Effects of Combined Treatment With Ibandronate and Pulsed Electromagnetic Field on Ovariectomy-Induced Osteoporosis in Rats

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Ibandronate (IBN) and pulsed electromagnetic field (PEMF) have each shown positive effects for treating osteoporosis, but no study has evaluated the relative effects of these treatments combined. This study investigated the effects of IBN + PEMF on bone turnover, mineral density, microarchitecture, and biomechanical properties in an ovariectomized (OVX) rat model of osteoporosis. Fifty 3-month-old rats were randomly apportioned to receive a sham-operation (n = 10), or ovariectomy (n = 40). The latter group was equally divided as the model (OVX control) or to receive IBN, PEMF, or IBN + PEMF. Beginning the day after surgery, the IBN and IBN + PEMF groups received weekly subcutaneous IBN; the PEMF and IBN + PEMF groups were given daily PEMF during the same 12 weeks. After 12 weeks of treatments, biochemical parameters, bone mineral density (BMD), microarchitecture parameters, biomechanical properties, and some metabolic modulators that are involved in bone resorption were compared. The L5 lumbar vertebral body BMDs of the IBN, PEMF, and IBN + PEMF groups were 121.6%, 119.5%, and 139.6%; maximum loads were 111.4%, 112.7%, and 121.9%; and energy to failure was 130.8%, 129.2%, and 154.9% of the OVX model, respectively. The IBN + PEMF group had significantly lower levels of serum tartrate-resistant acid phosphatase 5b, and greater improvement in BMD, bone microarchitecture, and strength of the lumbar spine compared with monotherapy groups. Results showed that IBN + PEMF had a more favorable effect on the lumbar spine in this osteoporosis model than did either monotherapy. Bioelectromagnetics. © 2016 Wiley Periodicals, Inc.

Keywords: pulsed electromagnetic field; ibandronate; combination treatment; osteoporosis; bone mass

INTRODUCTION

Osteoporosis is characterized by low bone mass and impairment of bone microarchitecture that results in skeletal fragility and increased susceptibility to fracture [Jeremiah et al., 2015]. Ibandronate (IBN), a nitrogen-containing bisphosphonate, has been a research target for oral and intravenous osteoporotic therapies for more than a decade. Bauss et al. [2002] showed that IBN treatment resulted in a dose-dependent increase in bone mineral density (BMD) in a rat model of osteoporosis. More preclinical studies using various animal models of osteoporosis indicated that IBN was associated with reduced bone turnover, increased BMD, and bone quality. A prospective clinical study in 2004 [Chesnut et al., 2004] of osteoporotic women supported the use of oral IBN to reduce the risk of vertebral fracture. Srividhya et al. [2015] showed that IBN was associated with a significantly greater increase in BMD compared with conventional hormone therapy in postmenopausal osteoporotic women.

Another promising and noninvasive treatment for osteoporosis is pulsed electromagnetic field (PEMF)
therapy. In various animal models of osteoporosis, PEMF was associated with higher BMD, bone strength, and less deterioration of bone microarchitecture [Jing et al., 2010, 2011, 2013, 2014]. Clinical studies with osteoporotic patients have also indicated that PEMF can increase BMD, alleviate pain, and improve quality of life without side effects [Liu et al., 2013a,b]. In our previous studies with rats, PEMF appeared to have positive effects in ovariectomy-induced osteoporosis [Zhou et al., 2012a, 2013] and streptozotocin-induced diabetic osteopenia [Zhou et al., 2015].

Some studies using ovariectomized (OVX) rats have indicated that certain drug combinations are associated with greater BMD and bone strength, relative to monotherapy [Sakai et al., 2012; Sugimoto et al., 2013]. However, to the best of our knowledge, no study has investigated a combination of drug and physiotherapy in OVX-induced osteoporosis. In particular, while the positive effects of either IBN alone or PEMF alone on osteoporotic bone have been documented, information is lacking regarding the effects of combining IBN and PEMF in rats with ovariectomy-induced osteoporosis.

In the present study, we investigated the effects of combined IBN and PEMF on BMD, bone microarchitecture, and bone strength in OVX rats. Furthermore, since the receptor activator of nuclear factor kappa-B/RANK ligand/osteoprotegerin (RANK/RANKL/OPG axis) has a key putative role in bone remodeling and disorders of mineral metabolism [Vega et al., 2007], we also investigated the effects of these therapies on mRNA expressions of OPG and RANKL. This study was approved by the Ethics Committee of the First Affiliated Hospital of University of South China.

