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Efficacy of Serotonin Inhibition in Mouse Models of Bone Loss

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ABSTRACT

In a proof-of-concept study it was shown that decreasing synthesis of gut serotonin through a small molecule inhibitor of Tph1 could prevent and treat ovariectomy-induced osteoporosis in young mice and rats. In this study, we define the minimal efficacy of this Tph1 inhibitor, demonstrate that its activity is improved with the duration of treatment, and show that its anabolic effect persists on interruption. Importantly, given the prevalence of osteoporosis in the aging population, we then show that Tph1 inhibition rescues ovariectomy-induced bone loss in aged mice. It also cures the low bone mass of *Lrp5*-deficient mice through a sole anabolic effect. Lastly, we provide evidence that inhibition of gut serotonin synthesis can work in concert with an antiresorptive agent to increase bone mass in ovariectomized mice. This study provides a more comprehensive view of the anabolic efficacy of Tph1 inhibitors and further establishes the spectrum of their therapeutic potential in the treatment of bone-loss disorders. © 2011 American Society for Bone and Mineral Research.

KEY WORDS: SEROTONIN; TPH1 INHIBITOR; ANABOLIC TREATMENT; OSTEOPOROSIS; BONE FORMATION

Introduction

Cell-specific loss- and gain-of-function mutations engineered in the mouse through homologous recombination in embryonic stem cells and biochemical assays in mice and humans have provided evidence that the molecular bases of osteoporosis pseudoglioma (OPPG) and of the high-bone-mass (HBM) syndrome may be an increase and a decrease in gut serotonin synthesis, respectively.[1,2] Serotonin would act as a hormone-inhibiting osteoblast proliferator following its binding to a specific receptor.[2] These data have been confirmed by measurements, performed in multiple laboratories around the world, of circulating serotonin levels in patients suffering from either HBM syndrome or OPPG.[1–4]

In view of these data, studying the role played by gut serotonin in bone physiology becomes an even more important issue to tackle from a medical perspective. As a matter of fact, the importance of gut serotonin as a regulator of bone formation was extended beyond these two rare genetic disorders when a proof-of-principle study showed that inhibiting the synthesis of gut serotonin pharmacologically through a small molecule taken orally once a day sufficed to prevent and cure, in mice and rats, ovariectomy-induced osteoporosis through a purely anabolic mode of action.[5]

Gut-derived serotonin biosynthesis is initiated following the hydroxylation of tryptophan by tryptophan hydroxylase 1 (Tph1), an enzyme expressed mainly in the enterochromaffin cells of the duodenum.[6] LP533401, the drug used in the aforementioned study, is an inhibitor of Tph1 enzymatic ability that does not cross the blood-brain barrier in any significant manner.[7] Therefore, it does not affect the synthesis of brain serotonin, which also regulates bone mass accrual.[5,7,8]

As important as it was in strengthening the biologic importance of the regulation of bone formation by gut serotonin, this initial study did not address all the questions that a potential anabolic treatment for osteoporosis ought to answer. This study was conducted precisely to address them in a model organism. We define here a minimal dose of LP533401 sufficient to decrease circulating serotonin levels and to increase bone-formation parameters. We also show that using this Tph1 inhibitor to decrease circulating serotonin levels cures, as it should, the low bone mass of *Lrp5*-deficient mice. Importantly, since osteoporosis usually affects older patients, we show that this treatment also rescues ovariectomy-induced bone loss in

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aged (12-month-old) mice. Lastly, we provide evidence that inhibition of gut serotonin synthesis can work in concert with an antiresorptive agent to increase bone mass in ovariectomized mice.

Materials and Methods

Animals and treatments

The generation of *Lrp5*-deficient mice was described previously.[9] C57BL6/J mice were purchased from the Jackson Laboratories (Bar Harbor, ME, USA) or from the National Institute on Aging Aged Rodent Colony (Bethesda, MD, USA). Then 6-week-old or 9-month-old virgin female mice were subjected to either bilateral ovariectomy or sham operation and were pair fed thereafter. LP533401 was purchased from Dalton Pharma Services (Toronto, Ontario, Canada), dissolved in polyethylene glycol and 5% dextrose (40:60; Sigma, St Louis, MO, USA), and administered daily by gavage at the indicated doses, as described previously.[5] Alendronate (60 μg/kg of body weight per week; Sigma) was dissolved in saline and injected intraperitoneally. Lithium chloride (200 mg/kg daily; Sigma) was dissolved in distilled water and administered by gavage. All mice were subjected to intraperitoneal injections of calcein (25 mg/kg in 0.15 M NaCl and 2% $NaHCO_3$ buffer; Sigma) 6 and 2 days prior to killing. All procedures were approved by the Columbia University Institutional Animal Care and Use Committee.

Bone analyses

Tibias and lumbar vertebrae (L_3 and L_4) were dissected, cleaned of excess soft tissue, and processed for micro–computed tomography (μCT) or histomorphometric analysis. For histomorphometry, specimens were fixed for 24 hours in 10% formalin, dehydrated in a graded series of ethanol, and embedded in methyl methacrylate resin. Von Kossa/van Gieson staining was performed on 7-μm sections for the quantification of bone volume over tissue volume (BV/TV) using the ImageJ software (Bethesda, MD, USA). Bone-formation rate (BFR) was analyzed on unstained 4-μm sections following calcein double labeling of the specimen.[10] For analysis of the parameters of osteoblasts and osteoclasts, 4-μm sections were stained with toluidine blue and tartrate-resistant acid phosphatase (TRACP), respectively. Bone histomorphometry was performed using the Osteomeasure Analysis System (Osteometrics, Atlanta, GA, USA), and parameters were measured according to international guidelines.[11] Trabecular bone architecture of the right proximal tibia was assessed using a μCT system (VivaCT 40, Scanco Medical AG, Bassersdorf, Switzerland). The tibial bone specimen was stabilized with gauze in a 2-mL centrifuge tube filled with 70% ethanol and fastened in the specimen holder of the μCT scanner. One hundred μCT slices, corresponding to a 1.05-mm region distal from the growth plate, were acquired at an isotropic spatial resolution of 10.5 μm. A global thresholding technique was applied to binarize gray-scale μCT images, where the minimum between the bone and bone marrow peaks in the voxel gray-value histogram was chosen as the threshold value. The trabecular bone compartment was segmented by a semiautomatic contouring method and subjected to a model-independent morphologic analysis by the standard software provided by the manufacturer of the μCT scanner. Bone mineral density (BMD) and 3D morphologic parameters, including model-independent measures by distance transformation (DT) of bone volume fraction (BV/TV), Tb.N* (trabecular number), Tb.Sp* (trabecular separation), and connectivity density (Conn.D), were evaluated.[12,13]

