ORIGINAL ARTICLE

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One Year of Abaloparatide, a Selective Activator of the PTH1 Receptor, Increased Bone Formation and Bone Mass in Osteopenic Ovariectomized Rats Without Increasing Bone Resorption

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ABSTRACT

Abaloparatide is a novel 34-amino acid peptide selected to be a potent and selective activator of the parathyroid hormone receptor (PTH1R) signaling pathway with 41% homology to PTH(1-34) and 76% homology to PTHrP(1-34). A 12-month treatment study was conducted in osteopenic ovariectomized (OVX) rats to characterize the mechanisms by which abaloparatide increases bone mass. Sprague-Dawley (SD) rats were subjected to OVX or sham surgery at age 6 months and left untreated for 3 months to allow OVX-induced bone loss. Ten OVX rats were euthanized after this bone depletion period, and the remaining OVX rats received daily subcutaneous injections of vehicle ($n = 18$) or abaloparatide at 1, 5, or 25 μg/kg/d ($n = 18$/dose level) for 12 months. Sham controls ($n = 18$) received vehicle daily. Bone densitometry and biochemical markers of bone formation and resorption were assessed longitudinally, and L_3 vertebra and tibia were collected at necropsy for histomorphometry. Abaloparatide increased biochemical bone formation markers without increasing bone resorption markers or causing hypercalcemia. Abaloparatide increased histomorphometric indices of bone formation on trabecular, endocortical, and periosteal surfaces without increasing osteoclasts or eroded surfaces. Abaloparatide induced substantial increases in trabecular bone volume and density and improvements in trabecular microarchitecture. Abaloparatide stimulated periosteal expansion and endocortical bone apposition at the tibial diaphysis, leading to marked increases in cortical bone volume and density. Whole-body bone mineral density (BMD) remained stable in OVX-Vehicle controls while increasing 25% after 12 months of abaloparatide (25 μg/kg). Histomorphometry and biomarker data suggest that gains in cortical and trabecular bone mass were attributable to selective anabolic effects of abaloparatide, without evidence for stimulated bone resorption. © 2016 American Society for Bone and Mineral Research.

KEY WORDS: ABALOPARATIDE; PTHrP; ANABOLIC AGENTS; BONE FORMATION; OSTEOPOROSIS

Introduction

Abaloparatide (BA058) is a novel 34-amino acid peptide selected to be a potent and selective activator of the parathyroid hormone receptor (PTH1R) signaling pathway, with 41% homology to PTH(1-34) and 76% homology to PTHrP(1-34). A phase 2 clinical study of daily abaloparatide (s.c.) in postmenopausal women with osteoporosis demonstrated significant increases from baseline in bone mineral density (BMD) and bone formation markers over a 24-week treatment period.[1] A phase 3 fracture trial of abaloparatide in postmenopausal women with osteoporosis showed that abaloparatide significantly reduced the risk of new vertebral fractures, nonvertebral fractures, clinical fractures, and major osteoporotic fractures,[1,2] and a 2-year extension of this trial is ongoing.[3] Nonclinical studies of abaloparatide are relevant for understanding its effects on bone. The current study in adult ovariectomized Sprague-Dawley (SD) rats was designed to assess the effect of abaloparatide on bone mass and to understand the cellular mechanisms by which abaloparatide increases bone mass.

At a molecular level, abaloparatide acts via agonism of the PTH1 receptor (PTH1R). Daily injection of other PTH1R agonists, including PTH(1-34), PTH(1-84), PTHrP, and other PTHrP analogs, can increase bone formation, bone mass, and bone strength in preclinical models.[4,5] PTH1R agonists typically promote the activation of bone remodeling,[6,7] but intermittent PTH administration is also capable of increasing bone mass in mice

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that lack the capacity for bone remodeling, indicating that bone resorption is not essential for bone anabolism via PTH1R activation.[8] Pro-resorption and pro-formation signals resulting from PTH1R activation derive from different molecular pathways,[9] suggesting the possibility of differentially activating anabolic versus catabolic responses. Significant efforts have been made to identify more selectively anabolic PTH1R agonists that increase bone formation with lesser stimulation of bone resorption,[10,11] while parallel efforts have examined the potential clinical benefits of combining PTH1R agonists with antiresorptive agents as a way of minimizing bone resorption and maximizing gains in bone mass.[12–14] In the current study, we tested the hypothesis that abaloparatide would increase bone formation and bone mass without increasing bone resorption, which may represent an effective and efficient means of increasing bone mass. Abaloparatide was shown to increase bone mass in a 16-month cynomolgus monkey study[15] and a 24-week clinical trial[1] in conjunction with significant, early, and sustained increases in bone formation parameters. A study conducted in postmenopausal women showed that abaloparatide at 80 μg/d caused lesser increases in the bone resorption marker serum carboxy-terminal cross-linking telopeptide of type I collagen (sCTX) in comparison with open-label PTH(1-34) (teriparatide).[1] Abaloparatide 80 μg also led to greater gains in total hip BMD versus PTH(1-34), suggesting that abaloparatide effects on BMD may be influenced by its lower propensity to stimulate bone resorption.

The current study examined the relative balance of bone formation and bone resorption in adult osteopenic OVX rats during a 12-month period of treatment with a wide range of abaloparatide doses. Several biochemical markers of bone formation and bone resorption were analyzed, and in vivo bone densitometry and trabecular and cortical bone histomorphometry were also assessed. The pharmacology findings of increased bone formation, lack of increased bone resorption, and substantial gains in bone mineral density along with increased trabecular and cortical bone volume support the continued development of abaloparatide for reducing fracture risk in patients with osteoporosis.

Materials and Methods

Animals and husbandry

All animal procedures and activities were approved by and performed in an AAALAC-accredited facility under Good Laboratory Practice (GLP) conditions (Charles River Laboratories, Montreal, Canada). A total of 115 female Sprague-Dawley rats, including 15 spare animals, were received from Charles River (Raleigh, NC, USA) at age 22 weeks and cared for in accordance with established guidelines.[16] Upon arrival, animals were individually housed in stainless-steel cages with automated watering and *ad libitum* access to PMI Nutrition International (Shoreview, MN, USA) Certified Rodent Chow No. 5CR4 (14% protein). Throughout the study, animals were housed on a 12-hour light/dark cycle, with a temperature range of 19°C to 25°C and a relative humidity range of 30% to 70%.