MATERIALS AND METHODS

All the procedures used in the animal studies were approved by the Ethics Committee at First Affiliated Hospital of University of South China (Hengyang, China) (reference no. 2014037), and were performed in accordance with ethics criteria contained in the bylaws of the committee.

Animals

Fifty 3-month-old female Sprague-Dawley rats were obtained from the Experimental Animal Center of the University of South China (Hengyang, China), and acclimatized for 1 week. Animals were housed in stainless-steel wire cages under standard laboratory conditions (12 h light/dark cycle, relative humidity of 55 ± 5%, 24 ± 2 °C) with free access to water and food. After 1 week of acclimatization, all rats were subjected to either bilateral ovariectomy or sham surgery, as we previously described [Zhou et al., 2012a,b, 2013, 2014].

Experimental Design

The rats were randomly divided into groups using SPSS version 18.0 statistical software (SPSS, Chicago, IL). Fifty 3-month-old rats were randomly apportioned to receive a sham-operation (n = 10) or ovariectomy (n = 40). The latter OVX model rats were randomly divided to receive IBN, PEMF, or IBN + PEMF. Beginning on the day after surgery, rats in the IBN group were treated with subcutaneous IBN (7 mg/kg, 1×/week for 12 weeks) [Yang et al., 2013]. Rats in the PEMF group were exposed to PEMF (whole body; 3.8 mT, 8 Hz, 40 min/day, 5 days/week for 12 weeks) as described below, and received isotonic sodium chloride solution. Rats in the IBN + PEMF group received both IBN and PEMF treatments simultaneously for the 12 weeks. Rats in the sham-operated and OVX model groups received isotonic sodium chloride and sham PEMF. Sham PEMF was administered as in the PEMF group, but without activating the “on” switch. After the 12-week intervention, all rats were killed by cervical dislocation.

Harvest of Tissues

All rats were killed by cervical dislocation 12 weeks after surgery. Blood samples were collected for biochemical analysis. The L3, L4, L5 vertebral bodies, femurs, and tibias were excised, and cleaned to remove soft tissues. The L5 vertebral bodies and right femurs were used for measurement of BMD and bone mechanical properties. The L4 lumbar vertebral bodies and proximal right tibias were used for analysis of microarchitecture parameters. The left femurs, left tibias, and L3 vertebral bodies were used for RT-PCR analysis.

Ovariectomy Surgery

All rats were anesthetized with 10% chloral hydrate by intraperitoneal injection. The sham operation involved exposure of ovaries and extraction of surrounding fatty tissue of the bilateral ovaries, leaving the ovaries intact, whereas ovariectomy involved full removal of bilateral ovaries.

PEMF Treatment

PEMF treatments were applied as previously described [Zhou et al., 2015]. Briefly, five rats were exposed (whole body) at the same time while in a single cage. PEMF was generated by the PEMF stimulation apparatus (Hunan Forever Elegance Technology, Loudi, China) with a frequency of 8 Hz and
an intensity of 3.82 mT. The waveform of the PEMF consisted of a pulsed burst (burst on, 25 ms; burst off, 100 ms; pulse on, 0.2 ms; pulse off, 0.05 ms) repeated at 8 Hz (Fig. 1).

**Determining Bone Resorption and Formation**

Serum levels of tartrate-resistant acid phosphatase 5b (TRACP5b) and bone-specific alkaline phosphatase (BALP) evaluated bone resorption and bone formation, respectively, using a commercial enzyme-linked immunosorbent assay (ELISA) system (Beijing Bioss Biological Technology, Beijing, China) as previously described [Zhou et al., 2015].

**Measurements of BMD**

Measurements of BMD were performed as previously described [Zhou et al., 2013]. Briefly, rats were killed by cervical dislocation, and L5 vertebral bodies and right femurs were uniformly immersed in water, 2 cm deep. These were routinely measured using dual-energy X-ray absorptiometry (DEXA) (Lunar, Madison, WI) with software for small animal research. After measuring BMD, the bone specimens were stored at $-20^\circ\text{C}$ until used for subsequent biomechanical examination.

