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Treatment with the combination of ibandronate plus eldecalcitol has a synergistic effect on inhibition of bone resorption without suppressing bone formation in ovariectomized rats

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ABSTRACT

Bisphosphonates are widely used in the treatment of osteoporosis and contribute to the reduction of bone fractures. Ibandronate (IBN) is a highly potent, nitrogen-containing bisphosphonate, which is administered orally or intravenously at extended dosing intervals. Vitamin D or active vitamin D_3 derivatives are also used in the treatment of osteoporosis, and are often used in combination with other drugs. In this study, we investigated the effect of treatment with the combination of once-monthly s.c. dosing of IBN plus once-daily oral eldecalcitol (ELD), an active vitamin D_3 derivative, using aged ovariectomized (OVX) rats. Treatment was started the day after OVX, and analyses were performed 4, 8, and 12 weeks thereafter by determination of bone markers, bone mineral density, biomechanical properties, and histomorphometry. The combination treatment showed a synergistic effect in increasing both lumbar and femoral BMD, and resulted in a significant increase in bone ultimate load. The combination of IBN plus ELD acted synergistically to reduce bone resorption, whereas bone formation did not decrease any more than with monotherapy with either IBN or ELD. Bone formation independent of bone resorption (a process known as 'minimodeling') was not changed in vehicle treated OVX rats despite the increase in bone turnover. ELD upregulated minimodeling, which was however not diminished in the combination treatment. In conclusion, treatment with the combination of IBN plus ELD was beneficial in the treatment of osteoporosis in aged OVX rats. It exhibited a synergistic inhibitory effect on bone resorption and keeps bone formation at the level of sham controls. This uncoupling of bone resorption/bone formation was affected, to some extent, by minimodeling-based bone formation which is independent of bone resorption. This combination regimen which showed synergistic effect on BMD and bone ultimate load without inhibition of bone formation may be beneficial in long-term osteoporosis treatment to prevent bone fractures.

1. Introduction

Ibandronate (IBN) is a highly potent inhibitor of bone resorption which was selected from among 300 bisphosphonate molecules [1]. IBN inhibits farnesyl diphosphate synthase (an enzyme correlated with efficacy of bone resorption) in osteoclasts more efficiently than alendronate, another widely used bisphosphonate [2]. IBN can be administered at extended between-dose intervals both intravenously and orally, and is widely used in the treatment of osteoporosis [3–5]. IBN exhibits its anti-osteoporotic effect in a dose-dependent manner [6], and the efficacy of treatment is related to the total dose of IBN, independent of the treatment regimen [7].

Abbreviations: IBN, ibandronate; ELD, eldecalcitol; OVX, ovariectomized; MCT, medium-chain triglyceride; OCN, osteocalcin; DPD, deoxypyridinorine; BV/TV, bone volume; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; Tb.N, trabecular number; Oc.S/BS, osteoclast surface; ES/BS, eroded surface; N.Oc/BS, osteoclast number; Ob.S/BS, osteoblast surface; N.Ob/BS, osteoblast number; MS/BS, mineralizing surface; OS/BS, osteoid surface; BFR/BS, bone formation rate; MAR, mineral apposition rate; Ac.f, activation frequency; N.Ml./BS, minimodeling number; Ml.BS/BS, minimodeling surface; Ml.BV/TV, minimodeling volume.

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Eldecalcitol (ELD) is an active vitamin D_3 derivative used for the treatment of osteoporosis in Japan [8]. Active vitamin D_3 derivatives such as ELD are frequently used in osteoporosis treatment in combination with other anti-osteoporotic drugs, not only because of their effect in correcting the uptake of calcium from the intestine but also because of their effect in reducing the incidence of bone fractures. However, even under appropriate treatment, many patients with osteoporosis nevertheless experience bone fractures, necessitating the establishment of a more effective regimen.

In this study, we investigated the effect of a combination treatment with IBN plus ELD on BMD, bone ultimate load, and bone turnover using aged ovariectomized (OVX) rats. We also attempted to explain a phenomenon of the uncoupling of bone formation/bone resorption that was observed in the combination treatment by focusing on minimodeling, which is defined as bone formation independent of bone resorption [9,10].

2. Materials and methods

2.1. Animal experiments

Female Wistar–Imamichi rats were purchased from the Institute for Animal Reproduction (Ibaraki, Japan) and sham-operated or ovariectomized (OVX) when 8 months old to make a model for the study of estrogen deficiency. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Chugai Pharmaceutical Co., Ltd. Animals were allowed free access to feed (CE-2; CLEA Japan, Tokyo, Japan) in stainless steel feeders. Drinking water was provided to the animals *ad libitum via* an automatic watering system. Glass water bottles fitted with sipper tubes were used during urine collection.

