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Original Article

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A comparison of the anabolic effects of rat and bovine parathyroid hormone (1-34) in ovariectomized rats

M. Li[1], D.R. Healy[1], Y. Li[1], H.A. Simmons[1], F. Gao[2], H.Z. Ke[1], B. Lu[1], T.A. Owen[1], D.D. Thompson[1]

[1]Osteoporosis Research, Department of Cardiovascular and Metabolic Diseases
[2] Mathematical and Statistical Sciences, Pfizer Global Research and Development, Groton, CT, USA

Abstract

The current study was designed to compare the skeletal effects of comparable doses of rat parathyroid hormone 1-34 (rPTH) and bovine parathyroid hormone 1-34 (bPTH) in ovariectomized (OVX) rats. Female Sprague-Dawley rats were OVX or sham-operated at 6 months of age and maintained untreated for 28 days after surgery. Baseline control and OVX rats were sacrificed at the beginning of treatment. Beginning 28 days post-OVX, the remaining rats were subcutaneously injected daily with rPTH or bPTH at 0, 5, 25, or 50 μg/kg/d for 28 days. Bone area, bone mineral content (BMC), and bone mineral density (BMD) of the distal femoral metaphyses were determined *ex vivo* using dual energy X-ray absorptiometry. Quantitative bone histomorphometry was performed on undecalcified longitudinal sections of the proximal tibia from each rat. Baseline OVX rats exhibited osteopenia as demonstrated by their significantly reduced femoral BMD and proximal tibia cancellous bone volume compared with those of baseline sham controls. Both rPTH and bPTH restored bone in OVX rats by markedly stimulating bone formation in a dose-dependent manner. However, a difference in potency between the two forms of PTH was evident. The percentage increases of BMC, BMD, cancellous bone volume, trabecular thickness, mineralizing surface, and bone formation rate in the OVX rats treated with bPTH at 5 μg/kg/d were the same as or above those treated with rPTH at the 25 μg/kg/d dose level. A relative potency analysis showed that bPTH was approximately 4- to 6-fold relatively more potent than rPTH in increasing distal femoral BMD as well as cancellous bone volume, mineralizing surface, and bone formation rate of proximal tibial metaphyses at comparable dose levels and a given time. These results may serve as a reference for *in vivo* study design when rPTH or bPTH are to be the agents for studies on bone anabolism.

Keywords: Ovariectomy, rPTH, bPTH, Bone Mass, Bone Formation

Introduction

Parathyroid hormone (PTH) is a promising therapeutic agent under development for the treatment of osteoporosis. In the past decades, numerous studies have been carried out in human subjects and in various animal models to examine the skeletal effects of PTH. These studies have clearly demonstrated that administration of PTH by daily injection stimulates bone formation and augments bone mass in humans and animals. Such anabolic skeletal effects have been shown in humans and rodents predominantly on cancellous enriched skeletal sites including metaphyseal and vertebral sites. The results from these studies have recently been extensively reviewed[1-4]. Recombinant human PTH (rhPTH) has been used in the majority of these studies and the dose response has been well documented. Rat PTH (rPTH) and bovine PTH (bPTH) are the two most commonly used forms of PTH utilized in pre-clinical studies to examine the skeletal effects of this hormone. Bone anabolism as indicated by increases in cancellous bone volume has been clearly shown in the rats treated with either rPTH or bPTH[5-10]. Tam et al.[10] reported that a daily dose of bPTH (1-84), ranging from 0.2 to 204 μg/kg/d for 12 days dose-dependently increased bone mineral apposition rate and trabecular bone volume in intact male rats. Lumbar spine BMD and cancellous bone volume were significantly increased after 15 or 60 days of treatment with bPTH at 40, 80, and 160 μg/kg/d in aged female rats without dose-related difference[9]. Treatment of OVX rats with 40 μg/kg/d of rPTH alone[6] or 30 μg/kg/d of rPTH in combination with 17β-estradiol[7] restored lost bone to the osteopenic skeleton. However, the dose response of the rat

Corresponding author: Dr. Mei Li, Department of Cardiovascular and Metabolic Diseases, Box MS8118W-208, Pfizer Global Research and Development, Groton, CT 06340, USA. E-mail: mei_li@groton.pfizer.com

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skeleton to rPTH treatments and the determination of relative potency of rPTH and bPTH *in vivo* are unclear. Therefore, the current study was designed to address these issues by analyzing the effects of rPTH and bPTH on cancellous bone in the osteopenic, OVX rat model.