**Micro-Computed Tomography (CT) Imaging**

To determine the effects of treatments on the trabecular microarchitecture of the L4 lumbar vertebral body and proximal right tibia, these tissues were scanned using a ZKKS micro-CT scanner (Guangzhou Zhongke Kaisheng Medical Technology, Guangzhou, China) as described previously [Zhou et al., 2015]. A 3-mm-thick volume of interest was selected 1 mm below the proximal growth plate, and the entire L4 vertebral body was selected for microarchitecture analysis. Parameters of the ZKKS micro-CT scanner included a tube voltage of 50 kV, tube current of 0.1 mA, slice thickness of 15 $\mu$m, and pixel size of 15 $\mu$m. Trabecular bone volume ratio (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) were examined.

**Bone Mechanical Properties**

To evaluate bone strength, the compression test for the L5 lumbar vertebral body and a 3-point bending test for the right femur were performed, using an AG-IS biomechanical testing system (Shimadzu, Kyoto, Japan) in accordance with our previous description [Zhou et al., 2012b]. Briefly, bone specimens were thawed gradually by warming to room temperature overnight. The load was applied at a speed of 2 mm/min until a fracture occurred. Maximum load and energy to failure were recorded and analyzed.

**Determination of OPG and RANKL mRNA Expression**

The left femur, left tibia, and L3 vertebral body were removed under aseptic conditions. After removal of the surrounding muscle and connective tissue, the marrow cavity was exposed and flushed with sterile PBS. Total RNA was extracted from the bones of each group, and each was crushed under liquid nitrogen using Trizol (Invitrogen, Carlsbad, CA). The total RNA was reverse-transcribed with a first-strand cDNA synthesis kit (MBI Fermentas, Vilnius, Lithuania), and real-time PCR was carried out in an FTC-2000 Real-Time PCR machine (Funglyn, Toronto, Canada) in accordance with our previous description [Zhou et al., 2013]. The primer sequences and expected RT-PCR products were: OPG (forward 5'-GTCCCTTGGCCCTGACTACTCT-3', reverse 5'-GACATCTTTTGCAACTGCTGTCT-3', product length, 190 bp), RANKL (forward 5'-GAGCGAAGACAGAACAGACT-3', reverse 5'-ACGAACCTTCCATCATAGCTG-3', product length, 137 bp), and $\beta$-actin (ACTB; forward 5'-GCCAACACGTGTGTCT-3', reverse 5'-AGGAGCAATGATCTTGATCTT-3', product length, 114 bp).

The OPG mRNA and RANKL mRNA were each normalized to ACTB mRNA. Data were analyzed with the $2^{-\Delta\DeltaCT}$ method.

**Statistical Analysis**

All data are presented as the mean ± standard deviation for each group. Statistical comparisons with results of multiple groups were analyzed using one-way ANOVA followed by Tukey’s post hoc test. Statistical comparisons were performed using SPSS Bioelectromagnetics
version 18.0 statistical software (SPSS, Chicago, IL). Differences between groups were considered significant at $P \leq 0.05$.

RESULTS

Serum TRACP5b and BALP

Serum TRACP5b levels were significantly higher in the OVX model group compared with the sham-operated group (Fig. 2). The IBN and PEMF groups had significantly lower levels of TRACP5b compared with that of the OVX model, and the IBN + PEMF group had significantly lower levels of TRACP5b compared with either the IBN or PEMF monotherapy groups. Serum levels of BALP of the OVX model were significantly higher than that of the sham-operated group (Fig. 2). The serum levels of BALP in the IBN, PEMF, and IBN + PEMF groups were statistically similar.

BMD

The BMDs of the L5 vertebral body or the right femur were significantly lower in the OVX model group compared with the sham-operated group (Fig. 3). However, the BMDs of the IBN, PEMF, and IBN + PEMF groups were 121.6%, 119.5%, and 139.6% of the OVX model, respectively, which is significantly higher for each group. Similarly, the BMDs of the right femur of the IBN, PEMF, and IBN + PEMF groups were 112.4%, 109.7%, and 113.5% that of the OVX model. In addition, the BMD of the L5 vertebral body of the IBN + PEMF group was significantly higher than that of either the IBN or PEMF groups ($P < 0.05$, both). The BMDs of the femurs of the IBN, PEMF, and IBN + PEMF groups were statistically similar.