2.1.1. A dose-finding experiment

From the day after surgery, OVX rats were treated with IBN (subcutaneously, once every 4 weeks, 3 times in total; 1, 3, 10, and 30 μg/kg) for 12 weeks. Isotonic sodium chloride solution (Otsuka Pharmaceutical Factory, Tokushima, Japan) was used as the vehicle. At 12 weeks after treatment, 24-h urine and serum from jugular vein blood were collected. Lumbar vertebrae and femurs were collected at the end of the experiment.

2.1.2. A combined therapy experiment

From the day after surgery, OVX rats were treated with either IBN (subcutaneously, once every 4 weeks, 3 times in total; 3 μg/kg), ELD (orally, once daily, 15 ng/kg), or their combination for 12 weeks. Isotonic sodium chloride solution was used as the vehicle for IBN and medium-chain triglyceride (MCT) (The Nisshin OilliO Group, Tokyo, Japan) was used as the vehicle for ELD. Every 4 weeks, 24-h urine and serum from jugular vein blood were collected. Seven days prior to necropsy, 20 mg/kg tetracycline (Sigma-Aldrich, MO, USA) was subcutaneously administered; 2 days prior to necropsy, 10 mg/kg calcein (Dojindo Laboratories, Kumamoto, Japan) was subcutaneously administered. Lumbar vertebrae and femurs were collected at the end of the experiment.

2.2. Serum and urine analysis

Calcium and phosphorus in serum and creatinine (Cr) in urine were measured using an automatic analyzer (Clinical Analyzer Model 7180; Hitachi High-Technologies Corporation, Tokyo, Japan). Serum osteocalcin (OCN) and urine deoxypyridinoline (DPD) were measured by ELISA (OCN: GE Healthcare Japan, Tokyo, Japan; DPD: DS Pharma Biomedical, Osaka, Japan). The concentration of urinary DPD was normalized to the concentration of urinary creatinine (Cr).

2.3. Bone mineral density measurement

Bone mineral density (BMD) of the second to fourth lumbar vertebrae (L2–L4) and right femur was determined by dual-energy X-ray absorptiometry (DCS-600EX-IIIR; Hitachi Aloka Medical, Tokyo, Japan). After dual-energy X-ray images were obtained, the long axis of the femur was divided into 10 parts numbered F1 to F10 from the proximal to distal ends, then BMD of the whole (F1–F10), proximal (F1–F3), middle (F4–F7), and distal (F8–F10) femur was calculated.

2.4. Bone mechanical property measurement

To assess the bone mechanical properties of trabecular and cortical bone, a compression test [11] for the L5 lumbar vertebra and a 3-point bending test [12] for the middle femur were performed. The vertebral arch and disk were removed from each L5 lumbar vertebra and vertebral bodies were trimmed to a length of 5 mm on the vertical axis. The trimmed vertebral bodies were placed in a bone strength tester (TK-252C; Muromachi Kikai, Tokyo, Japan), and compressive strength was measured by pressure at a speed of 2.5 mm/min. The 3-point bending test of the femur was performed by the same tester. The midpoint of the left femur was placed on a holding device, and the supports were located 12 mm apart. The bending force was calculated at a speed of 20 mm/min until fractures occurred. From the load–displacement curve, the ultimate load (N), stiffness (N/mm), and energy (mJ) were obtained.

2.5. Bone histomorphometry analysis

Bone histomorphometry analysis was performed as previously reported [13]. Briefly, the third lumbar vertebral body (L3) and right femur were fixed in 70% ethanol and stained according to the method of Villanueva [14]. After dehydration with ethanol and acetone, the samples were embedded in methyl methacrylate (Wako Pure Chemical Industries, Osaka, Japan). Midsagittal sections of L3, 5 μm thick, were obtained with a microtome (Supercut 2050; Reichert-Jung, Heidelberg, Germany). The cancellous bone within 1.7 mm from the growth plates was excluded from the measurements. The image obtained under a fluorescence microscope was recorded with a digital camera and the primary histomorphometric parameters were measured with an image analyzing system (Cosmozone 1SA; Nikon, Tokyo, Japan). Nomenclature, symbols, and units used in this study are those described in the Report of the American Society for Bone and Mineral Research Nomenclature Committee [15]. New formed bones on smooth cement lines were defined as minimodeling.

2.6. Statistical analysis

All data are presented as means ± standard deviation (SD). Statistical analysis was performed by analysis of variance (ANOVA) on the SAS statistical analysis software package (SAS institute Inc., Cary, NC, USA). Statistical differences between Sham and Vehicle groups were analyzed by unpaired t-test. In the dose-finding test, analysis of the sham group *vs.* other groups was performed by Dunnett's multiple comparison test. In the combination treatment test, analyses between the Vehicle group and all other groups were analyzed by Tukey's multiple comparison test. A value of $p < 0.05$ was considered significant for all statistical analyses.

3. Results

3.1. A dose-finding experiment

Prior to researching the IBN plus ELD combination treatment, we performed the dose-finding experiment of IBN in OVX rats. Urinary DPD, a bone resorption marker, and serum OCN, a bone formation