After a 4-week acclimation period, at which time their average weight was approximately 345 g, animals were allocated to 5 groups of 18 animals each (1 sham group and 4 OVX groups) that were balanced for body weight and lumbar spine areal BMD. An additional 10 OVX animals were assigned as pretreatment baseline animals and euthanized at the end of the bone depletion period. Animals were anesthetized with isoflurane and underwent OVX or sham surgery. Benzathine, penicillin G, and procaine were administered intramuscularly on the day of surgery and 2 days after surgery. Analgesia was provided by subcutaneous (s.c.) buprenorphine (0.05 mg/kg) and carprofen (5 mg/kg, s.c.). Animals were individually housed for up to 7 days to allow adequate healing, and thereafter were housed up to 3 per cage. Throughout the study, animals were observed for general health twice daily, and animals underwent detailed clinical observation once weekly. Food consumption was measured weekly for the first 14 weeks and monthly thereafter. Scheduled euthanasia at month 12 was by exsanguination from the abdominal aorta after isoflurane anesthesia. Animals were fasted overnight before terminal blood draw at necropsy. At necropsy, the uterus and vagina were weighed to assess ovarian status and the effects of ovariectomy.

A total of 13 rats were euthanized or found dead between study days 117 to 358 before study termination: 6 in the sham control group, 2 in the OVX-Veh group, 3 in the OVX + abaloparatide 1 μg/kg/d group, 1 in the OVX + abaloparatide 5 μg/kg/d group, and 1 in the OVX + abaloparatide 25 μg/kg/d group. For these animals' data, absolute values were reported if collected, and data based on % change from baseline were censored as required. Five deaths were likely secondary to complications from blood collection, whereas the remaining deaths were attributed to incidental age-related pathologies.

Study design and dose selection

After a 13-week postsurgical bone depletion period, one group of untreated OVX rats was euthanized as a pretreatment baseline group for histomorphometry data. The remaining groups were given daily s.c. injections of vehicle (Vehicle; 0.9% sodium chloride) or one of three dose levels of abaloparatide in a 0.1 mL/kg volume. Abaloparatide dose levels were 1 μg/kg/d (OVX-ABL1), 5 μg/kg/d (OVX-ABL5), and 25 μg/kg/d (OVX-ABL25), with dosing guided by weekly body weight measurements. Preliminary results from another rat study indicated that 6 weeks of abaloparatide at 1.25 μg/kg/d completely reversed OVX-induced bone loss (Radius Health, Inc., Waltham, MA, USA). This led to selection of 1 μg/kg as the low dose, and also 5- and 25-fold multiples of this dose to provide safety margins.

Bone densitometry

Bone area, areal BMD (aBMD), and aBMC were assessed in vivo using a Hologic Discovery A bone densitometer (Hologic, Inc., Bedford, MA, USA). Single scan measures were taken of the whole body, right femur, and L_1 to L_4 once before surgery, at the end of the bone depletion period, and at weeks 12/13, 25/26, and 51/52 of treatment; these time points are nominally referred to as month 3, month 6, and month 12, respectively.

Histomorphometry

Eight days and 3 days before death, animals received bicarbonate buffered calcein green (8 mg/kg, s.c.). The right tibia and L_3 vertebra were collected from the first 10 animals in each group that underwent scheduled month 12 necropsies and from baseline controls. Specimens were formalin-fixed for 3 days, transferred to 70% alcohol, and prepared without decalcification. Bones were dehydrated and embedded in methylmethacrylate. Unstained ground sections (two transverse levels) were prepared for evaluation of cortical bone at the

tibia-fibular junction (the diaphysis site). Goldner's trichrome-stained and unstained sections were prepared to evaluate cancellous bone in the vertebral body (1 sagittal level) and in the proximal tibial metaphysis (2 levels through the frontal plane). Histomorphometry was conducted using an Image-Pro Plus analyzer (Media Cybernetics, Rockville, MD, USA). Histomorphometry endpoints were in accordance with established guidelines.[17]

Clinical chemistry and bone turnover markers

Blood was collected by venipuncture from the jugular, and urine was collected overnight from metabolic cages. These collections were made at weeks 11/12, 30/31, and 47/48 of the treatment period, referred to nominally as months 3, 6, and 12. Animals were food-deprived overnight for blood and urine collections. Serum calcium and phosphorus were measured by colorimetric and UV methods, respectively (Hitachi P800-Module). Serum CTX was measured with the RatLaps assay (ImmunoDiagnostics Systems, Gaithersburg, MD, USA). Coefficients of variation (CVs) derived from 8 independent assay runs ranged from 2.8% to 15.8% for CTX concentrations from 2.1 to 170 ng/mL. Urine deoxypyrodinoline (uDPD) was measured with the MicroVue DPD EIA (Quidel, San Diego, CA, USA). CVs derived from 11 independent assay runs ranged from 2.8% to 10.7% for DPD concentrations from 0.67 to 29.4 ng/mL. Serum procollagen type I N propeptide (sPINP) was measured with a rat PINP enzyme immunoassay (ImmunoDiagnostics Systems, Gaithersburg, MD, USA). CVs derived from 10 independent assay runs ranged from 0.61% to 14.5% for sPINP concentrations from 0.72 to 79 ng/mL. Serum osteocalcin was measured by an assay from Biomedical Technologies Inc. (Madrid, Spain). CVs derived from 9 independent assay runs ranged from 2.4% to 11.4% for PINP concentrations from 0.7 to 79 ng/mL.

As an exploratory analysis, bone turnover markers were used to calculate anabolic windows. Anabolic windows were assessed by calculating abaloparatide-related changes in bone formation markers (PINP and osteocalcin) versus bone resorption markers (uDPD and sCTX). First, biomarker data were normalized by dividing individual values for the abaloparatide-treated animals by the average value of the OVX-Vehicle control group at each time point. Normalized uDPD and sCTX values for each animal were then subtracted from their normalized s-PINP and s-osteocalcin (sOC) values, respectively. These numerical differences were integrated over the entire treatment time course by area under the curve (AUC), which was designated as the anabolic window. Relationships between these anabolic windows versus the % change from baseline in whole-body areal bone mineral content (BMC) were examined via linear regression analysis.