Biochemistry and molecular biology

Blood samples were collected through cardiac puncture in Capiject tubes (Terumo, Tokyo, Japan) with gel barrier, kept at room temperature for 30 minutes, and centrifuged at 15,700*g* for 5 minutes at 4°C. Serotonin levels were quantified by ELISA (Serotonin Kit, Fitzgerald, Acton, MA, USA). Samples showing any signs of hemolysis were excluded from the serotonin assay. Serum carboxy-terminal collagen cross-links (CTX), 1,25-dihydroxyvitamin D_3 [$25(OH)_2D_3$], and procollagen type 1 N-terminal propeptide (P1NP) levels were measured with the Ratlaps, $1,25(OH)_2D_3$, and Rat/Mouse P1NP EIA kits (Immunodiagnostic Systems, Scottsdale, AZ, USA), respectively. Serum calcium and phosphate levels were measured with colorimetric assays (Biovision, Mountain View, CA, USA).

Real-time polymerase chain reaction (PCR) was performed on total RNA from flushed long bones and converted to cDNA using the Taq SYBR Green Supermix (Biorad, Hercules, CA, USA) with ROX on an MX3000 instrument; *Gapdh* amplification was used as an internal reference. Primers are available on request.

Statistical analysis

Statistical significance was assessed by Student's *t* test or one-way analysis of variance followed by the Newman-Keuls test for comparisons between more than two groups. Values of p less than .05 were considered significant. All values are expressed as mean ± SEM.

Results

Determination of a minimum effective dose of an inhibitor of gut serotonin synthesis necessary to affect bone mass

In the original study, inhibiting serotonin synthesis through daily gavage with LP533401 proved to be equally effective in rescuing ovariectomy-induced bone loss in mice and rats.[5] Therefore, with the goal to save time and limit costs, we used only the mouse as an animal model in this study.

It was shown previously that increasing the dose of LP533401 given daily to mice or rats up to 250 mg/kg enhances bone-formation parameters in a dose-dependent manner.[5] This experiment did not define, however, the minimum amount of LP533401 needed to treat ovariectomy-induced osteoporosis. To address this question, 6-week-old female mice were ovariectomized or sham-operated. Six weeks later, ovariectomized mice were treated once daily by gavage with either vehicle or LP533401 at doses ranging from 5 to 25 mg/kg for another 6 weeks (Fig. 1*A*). At the end of this treatment period, serum serotonin was measured, and bone histomorphometric analyses