Materials and methods

Six-month-old virgin female Sprague Dawley rats (Taconic Farms, Inc., Germantown, NY) weighing an average of 318 g were randomly divided into 10 groups (n=7-8/group). Each rat was anesthetized with an intraperitoneal injection of ketamine hydrochloride and xylazine at doses of 50 and 10 mg/kg body weight, respectively. Bilateral ovariectomy was performed in eight of the groups of rats and the remaining two groups of rats were subjected to sham surgeries. All rats were housed at 24^0C with a 12h light/12h dark cycle. All rats were allowed free access to water and commercial diet (Purina Laboratory Rodent Chow 5001, Purina-Mills, St. Louis, MO) containing 0.95% calcium, 0.67% phosphorus, and 4.5 IU/g vitamin D_3. The experiments were conducted according to Pfizer Inc. animal care-approved protocols, and animals were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

All rats were untreated for 4 weeks after surgery to allow for the development of osteopenia. At 4 weeks post-surgery, one group of sham-operated rats (BSL CON) and one group of OVX rats (BSL OVX) were sacrificed to serve as baseline controls. Another group of sham operated rats and one group of OVX rats were treated with vehicle (CON + VEH and OVX + VEH) for 4 weeks. The OVX rats in the remaining groups received daily subcutaneous (sc) injection with either rPTH (1-34) or bPTH (1-34) at 5, 25, or 50 μg/kg/d for 4 weeks. Thus, this study was comprised of the following groups: BSL CON, BSL OVX, CON + VEH, OVX + VEH, OVX + rPTH (5 μg), OVX + rPTH (25 μg), OVX + rPTH (50 μg), OVX + bPTH (5 μg), OVX + bPTH (25 μg), OVX + bPTH (50 μg). Synthetic rat or bovine PTH (Bachem Inc., Torrance, CA) was dissolved in a vehicle composed of acid saline (0.001N HCL) and 5% heat-inactivated OVX rat serum.

All rats were injected sc with calcein (Sigma Chemical Co., St. Louis, MO) both 12 and 2 days prior to sacrifice at a dose of 10 mg/kg body weight. This regimen resulted in deposition of a single or double fluorochrome label at bone surfaces that were actively mineralizing at the time of injections.

At the conclusion of 4 weeks of treatment with PTH, all rats were killed by CO_2 asphyxiation. Success of ovariectomy was confirmed by failure to detect ovarian tissue and by the observation of marked atrophy of the uterine horns at the time of necropsy. The right femurs and proximal tibiae were collected for bone mineral measurements and bone histomorphometric analysis, respectively.

The right femur of each rat was scanned using dual energy X-ray absorptiometry (QDR-4500/W, Hologic, Inc., Waltham, MA) equipped with regional high resolution scan software. Bone area, bone mineral content (BMC), and bone mineral density (BMD) of the distal femoral metaphysis, a skeletal site that is predominately composed of cancellous bone, were determined as previously described[11].

The right proximal tibia of each rat was dehydrated in graded concentrations of ethanol and embedded undecalcified in methyl methacrylate[12]. Frontal sections of proximal tibia were cut at 4- and 10-μm thickness using a Reichert-Jung Polycut S microtome (Cambridge Instruments, Heidelberg, Germany). The 4-μm sections were stained with modified Masson's Trichrome stain, and the 10-μm sections remained unstained for measurements of fluorochrome-based indices of bone formation.

All histomorphometric measurements were performed in cancellous bone tissue of the proximal tibia in the area between 1.2 and 3.6 mm distal to the growth plate-epiphyseal junction using an Image Analysis System (Osteomeasure, Inc., Atlanta, GA). Cancellous bone volume as a percentage of bone tissue area (BV/TV) and osteoclast surfaces as percentages of total cancellous perimeter (Oc.S/BS) were measured in 4-μm thick, stained sections. Trabecular number (Tb.N), thickness (Tb.Th) and separation (Tb.Sp) were calculated as described by Parfitt et al.[13]. Fluorochrome-based indices of bone formation including the percentage of cancellous bone surface with a double fluorochrome label (mineralizing surface, MS/BS) and mineral apposition rate (MAR) were measured in 10-μm thick, unstained sections. In addition, bone formation rate (bone surface referent) was calculated by multiplying mineralizing surface by mineral apposition rate (BFR/BS)[14]. Values for mineral apposition rate were not corrected for obliquity of the plane of section in cancellous bone[14].