Micro-CT Imaging and Analysis

Representative micro-CT images and microarchitecture parameters of the L4 vertebral body and proximal tibia are shown in Figure 4. In both the L4 vertebral bodies and tibias, the BV/TV, Tb.N, and Tb.Th of the OVX model were significantly lower compared with the sham-operated group, while Tb.Sp was significantly higher. Specifically with respect to the L4 vertebral bodies, in the IBN, PEMF, and IBN + PEMF groups, BV/TV was 122.0%, 116.4%, and 138.5%, respectively; Tb.N was 120.1%, 116.4%, and 132.2%; Tb.Th was 111.2%, 109.1%, and 113.0%; and Tb.Sp was 83.8%, 90.5%, and 79.8% of the OVX model. Concerning both L4 vertebral bodies and tibias, in the IBN, PEMF, and IBN + PEMF groups BV/TV, Tb.N, and Tb.Th were significantly higher compared with that of the OVX model, while Tb.Sp was significantly lower. In the combined IBN + PEMF group, in the L4 vertebral bodies BV/TV and Tb.N were significantly higher than that of either of the monotherapy groups, while Tb.Th and Tb.Sp were similar. However, in the tibias of the IBN, PEMF, and IBN + PEMF groups, all these parameters were similar.

Bone Mechanical Properties of the L5 Vertebral Body and Femur

The biomechanical compression test showed that the maximum load and energy to failure of the L5 vertebral body of the OVX model was significantly less than that of the sham-operated group ($P < 0.01$, both; Fig. 5). In the IBN, PEMF, and IBN + PEMF
groups the maximum loads were 111.4%, 112.7%, and 121.9%, respectively, of the OVX model, and energy to failure was 130.8%, 129.2%, and 154.9% of the OVX model. In the L5 vertebral bodies, the IBN + PEMF group had significantly higher maximum load and energy to failure values than did either of the monotherapy groups ($P < 0.01$, all). Similarly, the 3-point bending test showed that ovariectomy was associated with significantly less maximum load and energy to failure of the femur. In the femur, all treatments were associated with a significant preventive effect relative to the OVX model, but the maximum load and energy to failure were statistically similar.

**mRNA Expression for OPG and RANKL**

Compared with the sham-operated group, the normalized OPG mRNA expressions of the left femurs, tibias, and L3 vertebral bodies of the OVX model were significantly lower, while RANKL mRNA expressions were significantly higher (Fig. 6). In all these tissues, in all the treatment groups the OPG mRNA expressions were significantly higher compared with that of the OVX model, while RANKL mRNA expressions were lower. In the IBN + PEMF group, in the L3 vertebral body the OPG mRNA expression was significantly higher than that of either of the monotherapy groups.

**DISCUSSION**

In this study, we investigated the effects of combined IBN and PEMF on bone turnover, BMD, bone microarchitecture, and biomechanical properties in OVX rats. Results showed that the combination treatment had a more favorable effect on the lumbar spine in ovariecotmy-induced osteoporosis than did either monotherapy. However, combination therapy did not show greater beneficial effects on the femur or tibia.

Although a meta-analysis suggested that electromagnetic stimulation did not have a significant influence on delayed unions or non-united long-bone fractures, the study was limited by methodological factors and high between-study heterogeneity, leaving the question undecided [Mollon et al., 2008]. Another study showed that PEMF with 5-ms long bursts of 20 asymmetric biphasic magnetic field pulses (2 mT, 3.8 kHz), with bursts repeating at 15 Hz, had no effect on continuing substantial disuse bone loss [Spadaro et al., 2011]. However, there is evidence that PEMF provides real and meaningful benefits for osteoporosis patients; PEMF was found to increase BMD and improve balance in patients with osteoporosis [Liu et al., 2013]. Contradictory results of PEMF on osteoporosis may due to different stimulus parameters. Evidence has shown that there were obvious biological windows of stimulus parameters of PEMF therapy, identified by frequency, intensity, or their combinations, on various diseases [Markov, 2007]. Our research group has compared the effects of PEMF with different parameters (intensity: 0.77, 3.82, 9.87 mT; frequencies: 2, 8, 16 Hz; exposure time: 20, 40, 60 min) on OVX rats. We found the best stimulus parameters for treating OVX-induced osteoporosis in

**Fig. 3. Effects on bone mineral density (BMD).** BMD were determined using dual energy X-ray absorptiometry (DEXA) after 12 weeks of treatment with ibandronate (IBN) and pulsed electromagnetic field (PEMF), either alone or in combination (COM), in ovariectomized (OVX) rats. Data are expressed as mean ± SD ($n = 10$); $^aP < 0.01$ cf. Sham group; $^bP < 0.01$ cf. OVX group; $^cP < 0.05$ cf. OVX group; $^dP < 0.05$ cf. COM group.
rats: intensity of magnetism of 3.82 mT, field frequency of 8 Hz for 40 min/day [He et al., 2007; Huang et al., 2008; Yang et al., 2008]. So we selected the stimulus parameters in the present study. In the present study, PEMF showed positive effects on BMD, microarchitecture parameters, and biomechanical properties, which is consistent with our previous studies [Zhou et al., 2012a, 2013].