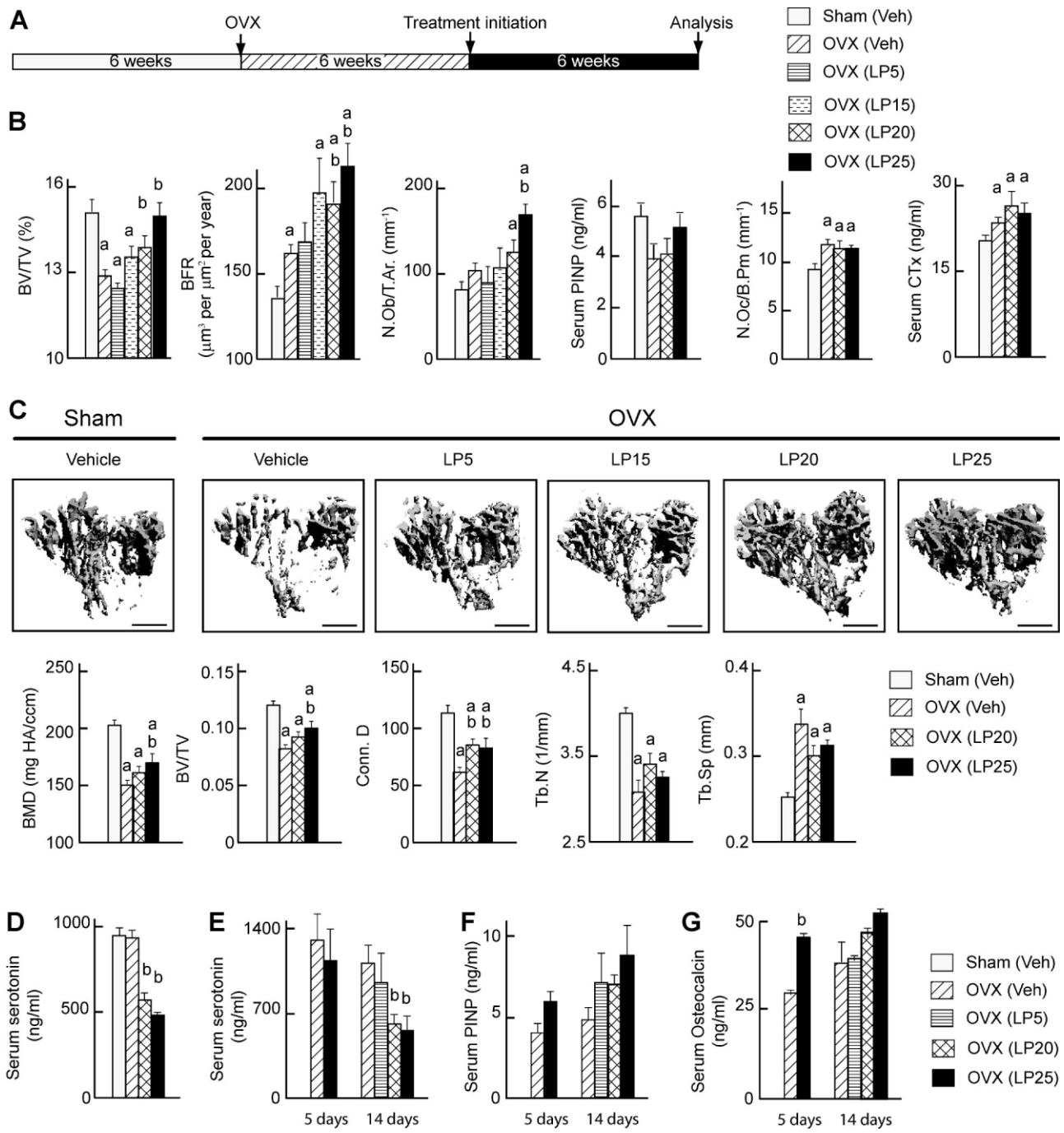

Fig. 1. Determination of a minimal dose of LP533401 reversing bone loss in ovariectomized mice. (*A*) Experimental regimen. Six-week-old mice were sham-operated or ovariectomized (OVX), left untreated for 6 weeks, and then treated orally with vehicle (Veh) or the indicated dose (5, 15, 20, or 25 mg/kg per day) of LP533401 from 6 additional weeks. (*B, C*) Histologic analysis of L_3–L_4 vertebrae, quantification of serum levels of P1NP and CTX (*B*), and μCT analysis of proximal tibia (*C*) at the end of the treatment period ($n > 8$ mice per group). BV/TV = bone volume over total volume; BFR = bone-formation rate; N.Ob/T.Ar = osteoblast number over trabecular area; N.Oc/B.Pm = osteoclast number over bone perimeter. Bars represent 500 μm. (*D, E*) Serum serotonin levels at the end of the 6-week treatment period (*D*) or after 5 or 14 days of treatment (*E*) with LP533401 or vehicle ($n > 5$ mice per group). (*F, G*) Serum P1NP (*F*) and osteocalcin (*G*) levels after 5 or 14 days of treatment with LP533401 or vehicle ($n > 5$ mice per group). Values are shown as means ± SEM. [a]$p < .05$ versus sham (Veh). [b]$p < .05$ versus OVX (Veh).

of lumbar vertebrae as well as μCT analyses of long bones were performed.

As shown in Fig. 1*B*, *C*, whether we looked at vertebrae or long bones, daily doses of LP533401 ranging from 5 to 15 mg/kg did not affect bone-formation parameters to a significant extent. On the other hand, dosages of both 20 and 25 mg/kg per day of LP533401 significantly increased bone-formation parameters in vertebrae, thereby increasing bone mass, as measured by the ratio of bone volume over total volume in vertebrae (BV/TV; Fig. 1*B*). As reported previously, LP533401 did not affect bone-

resorption parameters, adding further credence to the important notion that gut serotonin regulates only bone formation[5] (Fig. 1*B*). Of note, the same effect of these low doses of LP533401 was observed on long bones analyzed by μCT (Fig. 1*C*)

To verify that this bone anabolic effect was secondary to a decrease in serotonin biosynthesis, we measured circulating serotonin levels. As expected, we observed a dose-dependent decrease in circulating serotonin levels (Fig. 1*D*), with both the 20 and 25 mg/kg per day doses of LP533401 decreasing these levels between 40% to 50%. Next, to determine the minimum length of time needed to record an effect of LP533401, we measured serum serotonin levels in mice treated with LP533401 for only 5 or 14 days. Serum serotonin levels were already decreased after 5 days of treatment with 25 mg/kg per day of LP533401, although this did not reach statistical significance (Fig. 1*E*). In contrast, dosages of both 20 and 25 mg/kg per day of LP533401 significantly decreased serum serotonin levels after 2 weeks of treatment (Fig. 1*E*). Accordingly, the serum level of P1NP, a biomarker correlating with bone formation, was increased in a time-dependent manner in mice treated with LP533401 (Fig. 1*E*). These increases were not statistically significant because of a large variability between animals. In addition, the highest dose of 25 mg/kg per day of LP533401 used in this study raised serum osteocalcin levels significantly after only 5 days (Fig. 1*G*). Together these results indicate that a daily dose of 20 to 25 mg/kg of LP533401 is sufficient to efficiently increase bone-formation parameters and to cure an ovariectomy-induced osteoporosis because it quite rapidly decreases circulating serotonin levels by up to 50%.

Inhibiting gut serotonin synthesis rescues the low-bone-mass phenotype caused by *Lrp5* deficiency

It has been shown by others and by our group that the low bone mass observed in *Lrp5*-deficient mice and osteoporosis pseudoglioma patients is associated with an increase in circulating levels of serotonin.[2,4] To test whether this osteopenia could be treated by inhibiting serotonin synthesis in the gut, 6-week-old *Lrp5*-deficient mice were treated for 6 weeks with a daily dose of 25 mg/kg of LP533401 or vehicle (Fig. 2*A*). In a control experiment, we also treated a group of *Lrp5*-deficient mice with lithium chloride (LiCl), a plurispecific compound that was shown previously to increase bone mass in an Lrp5-independent manner.[14–17] Both treatments had a positive effect on the number of osteoblasts and bone-formation rate that resulted in an increase in bone mass, although P1NP serum levels