Data are expressed as the mean ± SEM for each group. Statistics were calculated using StatView 4.0 packages (Abacus Concepts, Inc., Berkeley, CA). Statistical differences between groups were evaluated with ANOVA followed by the Fisher PLSD test for multiple comparisons. Probabilities (p) less than 0.05 was considered significant unless otherwise indicated. Dose-dependent responses of rPTH and bPTH were determined by linear regression analysis using StatView 4.0 packages.

The relative potency of rPTH and bPTH was determined with the methods described by Tallarida and Murray[15]. Three identical doses of rPTH and bPTH were administered and their respective effects were measured. The values from these measurements were used for the relative potency analysis. Relative potency in this study refers to the ratio of the amount of rPTH to the amount of bPTH needed to produce the same specific effect assessed by DEXA scan or bone histomorphometric measurements. A relative potency ratio of 1 suggests the potency of the two treatments is the same. A value of the relative potency ratio greater than 1 implies that bPTH is relatively more potent than rPTH. A 95% confidence interval provides further determination if a relative potency value is statistically significant. Here we use BV/TV (%) as an example to demonstrate how the relative potency was determined. From the BV/TV (%) values, two

separate regression lines, each line representing dose-response curve of rPTH or bPTH treatment on BV/TV, were constructed. Statistical analysis showed that there was no difference between the slopes of the two regression lines when the dose is in log scale. Therefore two parallel lines were constructed by the least squares method. The two lines are:

$$rPTH: label\ (\%) = 14.8 + 6.6 \times log_{10}(dose)$$
$$bPTH: label\ (\%) = 19.8 + 6.6 \times log_{10}(dose)$$

These two lines and the data are plotted in Figure 1. The horizontal distance, *d*, between the two lines determines the relative potency:

$$relative\ potency = 10^{d}$$

In this particular case, the relative potency ratio was 5.62 with a 95% confidence interval of (1.15, 36800). The relative potency ratio suggested that 5.62 times as much of rPTH relative to the amount of bPTH is needed to produce the same effect measured by BV/TV in the dose range tested in this study. The lower bound of 95% confidence interval was above 1, indicating that the difference in relative potency between rPTH and bPTH on BV/TV was statistically significant.

The same procedure was applied to the relative potency analysis for all measurements except Tb.N, Tb.Sp, and MAR due to the lack of dose dependence for these variables. The results are summarized in Table 4.

Results

Body weight

Baseline OVX and vehicle-treated OVX rats weighed 13% and 20% more than baseline sham controls and vehicle-treated sham controls, respectively. The mean body weights of OVX rats treated with either rPTH or bPTH at any dose levels were not significantly different between each other or from that of vehicle-treated OVX rats (data not shown).

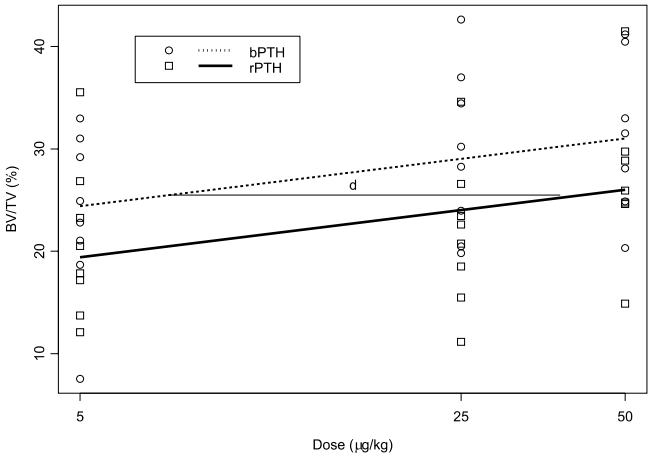

Figure 1. The individual value and dose-response lines based on BV/TV in OVX rats treated with rPTH (square and solid line) and bPTH (circle and dotted line).