Maintenance of bone mass is attributed to a balance between osteoclastic bone resorption and osteoblastic bone formation [Martin et al., 2009]. Thus, biochemical markers of bone turnover can be used to evaluate changes in bone resorption and formation. In the present study, because TRACP5b is an indicator of osteoclast activity [Ominsky et al., 2008] and BALP reflects osteoblast activity [Han et al., 2009], these were measured by ELISA. Evidence showed that serum TRACP5b [Li et al., 2009] and BALP activities in OVX model rats were higher than in sham-operated rats. This is consistent with previous studies [Han et al., 2009].

In the present study, significantly higher serum levels of TRACP5b and BALP in OVX model rats indicated that ovariectomy led to higher bone turnover. We also found that in each of the IBN, PEMF, and IBN + PEMF groups, serum TRACP5b was significantly lower than that of the OVX model and similar to control values. This suggests that all the treatments inhibited osteoclast function and bone resorption. In addition, the IBN + PEMF group had significantly lower levels of TRACP5b compared with either the IBN or PEMF monotherapy groups. Treatment with IBN and PEMF, either as monotherapies or in combination, was not associated with a change in serum BALP relative to the OVX model. This suggests that none of the treatments affected

**Fig. 4.** Effects on trabecular microarchitecture of lumbar spine and proximal tibia. Trabecular microarchitecture parameters were determined using Micro-CT after 12 weeks of treatment with ibandronate (IBN) and pulsed electromagnetic field (PEMF), either alone or in combination (COM), in ovariectomized (OVX) rats. Data are expressed as mean ± SD (n = 10), *P < 0.01 cf. Sham group; *P < 0.01 cf. OVX group; *P < 0.05 cf. OVX group; *P < 0.05 cf. COM group; *P < 0.01 cf. COM group.
osteoblast function or bone formation. These results indicate that combination therapy with IBN and PEMF suppressed bone turnover more so than either of the monotherapies.

Importantly, in the current study BALP did not fall below levels of the sham-operated group, and therefore the suppressive effect of the combined treatment on bone turnover was not excessive. We speculate that suppression of bone turnover, which was enhanced by the combination therapy, may contribute to additive effects of different therapies. Evidence has shown that over-suppression of bone turnover can lead to increased risk of atypical bone fractures [Odvina et al., 2005; Lenart et al., 2008]. In addition, combination therapy with anti-resorptive drugs can inhibit bone formation to levels that are below normal, harming bone quality [Visekruna et al., 2008]. Although serum markers for bone metabolism were investigated in the present study, we did not conduct histomorphometry double labeling with tetracycline (a bone-seeking fluorochrome) [Frost, 1969] to directly evaluate bone resorption and formation rate. This is a limitation of the study.

BMD measurement using DEXA is a standard tool in the diagnosis of osteoporosis, used to evaluate the degree of osteoporosis and drug efficacy [Kanis et al., 1994]. Additionally, BMD is considered a valid intermediate end point for judging the efficacy of fracture risk reduction [Bonnick and Shulman, 2006]. Consequently, therapies for osteoporosis are often assessed by BMD. The present study found that either IBN or PEMF monotherapy was associated with significantly higher BMD in the L5 vertebral body and femur of ovariectomized rats, which is in accord with previous studies [Bauss et al., 2002; Shahnazari et al., 2010; Zhou et al., 2012a, 2013]. Furthermore, the associated effects of IBN + PEMF on the BMD of the femur were not significantly different from that of...
either monotherapy. These results indicate that effects of combination therapy on the BMD of the femur were not additive. On the other hand, the BMD of the lumbar spine in the combination group was higher than that of either monotherapy group. These results suggest that, compared with monotherapy, combination therapy has a greater beneficial effect on the BMD of the lumbar spine.