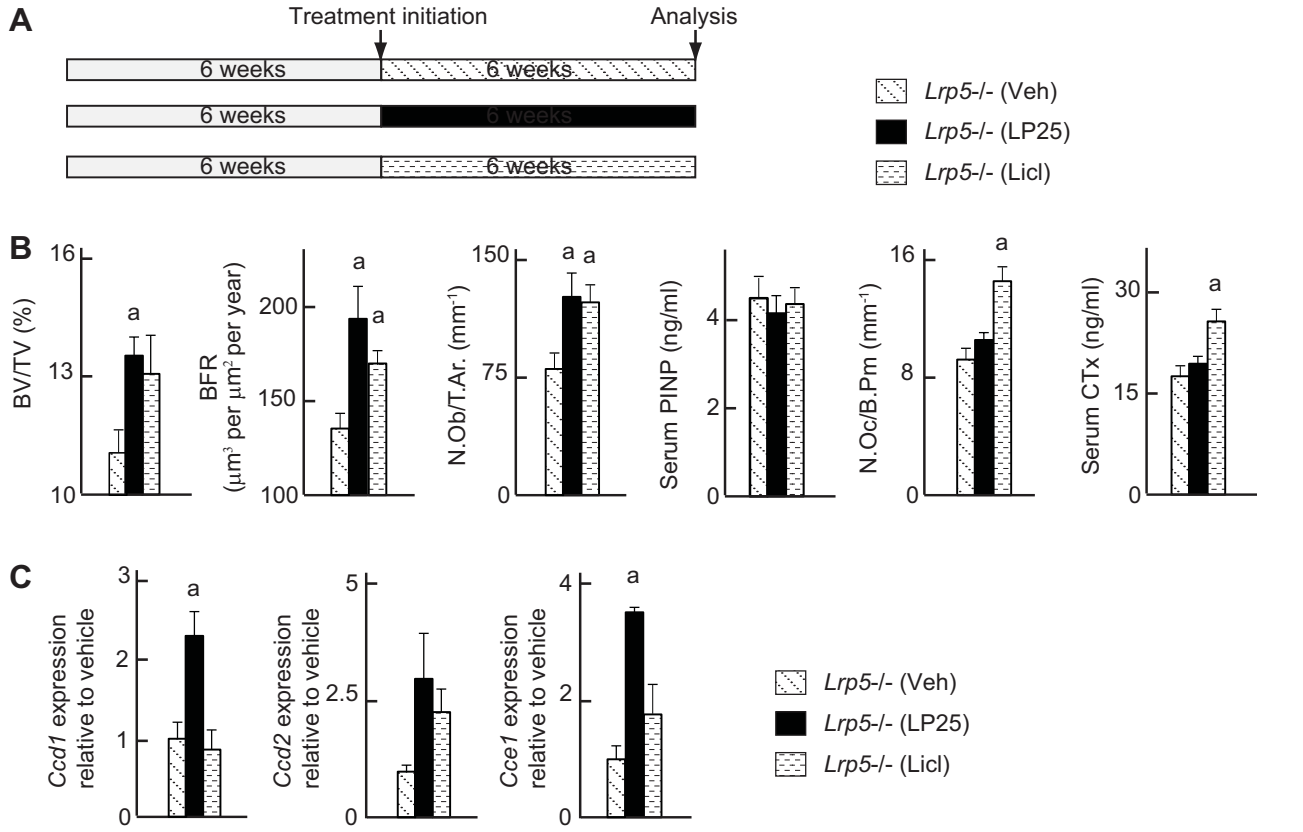

Fig. 2. Tph1 inhibition treats the osteopenia of *Lrp5*-deficient mice. (*A*) Experimental regimen. Six week-old *Lrp5*-deficient mice were treated orally with vehicle (Veh) or LP533401 (25 mg/kg per day) or LiCl (200 mg/kg per day) for 6 weeks. (*B*) Histologic analysis of L_3–L_4 vertebrae and serum levels of P1NP and CTX at the end of the treatment period ($n = 7$ mice per group). BV/TV = bone volume over total volume; BFR = bone-formation rate; N.Ob/T.Ar = osteoblast number over trabecular area; N.Oc/B.Pm = osteoclast number over bone perimeter. Bars represent 500 μm. (*C*) Expression of *cyclin D1* (*Ccd1*), *cyclin D2* (*Ccd2*). and *cyclin E1* (*Cce1*) in long bone quantified by real-time PCR ($n \geq 3$ mice per group). Values are shown as means ± SEM. $^{a}p < .05$ versus vehicle.

were not raised significantly in serum (Fig. 2*B*). The positive effect of LP533401 on osteoblast number, however, was consistent with increased expression in bone of *cyclin D1*, *D2*, and *E1*, the very genes whose expression is decreased in the absence of *Lrp5*,[2] and was at least twice more increased in LP533401-treated than in LiCl-treated *Lrp5*-deficient mice (Fig. 2*C*). We noted that osteoclast numbers and bone resorption, estimated by serum levels of CTX, were increased significantly in LiCl-treated mice, whereas they were unchanged by the LP533401 treatment (Fig. 1*B*). Thus these results suggest that inhibiting serotonin synthesis in the gut is potent enough to treat bone-loss conditions as rapid and severe as the one associated with *Lrp5* deficiency.

Long- versus short-term effect of inhibiting gut serotonin synthesis on the rescue of ovariectomy-induced bone loss

In order to evaluate the potential value of inhibiting gut serotonin synthesis in the treatment of low-bone-mass diseases, two additional questions need to be addressed. The first is to determine whether the beneficial effect of inhibiting gut serotonin synthesis endures, at least partially, at the arrest of treatment. The second is to know whether long-term treatment would be more beneficial than shorter-term treatment.

To address these two questions, mice ovariectomized at 6 weeks of age were divided into three groups. A first group received vehicle for 12 weeks, a second group received LP533401 (25 mg/kg per day) for 6 weeks and then vehicle for the following 6 weeks, whereas a third group received LP533401 (25 mg/kg per day) for 12 weeks (Fig. 3*A*). All mice were euthanized and analyzed at 18 weeks of age. As seen in Fig. 3*B*, *C*, a 6-week treatment with LP533401 induced a significant increase in both osteoblast number and bone-formation rate, and this increase was maintained even 6 weeks after the end of treatment. This anabolic effect was observed both in vertebrae analyzed by histomorphometry and in long bones analyzed by μCT (Fig. 3*B*, *C*). Not surprisingly, a 12-week-long treatment with LP533401 resulted in a more significant increase in osteoblast number, bone-formation rate, and bone volume at all locations analyzed without affecting bone-resorption parameters (Fig. 3*B*). Likewise, serum levels of vitamin D_3, calcium, and phosphate were not affected by the long-term treatment with LP533401, suggesting that at the dose used in this study it does not significantly interfere with intestinal absorption of nutrients (Fig. 3*D*). We also measured levels of circulating serotonin. As expected, serum serotonin concentration was decreased by 40% after 12 weeks of treatment. Importantly, this level was still lower than in vehicle-treated mice 6 weeks after treatment with LP533401 was discontinued (Fig. 3*E*). Taken together, these results indicate that the beneficial effect of inhibiting gut serotonin synthesis is increased with duration of treatment but is still present at least 6 weeks after its interruption.