Distal femoral BMC and BMD

The BMC and BMD of distal femoral metaphyses are shown in Figure 2A and B. A 7.7% decrease in BMC was observed in baseline OVX rats as compared with baseline sham controls at 4 weeks post-surgery, but this decrease did not achieve statistical difference. At 8 weeks post surgery, a significantly decreased BMC was seen in vehicle-treated OVX rats (-11%) relative to vehicle-treated sham controls. The mean values for BMD of distal femoral metaphysis of baseline OVX rats and vehicle-treated OVX rats were significantly lower than those of baseline sham controls and vehicle-treated sham controls, respectively. In contrast, distal femoral BMC and BMD were significantly increased in OVX rats treated with rPTH or bPTH at all dose levels as compared with vehicle-treated OVX rats with an exception of a non-significant increase in BMC of OVX rats treated with either 5 or 25 μg/kg/d of rPTH. The mean values of BMC and BMD for OVX rats treated with rPTH at 5 or 25 μg/kg/d or bPTH at 5 μg/kg/d were at the same level as vehicle-treated sham controls. In addition, the mean value of distal femoral BMC and BMD in OVX rats treated with 25 or 50 μg/kg/d of bPTH was significantly higher than that of vehicle-treated sham controls. However, in the OVX rats treated with rPTH such significant increases in BMC and BMD were only achieved at the 50 μg/kg/d dose level. The percentage increases over vehicle in BMC and BMD at the distal femur in the OVX rats treated with bPTH at 5 μg/kg/d were the same as or greater than those treated with rPTH at the 25 μg/kg/d dose level (Table 2). A linear regression analysis showed that there was a dose-dependent increase in BMC and BMD of the distal femurs in the OVX rats treated with either rPTH or bPTH (Table 3).

Proximal tibial cancellous bone histomorphometry

Bone histomorphometric data from the proximal tibial metaphysis are shown in Table 1. BV/TV and Tb.N were significantly decreased whereas Tb.Sp, MS/BS, MAR, and BFR/BS were increased in baseline and vehicle-treated OVX rats as compared to baseline sham and vehicle-treated sham controls, respectively. Oc.S/BS of baseline OVX rats was significantly greater than that of baseline controls.

BV/TV was significantly increased in rats treated with either rPTH or bPTH at all dose levels as compared with vehicle-treated OVX rats with the exception of rats treated with 5 μg/kg/d of rPTH (53.8% increase, p=0.0581). A significant increase in Tb.N was seen in OVX rats treated with bPTH at all dose levels while only 50 μg/kg/d dose of rPTH produced a significant difference compared with vehicle-treated OVX rats. Tb.Th of the OVX rats treated with 25 or 50 μg/kg/d of rPTH and bPTH was significantly increased as compared to vehicle-treated sham and OVX

Group	BV/TV (%)	Tb.N (#/μm)	Tb.Th (μm)	Tb.Sp (μm)	Oc.S/BS (%)	MS/BS (%)	MAR (μm/d)	BFR/BS ($\mu m^2/\mu m/d$)
BSL CON	35.6 ± 1.8	4.8 ± 0.2	75.2 ± 2.6	138.8 ± 10.1	1.87 ± 0.20	21.5 ± 2.4	1.32 ± 0.09	0.29 ± 0.05
BSL OVX	19.2 ± 1.5*	3.0 ± 0.2*	65.0 ± 2.6	282.6 ± 25.2*	3.04 ± 0.31*	31.8 ± 1.4*	1.69 ± 0.08*	0.53 ± 0.02*
CON + VEH	27.4 ± 2.4	4.4 ± 0.3	63.8 ± 5.8	168.8 ± 10.9	1.50 ± 0.23	9.3 ± 1.6	1.06 ± 0.09	0.10 ± 0.02
OVX + VEH	13.6 ± 1.9[a]	2.0 ± 0.2[a]	67.2 ± 4.5	451.3 ± 41.3[a]	2.10 ± 0.49	26.8 ± 2.6[a],	1.30 ± 0.09[a]	0.36 ± 0.05[a]
OVX + rPTH (5 μg)	20.9 ± 2.7	2.6 ± 0.2[a]	79.9 ± 6.7[a]	324.7 ± 35.2[a]	1.97 ± 0.39	33.5 ± 1.8[a,b]	1.43 ± 0.07[a]	0.48 ± 0.03[a,b]
OVX + rPTH (25 μg)	21.6 ± 2.5[b]	2.5 ± 0.2[a]	86.5 ± 4.7[a,b]	343.3 ± 43.2[a]	2.09 ± 0.30	37.2 ± 1.8[a,b]	1.42 ± 0.08[a]	0.53 ± 0.04[a,b]
OVX + rPTH (50 μg)	27.2 ± 3.0[b]	3.0 ± 0.2[a,b]	88.3 ± 6.4[a,b]	245.6 ± 20.1[a,b]	1.32 ± 0.22	44.2 ± 1.5[a,b]	1.52 ± 0.10[a]	0.67 ± 0.05[a,b]
OVX + bPTH (5 μg)	23.5 ± 2.9[b]	2.9 ± 0.2[a,b]	78.2 ± 6.2[a]	280.0 ± 36.4[a,b]	2.04 ± 0.24	37.1 ± 2.5[a,b]	1.45 ± 0.06[a]	0.54 ± 0.05[a,b]
OVX + bPTH (25 μg)	29.6 ± 2.9[b,c]	3.1 ± 0.2[a,b]	95.3 ± 4.2[a,b]	239.8 ± 24.0[b,c]	1.29 ± 0.28[c]	46.6 ± 1.9[a,b,c]	1.43 ± 0.05[a]	0.66 ± 0.02[a,b,c]
OVX + bPTH (50 μg)	31.4 ± 2.9[b,c]	3.0 ± 0.3[a,b]	106.9 ± 4.7[a,b,c]	251.6 ± 35.9[b]	1.00 ± 0.13[b,c]	55.0 ± 4.3[a,b,c]	1.62 ± 0.11[a,b]	0.89 ± 0.08[a,b,c]

