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The effect of switching from teriparatide to anti-RANKL antibody on cancellous and cortical bone in ovariectomized mice

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ABSTRACT

We examined the effect of teriparatide, and switching from teriparatide to anti-RANKL (receptor activator of nuclear factor κB ligand) monoclonal antibody, in ovariectomized mice. Twelve-week-old female C57BL/6 mice were ovariectomized or sham operated. Four weeks after surgery, ovariectomized mice were subjected to one of the following four treatments: phosphate-buffered saline (PBS) for 8 weeks; teriparatide for 4 weeks followed by PBS for 4 weeks (PTH4W group); teriparatide for 8 weeks (PTH8W group); or teriparatide for 4 weeks followed by anti-RANKL antibody (single subcutaneous injection of 5 mg/kg) (SWITCH group). Twelve weeks after the operation, bone mineral density was increased in PTH8W and SWITCH groups to broadly comparable levels, but these were significantly decreased in the PTH4W group after discontinuation of teriparatide. Histomorphometric analysis demonstrated that cancellous bone formation and resorption were profoundly suppressed in the SWITCH group. Bone formation was also suppressed on the endocortical surface of cortical bone but was maintained on the periosteal surface. Anti-RANKL antibody suppressed osteoclast activity immediately after treatment, while bone formation was only gradually decreased. These results suggest that anti-RANKL antibody may be a therapeutic option after discontinuation of teriparatide therapy.

1. Introduction

Skeletal homeostasis maintains the balance of bone formation by osteoblasts and bone resorption by osteoclasts [1,2]. This balance is disrupted in osteoporosis and bone resorption becomes dominant, increasing the risk of fragility fracture [3,4]. In addition to conventional therapeutic drugs, such as selective estrogen receptor modulators and nitrogen-containing bisphosphonates, more potent agents have recently become available, such as teriparatide, a recombinant human parathyroid hormone (PTH)(1-34) [5–7], and denosumab, a human anti-receptor activator of nuclear factor κB ligand (RANKL) antibody [8,9].

Intermittent administration of PTH(1-84) or teriparatide reportedly stimulates bone formation, increases bone mineral density (BMD) and prevents fragility fractures [5,7,10], although the underlying mechanisms remain unclear. Although teriparatide is a potent anti-osteoporosis agent, the treatment period is limited to 24 months, and the effect is diminished after discontinuation of treatment [11,12]. It has been reported that bone loss after discontinuing teriparatide can be prevented by anti-resorptive agents such as bisphosphonates [11,13]. Denosumab is an excellent candidate for continuation therapy after teriparatide. Administration of 60 mg denosumab every 6 months for 3 years significantly increased BMD and decreased fracture risk in postmenopausal women with osteoporosis in the FREEDOM study [14]. The BMD reinforcement effect afforded by denosumab is maintained for at least 8 years [15]. As for combination and switching therapies, it was recently reported that BMD increased more in patients treated with teriparatide and denosumab combination therapy than those treated with teriparatide or denosumab alone [16,17]. In addition, BMD continued to increase in postmenopausal women with osteoporosis who switched from teriparatide monotherapy to denosumab [18]. We previously investigated the effect of teriparatide, anti-RANKL antibody, and teriparatide and anti-RANKL antibody combination therapy on ovariectomized mice, and found that combination therapy had additive effects on BMD compared with monotherapy [19].

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In this study, we examined the effect of switching from teriparatide to anti-RANKL antibody in ovariectomized mice. Switching from teriparatide to anti-RANKL antibody markedly increased cancellous and cortical BMD. Although bone formation and resorption were strongly suppressed in cancellous bone and the endocortical surface of cortical bone, bone formation was maintained on the periosteal surface of cortical bone after anti-RANKL antibody treatment. In addition, a rapid reduction in bone resorption due to suppression of osteoclast activity was observed immediately after anti-RANKL antibody administration, while significant reductions in bone formation were only observed later. This temporal discrepancy may contribute to the potent therapeutic effects of anti-RANKL antibody.

2. Materials and methods

2.1. Reagents and animals

Anti-mouse monoclonal RANKL antibody (OYC1) was purchased from Orient Yeast Co. (Tokyo, Japan). Teriparatide was obtained from Sigma (Tokyo, Japan). Twelve-week-old virgin female C57BL/6 N mice were purchased from Sankyo Labo Service Co. (Tokyo, Japan). All mice were housed under specific pathogen-free conditions and exposed to a 12-h light, 12-h dark cycle and treated humanely, as directed by the Animal Care and Use Committee of The University of Tokyo.