Pharmacologic inhibition of gut serotonin synthesis cures gonadectomy-induced bone loss in aged mice

Osteoporosis is a disease developing after menopause; therefore, it is a disease of aging.[18] Hence any anabolic treatment of gonadal failure–induced osteoporosis needs to be effective in older mice, which may have a smaller pool of osteoblast progenitor cells.

To determine whether inhibiting gut serotonin synthesis could cure osteoporosis in older mice, we ovariectomized 9-month-old female mice, waited 6 weeks, and then treated them with either vehicle or LP533401 (25 mg/kg per day) for another 6 weeks (Fig. 4*A*). All animals were euthanized and analyzed at 12 months of age. As can be seen in Fig. 4*B*, daily gavage with LP533401 rescued the bone loss caused by gonadectomy in these 12-month-old mice. Again, this rescue was due to an increase in the number of osteoblasts and in the bone-formation rate, whereas osteoclast number and serum CTX were not affected by the treatment with LP533401 (Fig. 4*B*). A μCT analysis demonstrated that the beneficial effects of daily gavage with LP533401 were not restricted to vertebrae because it also could be observed in long bones (Fig. 4*C*). As in young mice, serum serotonin concentrations were decreased by 40% in LP533401-treated older mice (Fig. 4*D*). These experiments indicate that, at least in the mouse, inhibiting the synthesis of gut serotonin can cure, through an anabolic mechanism, gonadal failure–induced bone loss in older animals.

Additive effect of inhibiting gut serotonin synthesis and bone resorption in gonadal failure–induced osteoporosis

A last attribute that an anabolic treatment for osteoporosis ideally should have is that it should be able to act in concert with an inhibitor of bone resorption. To determine if the inhibition of gut serotonin synthesis could fulfill this criterion, mice were ovariectomized at 6 weeks of age and 6 weeks later were treated with either vehicle or LP533401 (25 mg/kg per day, gavage) or alendronate (60 μg/kg per week, intraperitoneally) or both drugs for an additional 6 weeks (Fig. 5*A*). These mice then were analyzed by bone histomorphometry and μCT for bone parameters while circulating serotonin levels were measured.

As expected, either LP533401 or alendronate could increase bone mass in ovariectomized mice, albeit through different cellular mechanisms (Fig. 5*B*, *C*). In mice treated with LP533401, this was achieved by increasing the number of osteoblasts and the bone-formation rate, whereas in mice treated with alendronate, this was achieved by decreasing the number of osteoclasts and bone resorption (Fig. 5*B*). We also observed that alendronate decreased the number of osteoblasts and the bone-formation rate significantly (Fig. 5*B*). Yet mice treated with both LP533401 and alendronate demonstrated a further increase in bone mass in vertebrae compared with mice treated with either drug (Fig. 5*B*). This effect was secondary to a combined increase in bone-formation parameters and a decrease in bone-resorption parameters (Fig. 5*B*). μCT analysis of proximal tibias failed to see an overt advantage of the combination treatment over alendronate alone (Fig. 5*C*). As expected, the alendronate treatment had no effect on the decrease in serum serotonin levels caused by Tph1 inhibition (Fig. 5*D*). These data suggest that the combination of a gut serotonin synthesis inhibitor and an antiresorptive agent could be a viable treatment option for low-bone-mass diseases.