BMD is a primary determinant of bone mechanical strength [Friedman, 2006], but microarchitecture, bone turnover, and other factors are also important [Teitelbaum, 2000; Seeman and Delmas, 2006; Seeman, 2008]. Trabecular bone microarchitecture correlates with bone mechanical strength [Ulrich et al., 1999; van der Linden et al., 2001]. Therefore, in the present study we investigated morphological changes in the trabecular bone. The micro-CT analysis of the trabecular bone of the tibia revealed that the ability of IBN + PEMF to increase BV/TV, Tb.Th, and Tb.N and decrease Tb.Sp were not significantly different from that of either monotherapy. This indicates that IBN + PEMF did not have a synergistic effect on the trabecular bone microarchitecture of the tibia. The micro-CT analysis of the trabecular bone of the lumbar spine revealed that IBN + PEMF was associated with an increase in BV/TV and Tb.N of the lumbar spine that exceeded that of either monotherapy. This suggests that the combination of IBN and PEMF may have a synergistic effect on bone microarchitecture in the lumbar spine.

The biomechanical quality of bone is an important factor that reflects the degree of bone fragility and fracture risk [Turner and Burr, 1993; Hernandez and Keaveny, 2006]. Biomechanical parameters can thus be used to evaluate osteoporotic bone. In the present study, biomechanical examination showed that ovariectomy was associated with deterioration in bone strength of the lumbar spine and femur. Monotherapy with IBN or PEMF was associated with higher maximum load and energy to failure of the lumbar spine and the femur, and these improvements were greater in the IBN + PEMF group for the lumbar spine. These results indicate that the combination treatment had a synergistic effect on bone mechanical properties of the lumbar spine. These effects on bone mechanical strength of the lumbar spine occurred coincidently with greater improvement in BMD and bone microarchitecture compared with either monotherapy. Therefore, the synergistic effect of combination treatment on bone strength in the lumbar spine of ovariectomized rats is at least partly attributable to improvement in BMD and bone microarchitecture.

However, these synergistic effects of the combination treatment were not demonstrated by bone strength of the femur and trabecular bone microarchitecture of the tibia. The tibia and femur have lower amounts of trabecular bone compared with the lumbar spine. Previous studies have shown that the extent of cortical and trabecular bone loss in ovariectomized rats differs; the procedure tended to cause

![Fig. 6. Effects on mRNA expressions of OPG and RANKL. Related mRNA expressions were evaluated by real-time PCR after 12 weeks of treatment with ibandronate (IBN) and pulsed electromagnetic field (PEMF), either alone or in combination (COM), in ovariectomized (OVX) rats. Data are expressed as mean ± SD (n = 10), aP < 0.01 cf. Sham group; bP < 0.05 cf. OVX group; cP < 0.01 cf. OVX group; dP < 0.05 cf. COM group.](image-url)
preferential loss of trabecular bone [Ominskey et al., 2008]. Bone loss rates of these different sites may, at least in part, account for the site-specific discrepancy in the effects of combination treatment on bone strength and trabecular bone microarchitecture.

Evidence has shown that the RANK/RANKL/OPG signaling pathway has a key role in osteoclastogenesis and osteoclast function [Boyce and Xing, 2008]. The binding of RANKL to RANK is essential for the formation, activation, and survival of osteoclasts [Burgess et al., 1999], whereas OPG inhibits the RANKL/RANK pathway via competition with RANKL [Simonet et al., 1997]. Greater expression of RANKL in bone microenvironment results in high bone resorption, while higher expression of OPG suppresses bone resorption. In the present study, in all the treatment groups OPG mRNA expression was significantly higher, and RANKL mRNA expression was lower, in the left femurs, tibias, and L3 vertebral bodies compared with the OVX model group. In addition, OPG mRNA expression in the L3 vertebral body in the IBN þ PEMF group was significantly higher than that of the IBN or PEMF groups. This occurred concurrently with greater improvement in BMD, bone microarchitecture, and bone strength of the L5 vertebral body in the IBN þ PEMF group. Therefore, the more favorable effect of the combination treatment on BMD, bone microarchitecture, and bone strength in the lumbar vertebral body may be, at least partly, attributable to the increase of OPG mRNA expression.

There are several limitations in the present study. First, the mechanisms underlying the synergistic efficacy of IBN þ PEMF are not fully understood, and further research is needed. Secondly, although osteoporosis can be successfully established using 3-month-old rats (mature but young) [Sehmisch et al., 2009; Yang et al., 2011; Zhou et al., 2012a,b, 2013, 2014], growth-dependent effects may have influenced the results.

In conclusion, the combined treatment with IBN and PEMF showed synergistic effects on BMD, bone microarchitecture, and mechanical strength of the lumbar spine. This may be attributable to the suppression of bone turnover without inhibition of bone formation. The results of this in vivo study warrant consideration of a combination treatment with IBN and PEMF in clinical applications for post-menopausal osteoporosis.

REFERENCES


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