Data are expressed as mean ± SEM.
*p<0.05 BSL OVX vs. BSL CON; [a]p<0.05 vs. CON + VEH; [b]p<0.05 vs. OVX + VEH; [c]p<0.05 vs. rPTH at the same dose.
BV/TV: cancellous bone volume; Tb.N: trabecular number; Tb.Th: trabecular thickness; Tb.Sp: trabecular separation; Oc.S/BS: osteoclast surface; MS/BS: mineralizing surface; MAR: mineral apposition rate; BFR/BS: surface-based bone formation rate.

Table 1. Cancellous bone histomorphometric variables in proximal tibial metaphysis.

rats. Tb.Sp was decreased in OVX rats treated with all dose levels of bPTH but for rPTH only the 50 μg/kg/d dose level showed a significant decrease. In addition, the mean values of Tb.Sp in OVX rats were returned back to the levels of vehicle-treated sham controls by the treatment with two higher doses of bPTH. A significant decrease in Oc.S/BS was observed only in the OVX rats treated with 50 μg/kg/d of bPTH. Treatment of OVX rats with rPTH or bPTH significantly increased MS/BS and BFR/BS compared with vehicle treatment. A trend of increased MAR was observed in OVX rats treated with rPTH or bPTH relative to OVX rats treated with vehicle, but this trend did not reach statistical significance except with 50 μg/kg/d of bPTH. The percentage increases over vehicle in BV/TV, Tb.N, MS/BS, and BFR/BS generated from the proximal tibia in the OVX rats treated with bPTH at 5 μg/kg/d were the same as or greater than those treated with rPTH at the 25 μg/kg/d dose level. The percentage increases over vehicle in BV/TV, Tb.Th, MS/BS, and BFR/BS in the OVX rats treated with 50 μg/kg/d of bPTH were higher than those treated with rPTH at the same dose level (Table 2). A linear regression analysis showed that there was a dose-dependent increase in BV/TV, Oc.S/BS, MS/BS and BFR/BS of the proximal tibia in the OVX rats treated with either rPTH or bPTH (Table 3).

