptide was 660 minutes following a 30 nmoles/kg subcutaneous dose. Our comparable 18.1 nmoles/kg dose of PTH-CBD resulted in a MRT of 279 minutes, or 42% of the PTH-Fc peptide estimate and with no apparent hypercalcemic effect out to 48 hours post-administration. Attempts were made to model the relationship between PTH(1-34) or PTH-CBD, and serum calcium concentrations using an indirect response model that was used to model the relationship between PTH(1-34) exposure and calcium in humans (9). Most likely due to strong homeostatic mechanisms that maintain serum calcium levels in a narrow range and the small number of animals in our studies (vs. > 100 patients in the Satterwhite et al. study), development of a relationship was not possible. Future studies using different PTH-CBD administration paradigms that incorporate a serum

calcium monitoring plan will be important towards understanding how to maximize bone growth and avoid hypercalcemia.

Secretion of PTH from the parathyroid gland is tightly coupled to serum calcium through calcium-sensing receptors located on the surface of parathyroid cells (17). This serum calcium – PTH response relationship is non-linear, with small declines in calcium resulting in disproportionate increases in serum PTH (18–19). Consistent with this relationship, the subtle changes in serum calcium observed following subcutaneous administration of PTH(1-34) and PTH-CBD, that is, < 0.1 mM decrease in the first 30 minutes following administration, a 2 to 3 fold increase in rPTH(1-84) over baseline (16 ± 3.4 mM) was observed (Figure 3). Endogenous PTH also returned to baseline by three hours post-administration for both peptides. Although hPTH(1-34) does not interfere with measurement of endogenous rat PTH(1-84), it's possible that PTH-CBD does such that the measured endogenous PTH levels following dimeric peptide administration are underestimated. Future studies, likely to occur with a more specific PTH-CBD ELISA assay, will need to address this possibility.

In conclusion, a single dose of PTH-CBD, that is mole equivalent to a PTH(1-34) dose that is anabolic if administered to rats and humans on a daily basis, resulted in an anabolic effect in ovariectomized rats of similar magnitude at 6 months post-administration. This effect occurs despite a serum pharmacokinetic profile that implies >95% elimination within 24 hours. Taken together, these results support a hypothesis of selective retention of a catalytic amount of the hybrid peptide in a biologically active form as a result of specific binding to collagen in bone that enables the peptide to appropriately stimulate PTH/PTHrP receptors on osteoblasts to achieve net bone growth. Based on these results, continued investigation of PTH-CBD as a bone targeted anabolic agent for the treatment of post-menopausal osteoporosis is supported.

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Abbreviations

AIC	Akaike Information Criterion
CBB	collagen binding buffer
CBD	collagen binding domain
NLME	non-linear mixed effects
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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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Human PTH(1-34) and PTH-CBD serum pharmacokinetic parameters measured following subcutaneous administration of 19.4 nmoles/kg PTH(1-34) or 18.1 nmoles/kg PTH-CBD, respectively. Results represent the mean and associated variability (%CV) from values obtained in individual animals (N = 4 rats).

Table 1

Parameter	Units	PTH		PTH-CBD	
		Mean	% CV	Mean	% CV
AUC ₀₋₃₆₀	nM* min	100	11.3	391	29.8
AUC _{0-infinity}	nM* min	101	10.9	527	40.5
C _{max}	nM	0.8	14.9	2.1	17.1
Terminal Half-Life*	min	59	---	159	---

* Geometric Mean

Table 2

PTH(1-34) and PTH-CBD population pharmacokinetic parameter estimates obtained from simultaneous modeling of PTH(1-34) or PTH-CBD serum concentrations observed following subcutaneous and intravenous administration of each peptide to female rats.

Parameter	Units	PTH(1-34)		PTH-CBD	
		Estimate	% SEE	Estimate	% SEE
V1	mL	958	12.0	265	16.4
V2	mL	---	---	896	17.9
CL	mL/min	24.9	8.8	4.1	11.6
Q	mL/min	---	---	7.4	19.3
Ka	1/min	0.016	6.4	0.013	18.7
F	%	47	12.4	40	12.4
Residual error		0.46	8.8	0.41	8.2

% SEE represents the % standard error of the estimate. Residual error was estimated using a log-additive residual error model.