Statistical analysis

Group variances were compared using Levene's test. If Levene's test was not significant at $p < 0.05$, a one-way ANOVA was implemented. If Levene's test was significant and at least one of the group variances was null, rank-transformed data were analyzed by ANOVA. Otherwise, an ANOVA model allowing for differing variance in each group was performed to account for heterogeneity of group variances. Significance in the retained model ($p < 0.05$) led to Dunnett's *t* test to compare group means of the OVX-Vehicle control group with each of the abaloparatide groups. Longitudinal bone densitometry and bone turnover marker data were submitted to repeated measures analysis. These statistical analyses were performed using release 8.2 of SAS System (SAS Institute, Cary, NC, USA) or subsequent version. Anabolic window data were analyzed by unpaired *t* tests of normalized sPINP versus normalized uDPD/Cr, and of normalized sOC versus normalized sCTX, using GraphPad Prism Version 6 (GraphPad, San Diego, CA, USA). Linear regression analysis of anabolic windows versus % change from baseline in whole-body aBMC were conducted using GraphPad Prism Version 6. For these regressions, anabolic windows were calculated as the AUC of numerical difference between the normalized formation versus resorption markers.

Results

General health

Body weights and food consumption for the abaloparatide-treated animals were comparable to OVX-Vehicle control animals throughout the study (Supplemental Fig. S3). Ovariectomy was confirmed in all animals by notable reductions of uterus and vagina weight compared with sham controls. Uterine and vagina weights of OVX rats were not influenced by abaloparatide treatment (Supplemental Fig. S4).

Bone densitometry by DXA

In vivo DXA-derived aBMD data described in this section are expressed as percent change from pretreatment (month 0) baseline values, which were determined 3 months after OVX or sham surgery (Fig. 1). For reference, absolute aBMD data at this baseline are presented in Supplemental Table S1, which indicates the catabolic effects of OVX and the consistency of aBMD values across the OVX groups before treatment. During the 12-month treatment phase, aBMD of the whole body, proximal femur, and femur diaphysis of OVX-Vehicle controls remained generally stable, whereas L_1 to L_4 spine BMD declined progressively. Abaloparatide treatment resulted in early (by month 3), dose-dependent, and progressive increases in aBMD of the whole body, L_1 to L_4 lumbar spine, proximal femur, and femur diaphysis (Fig. 1). These increases were statistically significant for all abaloparatide dose levels and for all time points relative to OVX-Vehicle controls. The 25 μg/kg abaloparatide dose level resulted in maximal aBMD increases of 25% for whole body, 36% for the L_1 to L_4 spine, 37% for the proximal femur, and 51% for the femur diaphysis relative to pretreatment baseline. Dual-energy X-ray absorptiometry (DXA) also revealed a significantly greater increase in femur diaphysis projection area for the 25 μg/kg abaloparatide dose level at month 12, consistent with a treatment-related increase in periosteal diameter. The percent increases in femur diaphysis projection area from baseline to month 12 were $9.4 \pm 1.8\%$ (mean, SEM) for sham controls, $5.9 \pm 1.8\%$ for OVX-Vehicle, $8.3 \pm 1.8\%$ for 1 μg/kg abaloparatide, $9.3 \pm 1.3\%$ for 5 μg/kg abaloparatide, and $13.8 \pm 1.4\%$ for 25 μg/kg abaloparatide ($p < 0.05$ for 25 μg/kg abaloparatide versus OVX-Vehicle, $p < 0.05$). Increased projection area in the 25 μg/kg abaloparatide group had a significant influence on the reported increase in aBMD: Whereas femur diaphysis aBMD in that group increased by 51% from month 0 to month 12, areal bone mineral content (aBMC) increased by 72% (data not shown).

Bone histomorphometry

Trabecular histomorphometry at the L_3 vertebral body revealed significant dose-dependent effects of abaloparatide on bone volume, architecture, and turnover (Fig. 2). All changes

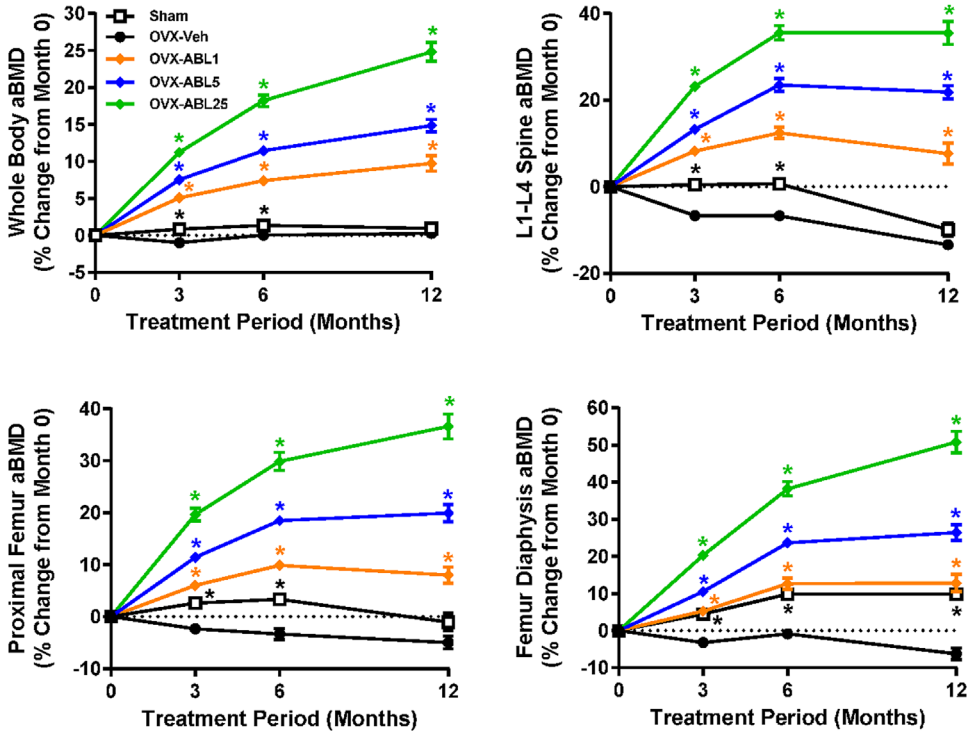

Fig. 1. Changes in areal bone mineral density (aBMD) versus pretreatment (month 0) baseline. ABL1, ABL5, and ABL25 refer to abaloparatide dose levels of 1, 5, and 25 μg/kg/d, respectively. Data represent group means ± SEM, $n = 12$–18/group. $^*p < 0.05$ versus OVX-Vehicle control.