Relative potency analysis

As shown in Table 4, the relative potency values of rPTH and bPTH based on the measurements of different components of bone mass (BMC, BMD, BV/TV), bone structure (Tb.Th), bone resorption (Oc.S/BS), and bone formation (MS/BS and BFR/BS) were all greater than 1. These data suggested that bPTH is likely to be more potent than rPTH at the same dose levels in altering those variables. The 95% confidence interval provided further determination if these values are statistically significant. The relative potency ratios for BMD, BV/TV, MS/BS and BFR/BS were 3.6, 5.6, 3.9, and 3.6, respectively, and the 95% confidence intervals were all above 1. These results indicated that bPTH in OVX rats is approximately 4 to 6 times relatively more potent than rPTH in increasing BMD, BV/TV, MS/BS, and BFR/BS. Such differences in the relative potency of rPTH

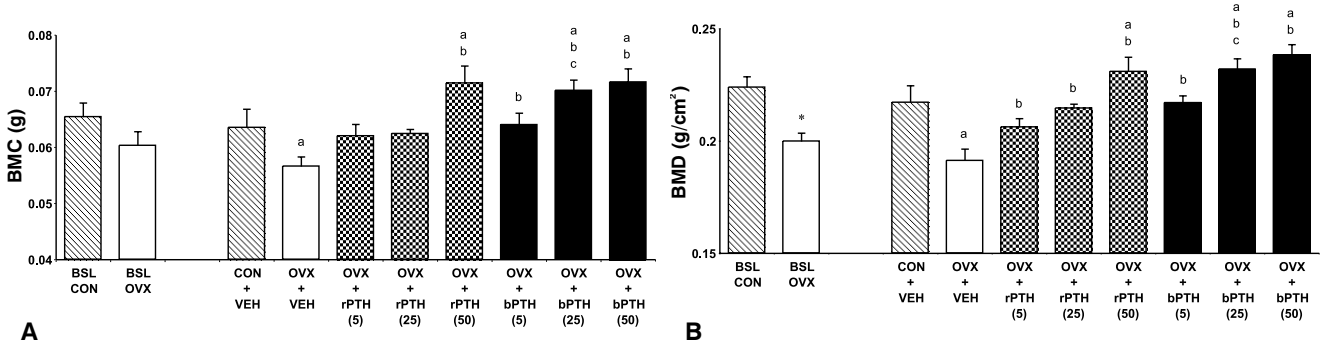

Figure 2. BMC (A) and BMD (B) of the distal femoral metaphyses from baseline control (BSL CON), baseline OVX (BSL OVX), sham-operated rats treated with vehicle (CON + VEH), OVX rats treated vehicle (OVX + VEH), OVX rats treated with rPTH or bPTH at 5, 25, or 50 μg/kg/d for 4 weeks. Data are expressed as mean ± SEM. *p<0.05 BSL OVX vs. BSL CON; [a]p<0.05 vs. CON + VEH; [b]p < 0.05 vs. OVX + VEH; [c]p<0.05 bPTH vs. rPTH at the same dose levels.

and bPTH reached statistical significance. Furthermore, the potency ratios for BMC, Tb.Th, and Oc.S/BS were 2.8, 2.8, and 2.9, respectively. These data suggested that the relative potency of the two peptides on these variables were in the same direction as the others mentioned earlier. However, the lower bounds of the 95% confidence intervals were below 1, suggesting that the differences in the potency ratios for these variables are not statistically significant.

Discussion

The purpose of this study was to determine the relative potency of rPTH and bPTH based on their *in vivo* ability to stimulate bone formation. The results indicated that both rPTH and bPTH at the highest dose used in this study were capable of completely restoring lost bone to osteopenic, OVX rats. However, bPTH stimulates bone formation to a greater extent at the proximal tibia of OVX rats relative to rPTH when the two peptides were compared at the same dose levels. For example, the percentage increases over vehicle treatment in bone formation rate by bPTH treatment were 49.1%, 82.6%, and 144.7% at 5, 25, and 50 μg/kg/d dose levels as compared to 31.8%, 46.1%, and 85.9% for rPTH, respectively. The greater bone formation induced by bPTH led to the greater increment in cancellous bone mass as compared with rPTH at the same dose levels. Furthermore, 25 μg/kg/d of bPTH treatment was shown to add additional bone at the distal femur and decrease trabecular separation at proximal tibia metaphysis whereas similar effects induced by rPTH were seen at 50 μg/kg/d dose level. Overall, the percentage increments produced by 5 μg/kg/d of bPTH treatment in OVX rats were similar to those induced by rPTH at 25 μg/kg/d dose level. All the data generated in this study suggest that bPTH was relatively more potent than rPTH in the OVX rat model.