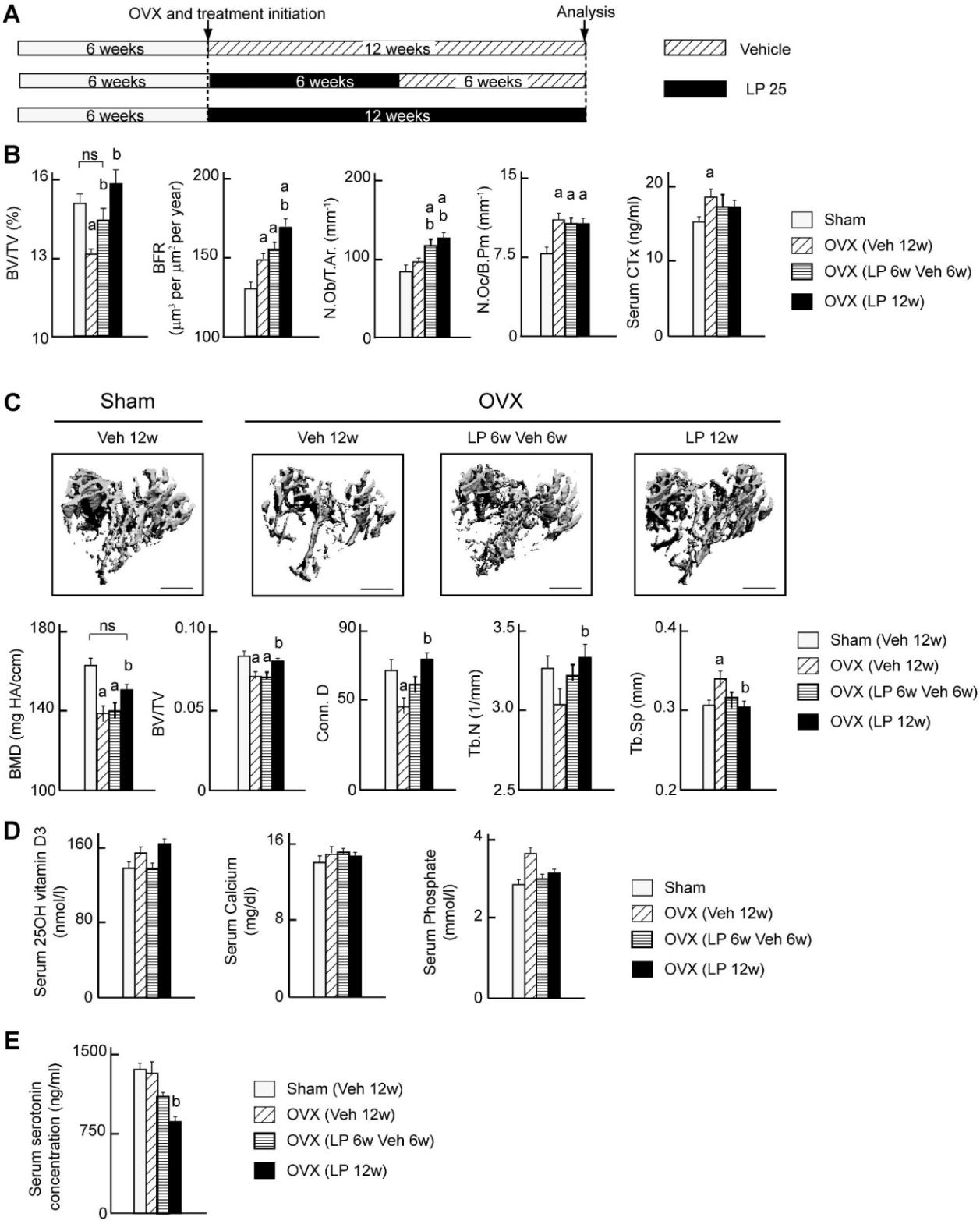

Fig. 3. The bone anabolic effect of Tph1 inhibition benefits from a long-term dosing and persists on treatment interruption. (*A*) Experimental regimen. Six-week-old mice were sham-operated or ovariectomized (OVX) and then treated orally for 12 weeks with either vehicle (Veh) or LP533401 (25 mg/kg per day) or for 6 weeks with LP533401 (25 mg/kg per day) followed by 6 weeks with vehicle. (*B, C*) Histologic analysis of L_3–L_4 vertebrae, quantification of serum levels of CTX (*B*), and μCT analysis of proximal tibia (*C*) at the end of the treatment period ($n \geq 8$ mice per group). BV/TV = bone volume over total volume; BFR = bone-formation rate; N.Ob/T.Ar = osteoblast number over trabecular area; N.Oc/B.Pm = osteoclast number over bone perimeter. Bars represent 500 μm. (*D*) Serum levels of vitamin D_3, calcium, and phosphate at the end of the 12-week treatment period. (*E*) Serum serotonin levels at the end of the 12-week treatment period. Values are shown as means ± SEM. [a]$p < .05$ versus sham (Veh). [b]$p < .05$ versus OVX (Veh).