described in this section are in relation to, and significantly different from, the OVX-Vehicle control group. After 12 months of treatment, L_3 trabecular bone volume (BV/TV) was 80%, 130%, and 150% greater in the abaloparatide 1, 5, and 25 μg/kg groups, respectively, versus OVX-Vehicle controls (all $p < 0.001$). L_3 osteoblast surface (Ob.S/BS) was approximately twofold greater in the abaloparatide 5 and 25 μg/kg groups (both $p < 0.05$), whereas osteoclast surface (Oc.S/BS) was similar in the abaloparatide and OVX-Vehicle groups. L_3 bone formation rate (BFR/BS) increased dose-dependently in the abaloparatide groups, reaching a level nearly threefold greater than OVX-Vehicle controls at the 25 μg/kg dose level ($p < 0.001$). Increased BFR/BS was attributable to increases in mineralizing surface (MS/BS), whereas mineral apposition rate (MAR) was generally unaffected by abaloparatide. Improvements in trabecular microarchitectural parameters were observed with all three doses of abaloparatide, including increased trabecular thickness (Tb.Th), decreased trabecular spacing (Tb.Sp), and increased trabecular number (Tb.N). An increase in activation frequency was observed for L_3 at the 25 μg/kg dose level. L_3 osteoid thickness (O.Th) and wall thickness (W.Th) were not significantly influenced by abaloparatide (Fig. 2). There were no significant differences between the abaloparatide group versus OVX-Vehicle controls for L_3 osteoid volume (OV/TV), osteoid thickness (O.Th), osteoid maturation time (Omt), formation period (FP), or resorption period (Rs.P), whereas osteoid surface (OS/BS) was significantly greater in the 5 and 25 μg/kg abaloparatide groups versus OVX-Vehicle (Supplemental Table S2).

Similar to effects in L_3, abaloparatide had favorable effects on most trabecular bone parameters measured at the proximal tibial metaphysis (Supplemental Fig. S1), including dose-dependent effects on BV/TV, Ob.S/BS, MS/BS, BFR/BS, OS/BS, Tb.Th, Tb.Sp, and Tb.N; these effects were statistically significant at the 25 μg/kg dose level, and in most cases at the lower dose levels as well. As was found at L_3, abaloparatide had no significant effects on Oc.S/BS or O.Th at the proximal tibial metaphysis. The tibial metaphysis exhibited significantly decreased OV/TV and osteoid maturation time (Omt) at the 25 μg/kg abaloparatide dose level, which was not observed at the L_3 site.

Cortical histomorphometry was assessed at the tibial diaphysis after 12 months of abaloparatide treatment (Fig. 3). Cortical width and cortical area increased dose dependently with abaloparatide, with statistical significance at all three doses and maximal increases of 80% and 50%, respectively, relative to OVX-Vehicle controls. Periosteal perimeter (Ps.Pm) also increased dose-dependently with abaloparatide, achieving significance at the 25 μg/kg dose. This increase was accompanied by significantly greater periosteal labeled perimeter (Ps.L.Pm/Ps.Pm) with all three abaloparatide dose levels, and increased periosteal bone formation rate (BFR/BS), with no effect on periosteal mineral apposition rate (MAR). Abaloparatide also promoted bone apposition on endocortical surfaces, as shown by significant and dose-dependent reductions in medullary area and endocortical perimeter (Ec.Pm) with all three abaloparatide dose levels ($p < 0.05$ versus OVX-Vehicle controls). These decreases were associated with significantly greater endocortical labeled perimeter (Ec.L.Pm/Ec.Pm), whereas endocortical MAR and endocortical BFR/BS were not significantly altered by abaloparatide at month 12. Endocortical eroded perimeter (Ec.E.Pm/Ec.Pm), an indicator of endocortical bone resorption, was not affected by abaloparatide (Fig. 3).