More convincingly, this finding is supported by the results from the relative potency analysis based on the variables derived from not only a single measurement but also various measurements performed in this study. These components included bone mass (BMC, BMD, BV/TV), bone structure (Tb.Th), bone resorption (Oc.S/BS), and bone formation (MS/BS and BFR/BS). Although the relative potency values for BMC, Tb.Th and Oc.S/BS were not statistically significant, they were in agreement with the trends from other measurements to suggest that bPTH is relatively more potent than rPTH in OVX rats. Importantly, the potency values of bPTH relative to rPTH based on the key measurements such as BMD, BV/TV, MS/BS, and BFR/BS in the present study were between 3.6 to 5.6 and the 95% confidence intervals for these variables were all above 1. These data indicated that bPTH is approximately 4 to 6 times relatively more potent than rPTH in stimulating bone formation and restoring bone in OVX rats at comparable dose levels and a given treatment period. However, the limitations of this study must be noted. The minimum and maximum effective doses for either rPTH or bPTH were not defined in this study. In addition, the relative potency of rPTH and bPTH on the mechanical property of bones and the serum pharmacokinetics of the peptides were not investigated in the current study. Nevertheless, results from the current study suggest that investigators need to take species specific potency differences into consideration when designing experiments with recombinant PTH.

The reason for the differences between the skeletal effects of rPTH and bPTH in rats observed in the current study is unclear. We have demonstrated that both rat and bovine PTH 1-34 bind with equal affinities to a recombinant human PTH receptor overexpressed in HEK293 cells and that both peptides are able to equally stimulate the accumulation of intracellular cyclic AMP (cAMP) in these transfected HEK293 cells as well as in ROS 17/2.8 cells which possess a native rat PTH receptor (Lu and Owen, unpublished data). It is quite possible that the interactions of these similar, but not identical, ligands (29/34 amino acid identity) with the rat PTH receptor induce slightly altered receptor conformations following binding, ultimately resulting in differential secondary signaling. It has been reported that amino acids in the C-terminal region (residues 15-34) of PTH are those which interact with the receptor to facilitate binding. Between rPTH and bPTH, residues 16, 18, 21, and 22 are not identical, potentially explaining the equivalent binding affinities but induction of slightly different receptor conformations following ligand binding[16]. In addition, residues in the N-terminal

Parameters	rPTH			bPTH		
	5 μg	25 μg	50 μg	5 μg	25 μg	50 μg
BMC	9.5	10.1	26.1	12.9	23.7	26.5
BMD	7.8	12.2	20.7	13.4	21.3	24.6
BV/TV	53.8	59.4	100.2	73.2	118.0	130.9
Tb.N	30.4	25.8	53.5	48.0	54.9	48.8
Tb.Th	18.8	28.6	31.3	16.3	41.7	59.0
MS/BS	25.1	38.7	65.0	38.3	73.9	105.0
MAR	7.7	6.9	14.5	9.1	7.6	22.3
BFR/BS	31.8	46.1	85.9	49.1	82.6	144.7

BMC: bone mineral content; BMD: bone mineral density; BV/TV: cancellous bone volume; Tb.N: trabecular number; Tb.Th: trabecular thickness; MS/BS: mineralizing surface; MAR: mineral apposition rate; BFR/BS: surface-based bone formation rate.

Table 2. Percentage increase in densitometric and histomorphometric parameters for the OVX rats treated with PTH compared with vehicle.

domain of PTH are believed to be involved in the induction of secondary signaling[17]. In this part of the peptide, residue 7 differs between rPTH and bPTH, again possibly affecting receptor signaling. Most of the reported data concerning interactions of PTH with its receptor are for the human PTH and the human PTH receptor. It would be quite informative to perform similar site-directed mutagenesis studies on rPTH and the rat PTH receptor, given the extensive use of this species as a model in many bone-related studies. Furthermore, the potential differences in bioavailability and metabolism of rPTH and bPTH that might have contributed to the differences in the relative potency of these peptides in stimulating bone formation and increasing bone mass in OVX rats requires further investigation.