2.2. Treatment protocols

A schematic of the study design is shown in Fig. 1. 30 mice were assigned to five equal groups. Four groups were ovariectomized (OVX) and one group was sham operated (SHAM group). Four weeks after surgery, mice in the OVX groups were further subdivided into the following groups according to their subsequent treatment: phosphate-buffered saline (PBS) for 8 weeks (OVX group); teriparatide (80 μg/kg/day) for 4 weeks followed by PBS for 4 weeks (PTH4W group); teriparatide for 8 weeks (PTH8W group); or teriparatide for 4 weeks followed by anti-RANKL antibody (single injection of 5 mg/kg) (SWITCH group). The frequency and dose of teriparatide are equivalent to once a week in humans, and the frequency and dose of anti-RANKL antibody are equivalent to once every half a year in humans. All mice were euthanized 12 weeks after surgery and subjected to BMD and histomorphometric analysis. The BMD in the femur and lumbar spine was measured using a bone mineral analyzer (PIXImus Densitometer; GE Medical Systems, Waukesha, WI).

We also examined the early effects of anti-RANKL antibody in 28 12-week-old female C57BL/6 mice treated with anti-RANKL antibody 4 weeks after ovariectomy (single injection of 5 mg/kg). We sacrificed them 1, 2, 3, 4, 5 and 6 days after treatment (Days 1, 2, 3, 4, 5 and 6). A control group of 4 mice sacrificed before injection was included (Day 0). Hind limbs and lumbar spines were subjected to histological and histomorphometric analysis.

2.3. Histomorphometric analysis

Histomorphometric measurements of cancellous bone were made at ×400 magnification in the secondary spongiosa area of the proximal tibia and lumbar spine. Cortical bone measurements were performed in the femoral diaphysis. For the analysis of bone mineralization, mice were injected subcutaneously with 20 mg/kg tetracycline hydrochloride on Day 5 and 16 mg/kg calcein on Day 2 before sacrifice.

2.4. Electron microscopy analysis

Following fixation, left tibiae were dissected free of soft tissues and immersed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde solution for 24 h at 4 °C for transmission electron microscopic (TEM) observation. The specimens were decalcified with 5% disodium ethylenediaminetetraacetic acid solution, post-fixed with osmium tetroxide, dehydrated through a graded series of acetone and embedded in epoxy resin (Epon 812, Taab, Berkshire, UK). Ultra-thin sections cut with an ultramicrotome (Sorvall MT-5000; Ivan Sorvall, Inc., Norwalk, CT), and stained with uranyl acetate and lead citrate. These specimens were observed under TEM (Hitachi H-7100, Hitachi Co., Tokyo, Japan) at 80 kV.

2.5. Statistical analysis

Each series of experiments was repeated at least six times. The results are expressed as the mean ± standard deviation. Statistical analyses were performed using analysis of variance (ANOVA) and the Tukey honest significant differences post-hoc test. P values < 0.05 were considered statistically significant. All analyses were undertaken using JMP®12 (SAS Institute Inc., Cary, NC, USA).

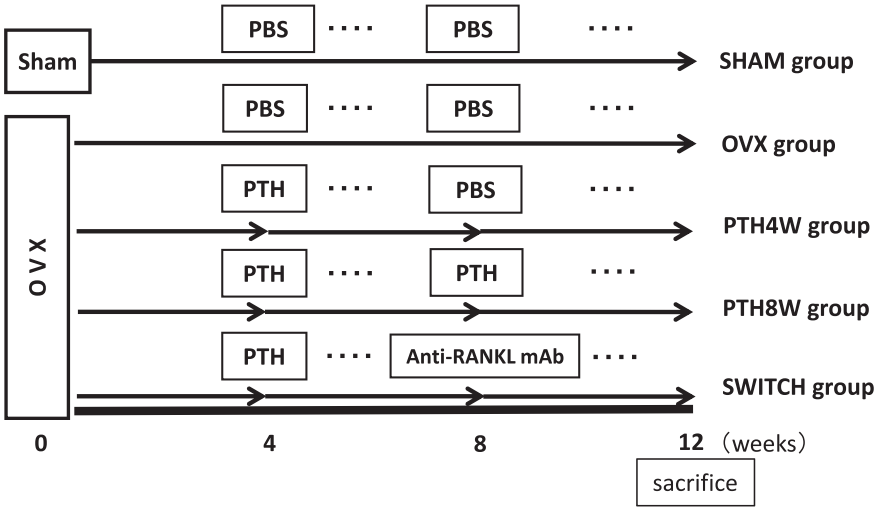

Fig. 1. Experimental protocol. Mice were divided into five groups ($n = 6$). Four groups were ovariectomized, and one group was sham-operated. Four weeks after surgery, ovariectomized mice were treated with either phosphate-buffered saline (PBS) for 8 weeks (OVX group), teriparatide 80 μg/kg/day subcutaneously, three times per week for 4 weeks followed by PBS for 4 weeks (PTH4W group), teriparatide 80 μg/kg/day subcutaneously, three times per week for 8 weeks (PTH8W group), or teriparatide for 4 weeks followed by subcutaneous anti-RANKL antibody (5 mg/kg) (SWITCH group). All mice were sacrificed 8 weeks after surgery.