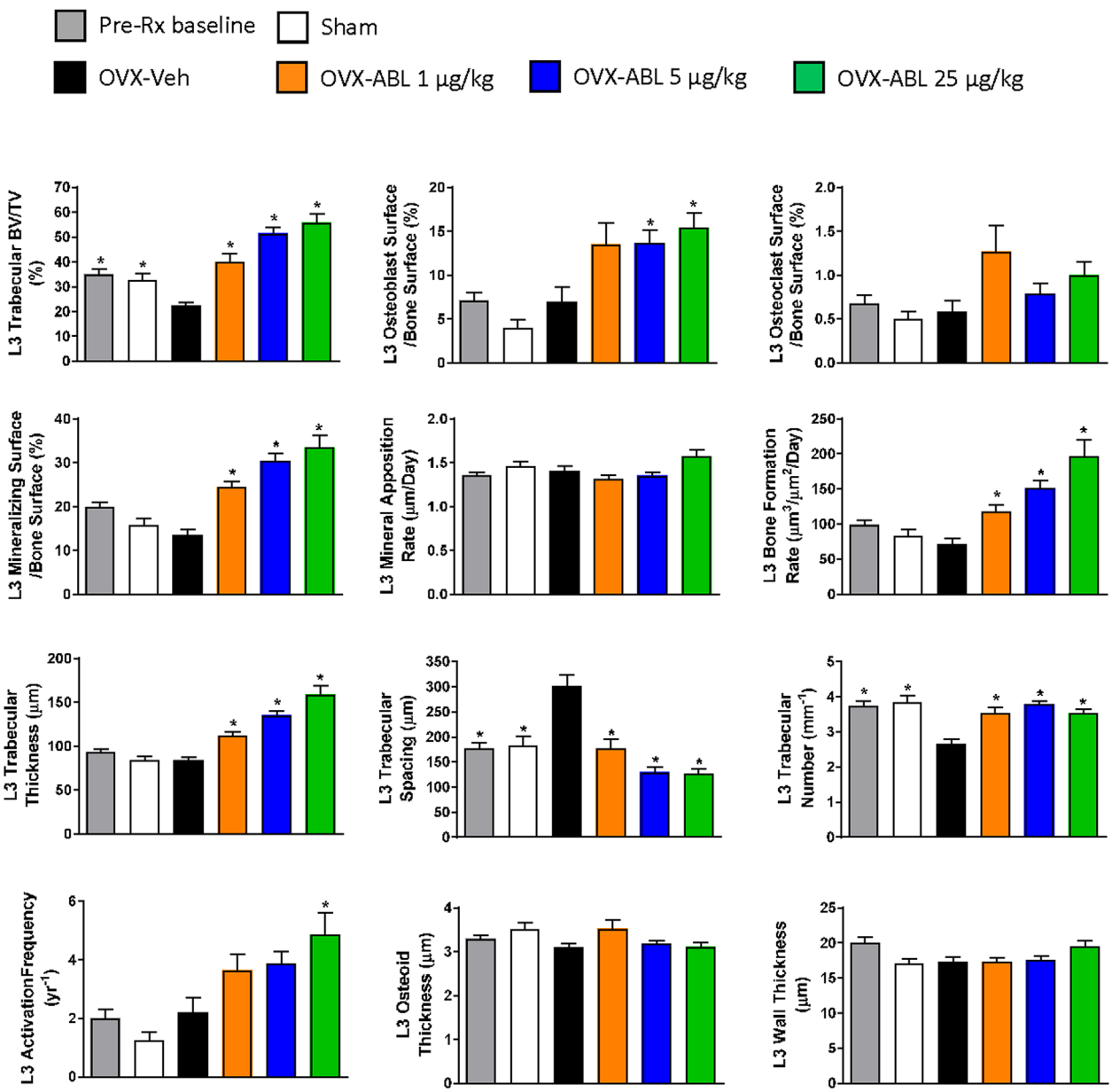

Fig. 2. Trabecular histomorphometry for the L_3 vertebral body. The Pre-Rx group refers to pretreatment OVX animals that were euthanized after a 3-month bone depletion period, just before the 12-month treatment phase. Data represent means ± SEM, $n = 10$/group. * < 0.05 versus OVX-Vehicle controls.

Bone turnover biochemical markers and serum chemistry results

The bone formation marker serum osteocalcin was significantly increased in the OVX-Vehicle group relative to sham controls at the pretreatment baseline (month 0), consistent with increased bone turnover resulting from OVX (Fig. 4). This increase persisted through month 6 of treatment. Abaloparatide significantly increased sOC from month 3 through month 12 at the 25 µg/kg dose level, and from month 6 through month 12 at the 5 µg/kg dose level ($p < 0.05$ versus OVX-Vehicle). Abaloparatide at a dose of 25 µg/kg also increased the bone formation marker sPINP at months 3 and 12 ($p < 0.05$ versus OVX-Vehicle; Fig. 4). The bone resorption marker sCTX was significantly greater in the OVX-Vehicle group versus sham controls at months 0, 3, and 6. Abaloparatide had no significant effects on sCTX at any time or dose level (Fig. 4). The bone resorption marker uDPD/Cr was also significantly greater in the OVX-Vehicle group versus sham controls at each time point. Abaloparatide treatment of OVX rats did not increase uDPD/Cr, and at month 6, the 5 µg/kg abaloparatide group had significantly lower uDPD/Cr relative to OVX-Vehicle controls (Fig. 4).

Bone turnover markers were also used to calculate anabolic windows, a quantitative exploratory approach for assessing treatment-related changes in the relative balance of bone formation and bone resorption. Anabolic windows were evident based on normalized sPINP values (adjusted for group mean values of OVX-Vehicle controls at each time point) that