In addition, the current study demonstrated that both rPTH and bPTH restore lost bone to osteopenic bone sites of OVX rats in a dose-dependent manner. The lowest dose (5 μg/kg/d) tested in this study for both rPTH and bPTH was effective in stimulating bone formation and increasing bone mass in OVX rats. These anabolic effects were evident by a significant increase in cancellous mineralizing surface, bone formation rate, and bone volume at the proximal tibia, and a significant increase in distal femoral BMC and BMD in OVX rats after 28 days of treatment with either rPTH or bPTH at this dose level. However, trabecular number, thickness, or separation in OVX rats treated with 5 μg/kg/d of rPTH or bPTH were not significantly different from those of OVX rats treated with vehicle. These data indicated that the treatment with rPTH or bPTH at 5 μg/kg/d for 4 weeks was not sufficient to improve trabecular structure despite the increment in bone mass at a skeletal site with established osteopenia. At 25 μg, rPTH significantly increased distal femoral BMD and proximal tibial BV/TV compared with vehicle treatment. However, these parameters did not differ significantly between 25 μg and 5 μg rPTH treatments. The increment of BMD and BV/TV induced by PTH treatment at 25 μg was significantly lower in the rPTH treated group compared with the bPTH treated group. The magnitude of increase in cancellous mineralizing surface and bone formation rate induced by treatment with rPTH or bPTH linearly increased with increasing doses. The significant stimulation of bone formation induced by rPTH or bPTH resulted in a complete restoration of cancellous bone mass and an improved trabecular structure at the proximal tibiae of OVX rats treated with the highest dose (50 μg/kg/d) used in this study. In addition, distal femoral BMC and BMD of OVX rats treated with rPTH or bPTH at this dose level were restored to the level above vehicle-treated sham controls, indicating that such treatments were able to add additional bone to this skeletal site. At the highest dose, bPTH had significantly higher values for proximal tibial BV/TV, Tb.Th, MS/BS, and BFR/BS compared with rPTH. These data suggest that at both 25 and 50 μg dose levels, bPTH is more effective than rPTH in stimulating bone formation and increasing bone mass in OVX rats with established osteopenia.

Although rPTH or bPTH at higher doses were able to completely restore bone mass, trabecular number was only partially restored by the treatment in OVX rats with established osteopenia. These findings were consistent with the previous reports in rats treated with PTH and indicate that the increment of cancellous bone was due primarily to the thickening of existing trabeculae[18-21]. With this limitation, PTH treatment failed to completely restore cancellous bone mass and structure in the OVX rats at a skeletal site with severe osteopenia[22]. Based on the findings in the OVX rats, the effectiveness of restoring cancellous bone mass and structure with PTH is indirectly related to the severity of bone loss at the beginning of treatment.

Variables	rPTH		bPTH	
	r	p	r	p
BMC	0.651	0.0001	0.643	0.0001
BMD	0.737	0.0001	0.719	0.0001
BV/TV	0.462	0.0101	0.559	0.0013
Tb.N	0.370	0.0442	0.225	0.2310
Tb.Th	0.399	0.0290	0.256	0.1720
Oc.S/BS	0.740	0.0001	0.471	0.0086
MS/BS	0.739	0.0001	0.774	0.0001
MAR	0.288	0.1224	0.461	0.0103
BFR/BS	0.672	0.0001	0.789	0.0001

BMC: bone mineral content; BMD: bone mineral density; BV/TV: cancellous bone volume; Tb.N: trabecular number; Tb.Th: trabecular thickness; Oc.S/BS: osteoclast surface; MS/BS: mineralizing surface; MAR: mineral apposition rate; BFR/BS: surface-based bone formation rate.

Table 3. Dose response for variables determined by linear regression analysis.

Variables	Potency Ratio	95% Confidence Interval
BMC	2.76	(0.934, 20.9)
BMD	3.60	(1.67, 12.2)
BV/TV	5.62	(1.15, 36800)
Tb.Th	2.79	(0.865, 29.8)
Oc.S/BS	2.93	(0.713, 126)
MS/BS	3.85	(1.85, 12.1)
BFR/BS	3.63	(1.58, 14.6)

BMC: bone mineral content; BMD: bone mineral density; BV/TV: cancellous bone volume; Tb.Th: Trabecular thickness; Oc.S/BS: osteoclast surface; MS/BS: mineralizing surface; BFR/BS: surface-based bone formation rate.

Table 4. Relative potency of rPTH and bPTH on densitometric and histomorphometric variables.

In summary, our results indicate that bovine PTH is relatively more potent than rat PTH in causing bone anabolism in OVX rats. We also confirmed that both rPTH and bPTH dose dependently stimulate bone formation and augment bone mass, resulting in complete restoration of lost bone mass in OVX rats with established osteopenia.

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