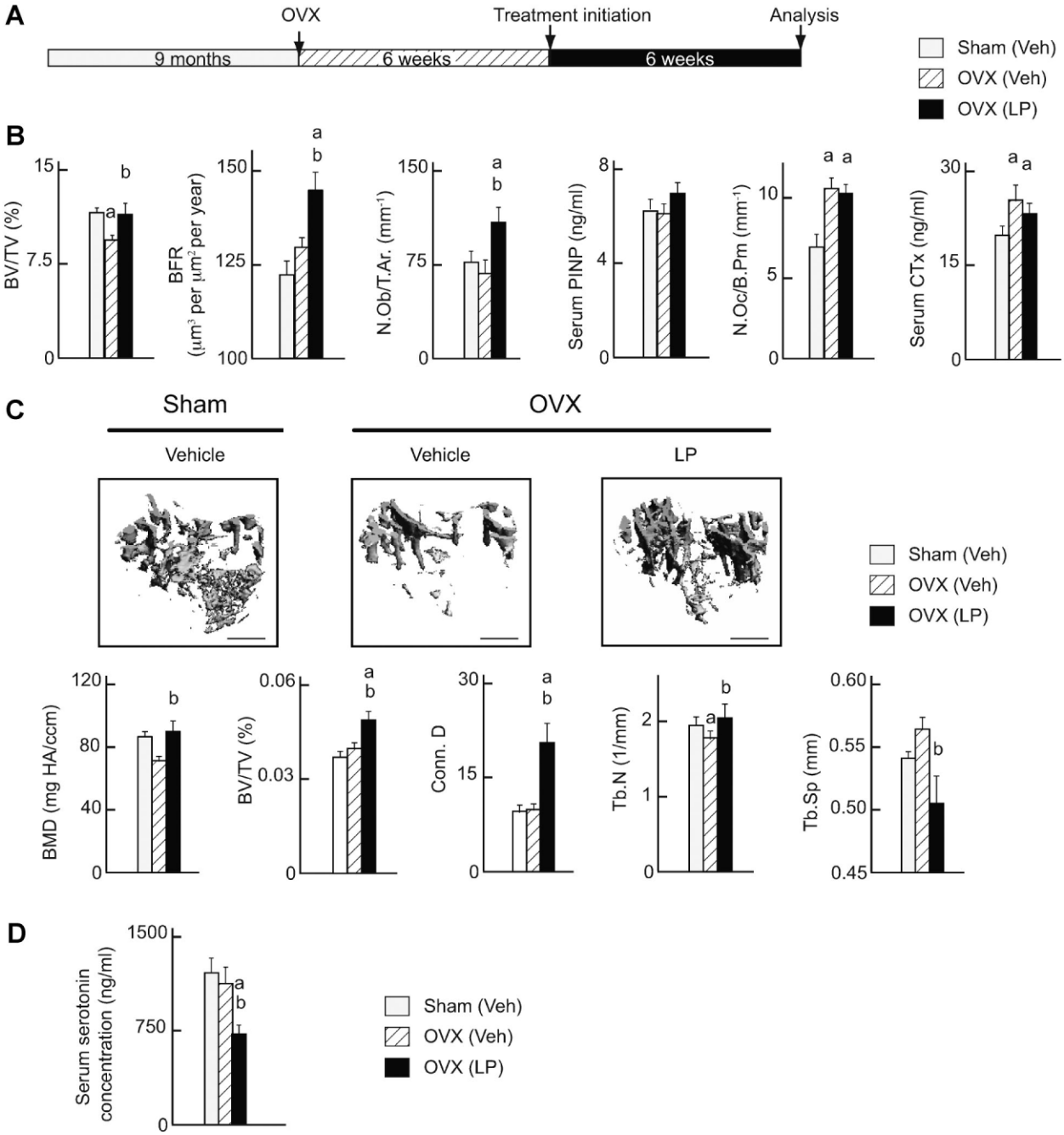

Fig. 4. LP533401 rescues the bone loss caused by ovariectomy in aged mice. (*A*) Experimental regimen. Nine-month-old mice were sham-operated or ovariectomized (OVX), left untreated for 6 weeks, and then treated orally with vehicle (Veh) or 25 mg/kg per day of LP533401 from 6 additional weeks. (*B, C*) Histologic analysis of L_3–L_4 vertebrae, quantification of serum levels of P1NP and CTX (*B*), and μCT analysis of proximal tibia (*C*) at the end of the treatment period ($n > 8$ mice per group). BV/TV = bone volume over total volume; BFR = bone-formation rate; N.Ob/T.Ar = osteoblast number over trabecular area; N.Oc/B.Pm = osteoclast number over bone perimeter. Bars represent 500 μm. (*D*) Serum serotonin levels at the end of the 6-week treatment period ($n \geq 8$ mice per group). Values are shown as means ± SEM. [a]$p < .05$ versus sham (Veh). [b]$p < .05$ versus OVX (Veh).

Discussion

Osteoporosis is a major health concern and a growing burden for health care systems throughout the western hemisphere.[19] The progressive aging of the general population has further propelled the treatment of this disease to the level of a major issue in modern biomedical research. Over the past two decades, antiresorptive drugs, mostly of the bisphosphonate class, have been the treatment of choice for osteoporosis, and to this day, there is still a large effort to develop novel antiresorptive treatments.[20–22] While this therapeutic strategy is successful in preventing bone loss, it does not rebuilt bone architecture; hence a major challenge therefore has been to identify anabolic agents.[21] The identification of gut-derived serotonin as a

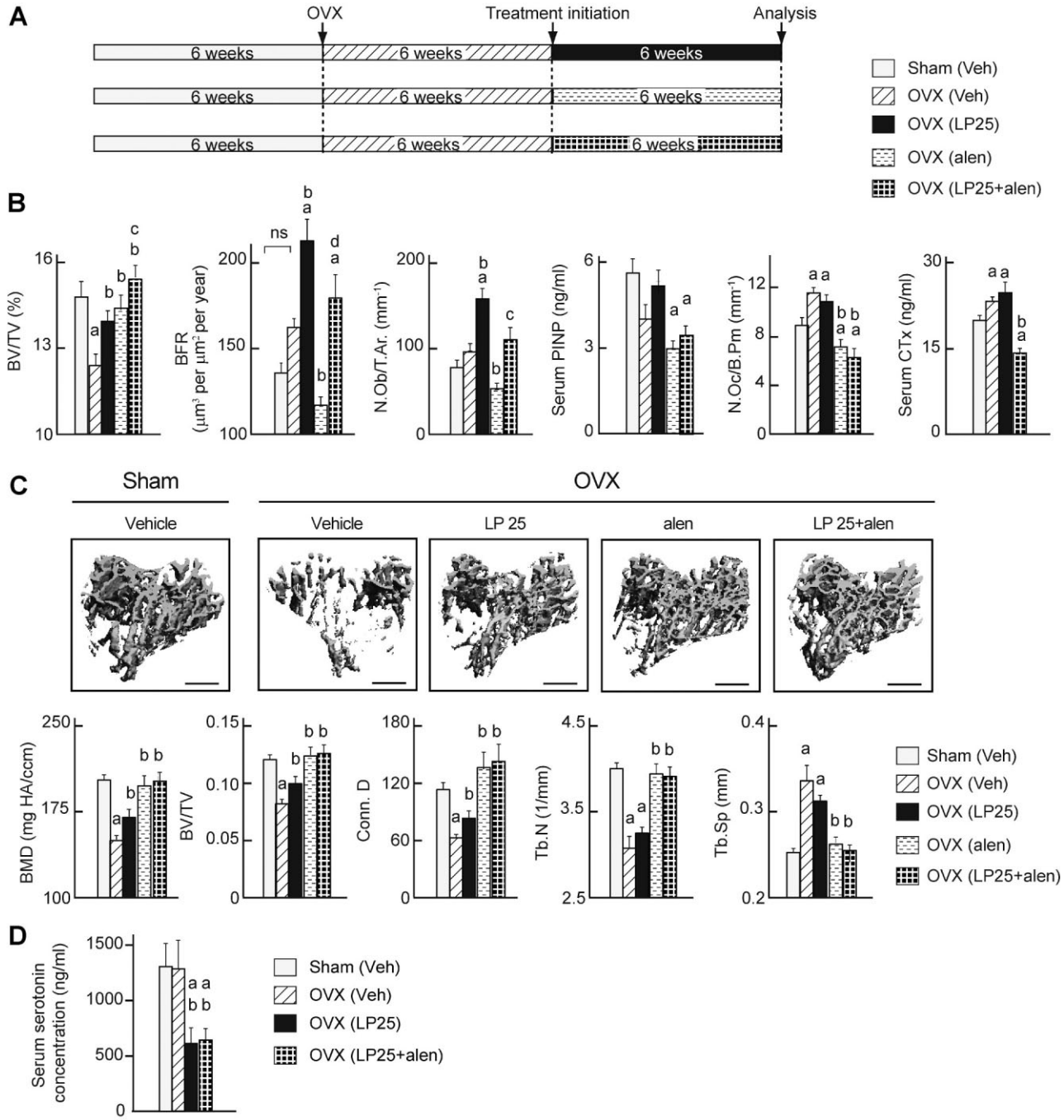

Fig. 5. Combination therapy of LP533401 and alendronate shows additive effect. (*A*) Experimental regimen. Six-week-old mice were sham-operated or ovariectomized (OVX), left untreated for 6 weeks, and then were treated for 6 weeks with vehicle (Veh) or LP533401 (gavage, 25 mg/kg per day) or alendronate (intraperitoneally, 60 μg/kg per day) or both LP533401 (gavage, 25 mg/kg per day) and alendronate (intraperitoneally, 60 μg/kg per day). (*B, C*) Histologic analysis of L_3–L_4 vertebrae, quantification of serum levels of P1NP and CTX (*B*) and μCT analysis of proximal tibia (*C*) at the end of the treatment period ($n \geq 8$ mice per group). BV/TV = bone volume over total volume; BFR = bone-formation rate; N.Ob/T.Ar = osteoblast number over trabecular area; N.Oc/B.Pm = osteoclast number over bone perimeter. Bars represent 500 μm. (*D*) Serum serotonin levels at the end of the 6-week treatment period ($n \geq 8$ mice per group). Values are shown as means ± SEM. [a]$p < .05$ versus sham (Veh). [b]$p < .05$ versus OVX (Veh). [c]$p < .05$ versus OVX (LP25). [d]$p < .05$ versus OVX (alen).