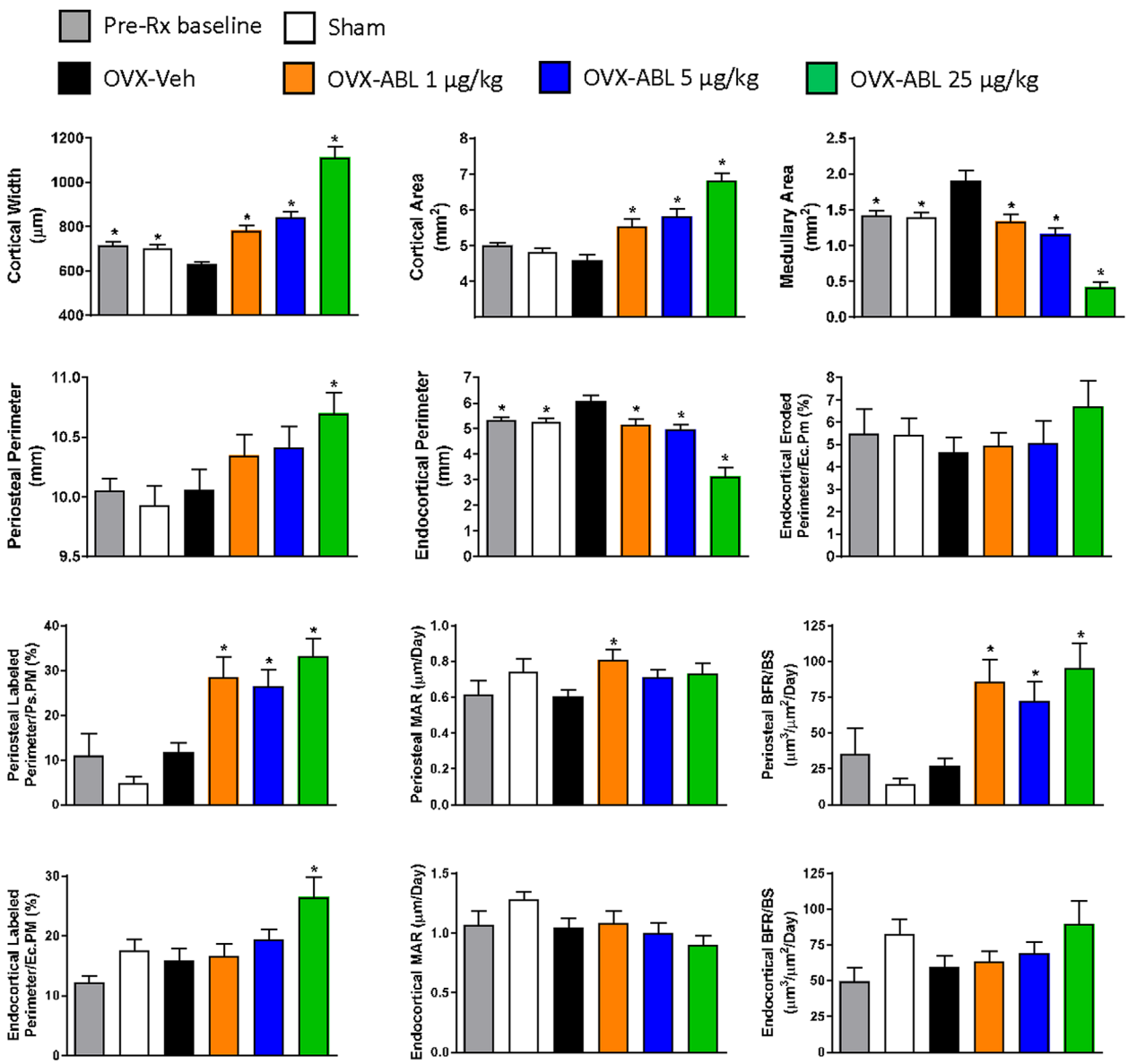

Fig. 3. Cortical histomorphometry for the tibial diaphysis. The Pre-Rx group refers to pretreatment OVX animals that were euthanized after a 3-month bone depletion period, just before the 12-month treatment phase. Data represent means ± SEM, $n = 10$/group. $^{*}p < 0.05$ versus OVX-Vehicle controls.

significantly exceeded normalized uDPD/Cr values during the treatment period for all abaloparatide dose levels (Fig. 5). Similarly, normalized sOC exceeded normalized sCTX values during the study for all dose levels of abaloparatide (Supplemental Fig. S2). The remaining permutations, namely sOC versus uDPD and sPINP versus sCTX, also demonstrated anabolic windows with abaloparatide (data not shown). Whether anabolic windows might reflect the relative positive bone balance was further assessed by expressing the numerical differences between normalized values of sPINP minus uDPD, or of sOC minus sCTX, versus percent change at month 12 in whole-body aBMC. Linear regression analyses showed significant positive correlations for sPINP minus uDPD versus percent change in BMC ($p < 0.0001$; Fig. 5), and for sOC minus sCTX versus percent change in BMC ($p < 0.01$; Supplemental Fig. S2).

The OVX-Vehicle group had significantly lower serum calcium relative to sham controls at months 6 and 12 (Fig. 4). Abaloparatide had minimal effects on serum calcium, with a statistically significant but marginal (+2.6%) increase at month 6 in the 25 µg/kg dose relative to OVX-Vehicle controls. The highest recorded serum calcium value in the abaloparatide groups was 11.7 mg/dL in a 5 µg/kg animal at month 6. This value remained near the middle of the normal serum calcium range (10.6 to 12.4 mg/dL), as derived from the mean values of sham control rats ± 2 standard deviations. Of 17 rats from the 25 µg/kg group for which serum calcium data were available, only 2 exhibited serum calcium values (11.6 mg/dL for both) that exceeded the group mean value for sham controls (11.5 mg/dL). Serum phosphorus was not significantly influenced by OVX, and there were no meaningful or consistent effects of abaloparatide on serum phosphorus apart from a modest (+9%) increase for

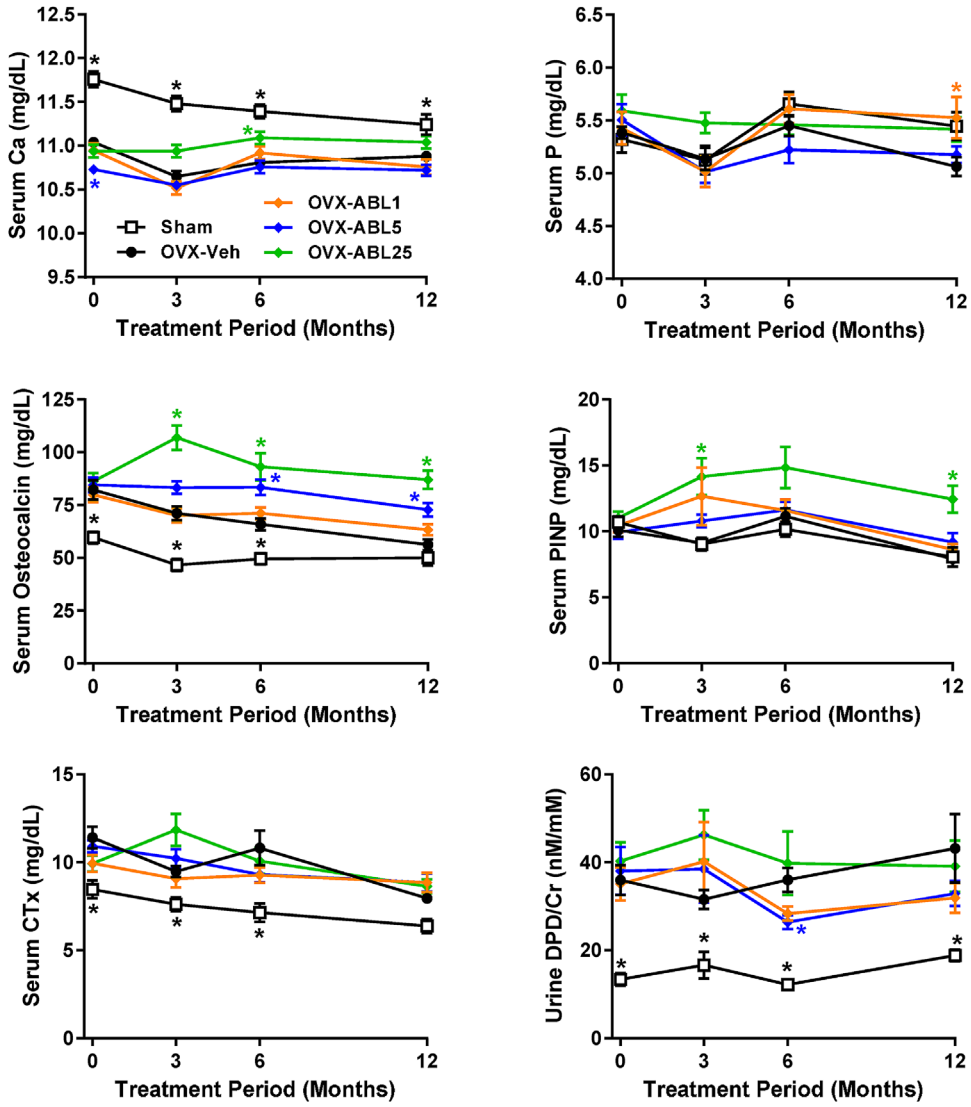

Fig. 4. Changes in serum calcium (Ca), serum phosphorus (P), and bone turnover markers. ABL1, ABL5, and ABL25 refer to abaloparatide dose levels of 1, 5, and 25 μg/kg/d, respectively. Data represent group means ± SEM. For serum calcium and phosphorus data, $n = 15$–18/group; for bone turnover marker data, $n = 9$–18/group. $^{*}p < 0.05$ versus OVX-Vehicle control.

the 1 μg/kg abaloparatide group at month 12 ($p < 0.05$ versus OVX-Vehicle). This value was similar to average values for sham controls.

Discussion

This study examined the effects of 12 months of abaloparatide daily administration at three dose levels on bone mineral density, histomorphometry, volume, architecture, and turnover in osteopenic OVX rats. Abaloparatide reversed the skeletal catabolic effects associated with 3 months post-OVX and prevented the progressive bone loss found in OVX-Vehicle controls 12 months later, with bone volume and density increasing dose-dependently. These structural improvements were associated with sustained increases in bone formation indices on trabecular, endocortical, and periosteal surfaces. There was no evidence for increased bone resorption with abaloparatide or meaningful increases in serum calcium.

Abaloparatide is a novel synthetic peptide activator of the PTH1 receptor selected for its ability to stimulate bone formation and increase bone mass with minimal stimulation of bone resorption. Native PTHrP can stimulate bone resorption and promote hypercalcemia pathologically and pharmacologically,[18,19] but data from mice indicate that the primary physiological role of PTHrP in the postnatal skeleton is attainment and maintenance of bone mass via stimulation of bone formation.[20–22] Consistent with that role, abaloparatide stimulated bone formation indices on all surfaces and skeletal sites examined, an effect that was mediated primarily by increases in the extent of mineralizing surfaces. Stimulation of bone formation was sustained for the full 12 months of abaloparatide treatment. Another OVX rat study showed that daily PTH(1-84) caused sustained increases in bone formation through 12 months of treatment.[5] The sustained stimulation of bone formation with abaloparatide and other PTH1R agonists appears to contrast with the more transient stimulation of bone

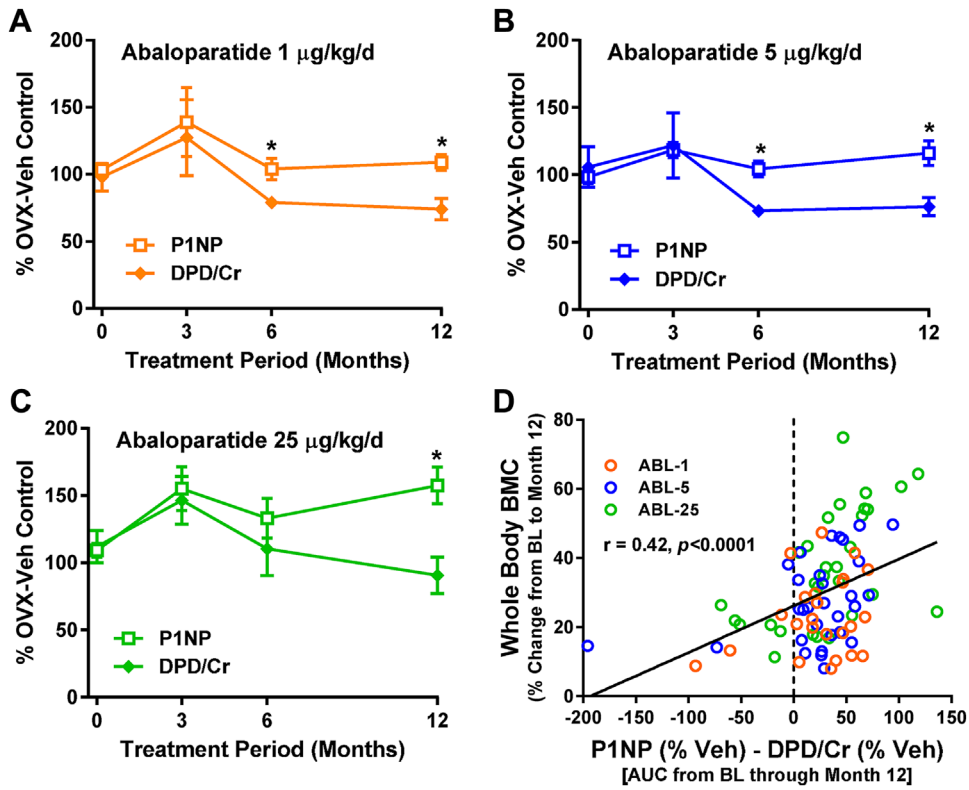

Fig. 5. Anabolic windows during 12 months of abaloparatide administration. Serum PINP and uDPD/Cr values from animals for each abaloparatide dose level were divided by OVX-Vehicle group mean values and plotted over time (*A–C*). Data represent group means ± SEM, $n = 9–18$/group. *Serum PINP significantly greater than uDPD/Cr, $p < 0.05$. Panel *D* illustrates linear regression analysis of area under the curve (AUC) for normalized sPINP minus normalized uDPD/Cr versus the percent change in whole-body BMC from baseline to month 12. The regression was statistically significant ($p < 0.0001$).