selective inhibitor of bone formation holds great promise that Tph1 inhibitors could represent a novel class of bone anabolic drugs.[2,5] Accordingly, in an initial proof-of-concept study, we showed that such a compound could treat the bone loss associated with gonadal failure in mice and rats.[5] This study extends these initial findings in several directions.

The bone anabolic effect of Tph1 inhibition relies on an increase in osteoblast proliferation,[5] raising the legitimate concern that it could be hindered in conditions where the pool of osteoblast progenitors is decreased. Among those, one can cite aging, whose importance is exemplified by the fact that osteoporosis is more generally a disease of adults over 55 years

of age and, in a smaller population, Lrp5 deficiency, which leads to severe bone loss in mice and humans.[9,23] In both cases, our study shows that Tph1 inhibition remains an efficient bone anabolic therapy. Indeed, whether in 6-week-old or 12-month-old mice, a 6-week treatment with 25 mg/kg per day of LP533401 restored bone volume in ovariectomized mice to the level seen in sham-operated animals. Likewise, despite the fact that *Lrp5*-deficient mice have a very low number of osteoblasts,[2,9] Tph1 inhibition was remarkably effective in increasing this number and thereby bone volume. A previously reported LiCl,[14] a multifunctional molecule,[15–17] also was able to increase bone mass in *Lrp5*-deficient mice. We note that while both molecules could increase BV/TV and osteoblast number equally well, when we looked at expression of the proliferation markers that are affected by *Lrp5* deficiency, that is, the *cyclin D1*, *D2*, and *E* genes, LP533401 was more effective than LiCl for two of them. This observation suggests that LiCl regulates the expression of different proliferation markers than Lrp5 and gut serotonin in osteoblasts. Even more important is the fact that LiCl, but not LP533401, significantly increased bone resorption and osteoclast numbers. An increase in osteoclast number also had been observed previously, although it did not reach statistical significance in this case probably because the mice were treated for a shorter time.[14] From a mechanistic point of view, since *Lrp5* mutations affect only the bone-formation arm of bone remodeling, the proresorptive effect of LiCl is inconsistent with the notion that LiCl would target Lrp5 function exclusively. This aspect of LiCl pharmacology could be explained, however, if one takes into consideration the fact that this molecule, which is used primarily to treat specific mental disorders, inhibits the synthesis of brain serotonin.[15,16] Since brain serotonin decreases bone resorption, this inhibition by LiCl should have a positive effect on this arm of bone remodeling.[24] In contrast to LiCl, the Tph1 inhibitor induced a sole anabolic effect, even when the treatment was extended up to 3 months. In any case, taken at face value and given the severity of the bone loss observed in osteoporosis pseudoglioma, these results suggest that Tph1 inhibition is a therapeutic avenue worth testing in these patients.

How does Tph1 inhibition compare with intermittent injections of parathyroid hormone (PTH), the only anabolic treatment currently approved for osteoporosis? This is a difficult question to address because PTH, but not Tph1 inhibition, has been tested in patients. Our previous comparison of the anabolic effect of Tph1 inhibition and intermittent PTH in ovariectomized rats showed that LP533401 was not less efficient than PTH in correcting BV/TV values both in long bones and vertebrae as well as femur cortical thickness.[5] This being acknowledged, we note that PTH therapy requires daily injections.[25–27] That Tph1 inhibitors are small molecules delivered orally is therefore a potential asset. Further, in a combination of PTH and alendronate, the anabolic efficacy of PTH appeared blunted by the antiresorptive treatment.[27–29] In contrast, the gain of bone mass resulting from inhibiting gut-derived serotonin production is not significantly affected by a concurrent treatment with alendronate, and Tph1 inhibition does not impair the effect of alendronate. We are fully aware that these results have to be interpreted cautiously because they were obtained only in rodents and in a small number of animals. Nevertheless, this result suggests that if confirmed and extended, a dual anabolic/antiresorptive therapy could be possible using Tph1 inhibitors.

Unlike PTH,[27] Tph1 inhibition shows a strict anabolic effect; even after 3 months of treatment with LP533401, CTX serum levels and osteoclast numbers were unchanged. This specific effect on bone formation raises the possibility that the therapeutic window of Tph1 inhibitors could be longer than the one of intermittent PTH. In addition, our data show that serum serotonin levels are still decreased compared with control mice 6 weeks after interrupting the treatment with LP533401, causing the bone-formation rate to remain higher than normal. These latter observations suggest that discontinuing the use of Tph1 inhibitors would not necessarily need to be followed by an antiresorptive treatment.

The potential use of Tph1 inhibitors to treat bone loss disorders is, of course, contingent to clinical investigations showing that they are safe to use in human. Although the long-term safety of this type of compound is not yet known, phase 2 trials have already demonstrated a good tolerance and no dose-dependent toxicity.[30] It is worth highlighting that these clinical trials were performed with doses up to 1000 mg four times a day. According to the mouse/human dose conversion recommended by the US Food and Drug Administration,[31] this dose represents more than 30 times the dose defined by this study as sufficient to treat ovariectomy-induced bone loss. Provided that this anabolic window accurately translates from rodents to humans, these clinical data bear good promise as to the safety of these compounds.

Disclosures

GK is a member of the scientific advisory board of Karos. All the other authors state that they have no conflicts of interest.

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Authors' roles: HI, VKY, and BZ performed the experiments; VKY, PD, and GK designed the experiments; XEG, PD, and GK reviewed the data; PD and GK provided funding and wrote the paper.

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