formation observed with sclerostin antibodies.[23,24] Sclerostin antibodies increase BMD by promoting activation Wnt signaling in bone,[25] but the early increases in bone formation with this therapeutic approach begin to attenuate after the first few months of initiating treatment.[23,24,26] In a recent OVX rat study, the attenuation of bone formation with chronic sclerostin antibody administration was accompanied by reduced numbers of mature osteoblasts, bone lining cells, and peritrabecular osteoprogenitor cells, whereas each of these cell populations remained elevated at similarly high levels with long-term PTH (1-34) administration.[23] Molecular explanations for these differential cellular responses are currently unclear but may involve Dkk1, an osteocyte-derived factor that inhibits bone formation by reducing Wnt signaling.[25] Sclerostin antibody administration to OVX rats increased skeletal *Dkk1* mRNA expression by nearly tenfold,[27] whereas various PTH1R agonists have been shown to reduce skeletal *Dkk1* mRNA expression in rats and in osteoblast cultures.[28,29] And while Dkk1 can limit the bone-building potential of sclerostin antibody therapy in rats,[30] Dkk1 overexpression in transgenic mice did not attenuate the bone-building effects of PTH(1-34).[29] These and other findings[23] indicate that PTH1R agonists and sclerostin inhibitors have several distinct biological effects on bone, but some evidence suggests the bone-building effects of PTH1R agonists may be at least partially mediated by the downregulation of sclerostin.[31–35] Other evidence from human and animal studies indicated that PTH1R agonists did not downregulate sclerostin,[36–38] and PTH1R agonists can robustly increase bone formation and bone mass in animals independent of any changes in sclerostin.[39,40] Combination therapy with intermittent PTH(1-34) plus sclerostin antibody in mice led to additive gains in BMD, providing additional evidence that PTH1R agonists have sclerostin-independent effects on bone.[41] A treatment-induced increase in T-cell–derived Wnt10b has been implicated as a potential contributor to the bone anabolic effects arising from PTH1R agonism,[41,42] which would suggest a potential contribution of Wnt pathway activation that is independent of sclerostin downregulation.

In contrast to the strong and sustained bone formation response to abaloparatide, bone resorption indices were not increased by abaloparatide. Bone resorption markers sCTX and uDPD/Cr remained similar in abaloparatide and vehicle control groups throughout the study, and osteoclast surface and eroded surface were also unaffected by abaloparatide at the study's endpoint. Consistent with lack of increased bone resorption, serum calcium in the abaloparatide groups remained within normal ranges throughout the study, with negligible increases relative to vehicle controls suggesting low calcium mobilization with abaloparatide. Studies in rats subjected to long-term administration of other PTH1R agonists indicated increases in bone resorption indices, including uDPD/Cr, osteoclast surface, and eroded surface.[4,5,43,44] Whether those findings directly contrast with effects of abaloparatide is unclear at this time because of the lack of head-to-head comparisons in rats. However, recent in vitro data showed that in comparison with PTH(1-34), abaloparatide binds with greater selectivity to a

PTH1R conformation ("RG") that is associated with more transient cAMP responses, providing a possible mechanism by which abaloparatide causes lesser activation of bone resorption.[45] A clinical study showed that abaloparatide caused greater increases in total hip BMD compared with PTH(1-34) over 18 months of treatment, while causing numerically smaller increases in bone resorption (and bone formation) markers.[1] These preclinical and clinical data suggest that minimal bone resorption responses allow abaloparatide to increase BMD with relatively less stimulation of bone formation.

Whether more selective anabolism with abaloparatide contributes to its overall ability to increase bone mass was explored by using bone turnover markers to assess anabolic windows. The anabolic window concept for PTH1R agonists was originally predicated on clinical data indicating an early increase in biochemical markers of bone formation that preceded a later increase in bone resorption markers.[46] This profile is thought to reflect an early period of relatively unopposed anabolism that may contribute to early BMD gains. Several subsequent studies evaluated anabolic windows for PTH-based combination or sequential therapies to assess whether such approaches might favorably alter the relative balance of formation and resorption compared with PTH(1-84) or PTH(1-34) monotherapy.[47–49] The extent to which anabolic windows contribute to increments in bone mass has not been previously described. To further explore their potential clinical utility, we calculated anabolic windows in abaloparatide-treated rats and related the overall size of the windows to 12-month changes in whole-body bone mass. The existence of anabolic windows was demonstrated by significantly greater abaloparatide treatment effects on bone formation markers (sPINP and sOC) relative to the effects on resorption markers (uDPD and sCTX). Regression analyses indicated that anabolic windows based on sPINP versus uDPD, and for sOC versus sCTX, were positively correlated with percent change in whole-body bone mass, suggesting these windows may indicate the extent to which abaloparatide induces a positive bone balance.

This study has several strengths, including a large sample size, a long duration of treatment, and a wide range of abaloparatide dose levels that provided robust exposure multiples relative to that anticipated in clinical settings. The study also has limitations, including the lack of an active bone anabolic comparator and the lack of data in male rats.

In summary, daily abaloparatide administration to osteopenic OVX rats for 12 months reversed OVX-related bone loss via dose-dependent increases in bone formation on trabecular and cortical surfaces. This anabolic effect, which was not accompanied by increases in bone resorption indices, resulted in improved trabecular microarchitecture and cortical geometry. These preclinical data support the continued development of abaloparatide as a treatment to reduce fracture risk in patients with osteoporosis.

Disclosures

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