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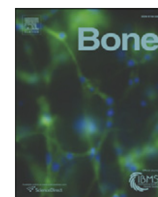

Original Full Length Article

Effects of minodronate on cortical bone response to mechanical loading in rats

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ABSTRACT

The effects of BPs on bone formation during mechanical loading are still unknown. In this study, we evaluated the effect of minodronate on the cortical bone response to mechanical loading applied using a 4-point bending device. We used six-month old female Wistar rats and randomized into five groups ($N=10$/group): Vehicle administration (VEH), low dose minodronate administration (MIN-L, 0.01 mg/kg BW), middle dose minodronate administration (MIN-M, 0.1 mg/kg BW), high-dose minodronate administration (MIN-H 1 mg/kg BW), and very high-dose minodronate administration (MIN-VH, 10 mg/kg BW). Minodronate or vehicle was administered orally using the feeding needle at a dosage 3 times/week for 3 weeks. Loads on the right tibia at 38 N for 36 cycles at 2Hz were applied in vivo by 4-point bending on the same day for 3 weeks. After calcein double labeling the rats were sacrificed and tibial cross sections were prepared from the region with maximal bending at the central diaphysis. Histomorphometry was performed at the entire periosteal and endocortical surface of the tibiae, dividing the periosteum into lateral and medial surfaces. The formation surface was reduced significantly in MIN-H and MIN-VH groups at the medial surface, and in MIN-VH group at the endocortical surface of the loaded tibia ($p<0.01$ vs. VEH). The mineral appositional rate was reduced significantly in MIN-H and MIN-VH groups at the endocortical surface of the loaded tibia ($p<0.01$ vs. VEH). The bone formation rate was significantly reduced in MIN-H group at the medial surface, and in MIN-H and MIN-VH groups at the endocortical surface of the loaded tibia ($p<0.01$ vs. VEH). However, no significant differences were observed in any parameters between the VEH group and either the MIN-L or MIN-M groups for both the loaded and non-loaded tibiae. Based on previous preventive studies in OVX rats, the optimal dose of minodronate for the treatment of osteoporosis would be 0.03 mg/kg (0.21 mg/kg/week). Therefore, we used 0.1 mg/kg of minodronate 3 times/week (0.30 mg/kg/week) that was close to 0.21 mg/kg/week. In conclusion, minodronate does not reduce the cortical bone response to mechanical loading at the optimal dose for the treatment of osteoporosis in rat model.

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Introduction

Bisphosphonates (BPs) are the most widely used drugs in the treatment of bone diseases associated with increased bone resorption, such as osteoporosis. BPs suppress bone resorption, normalize bone turnover, increase bone mineral density (BMD), and prevent fragility fracture [1–4]. Minodronate, a third-generation BP with an imidazopyridine ring side chain, is currently marketed in Japan for the treatment of osteoporosis [5,6]. Preclinical studies have shown that minodronate is at least 10 times more potent than alendronate in inhibiting bone resorption in vivo and in vitro [7], and possesses intermediate mineral-binding affinity [8]. Minodronate has been experimentally demonstrated to have anti-bone resorption activity that is more than 10,000 times stronger than that of etidronate and 10–100 times stronger than that of alendronate [9]. The nitrogen-containing bisphosphonates (N-BPs) inhibit bone resorption by preventing protein prenylation in osteoclasts [10,11], owing to inhibition of farnesyl pyrophosphate synthase, an enzyme in the mevalonate pathway [12]. Among the N-BPs, minodronate most potently inhibits bone resorption by osteoclasts [7,13,14]. On the other hand, the mineral binding affinity of minodronate is equivalent to that of risedronate, and is less than those of alendronate and zoledronate [15].

A phase III trial conducted to examine the effect of daily oral doses of 1 mg minodronate in women with postmenopausal osteoporosis showed total increases in BMD of 5.9%/year at the lumbar spine and 3.5%/year at the femur [5]. After 2 years of treatment, the risk of vertebral fractures was 24.0% in the placebo group compared with 10.4% in the minodronate (1.1 mg/day) group, a statistically significant reduction. Relative risk of vertebral fracture following minodronate treatment was 0.41, [6]. In addition, its usefulness has also been demonstrated in a 3-year extension study [16].

N-BPs affect bone formation by inhibiting osteoclastic bone resorption, thereby reducing activation frequency. In a study employing

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fracture healing models, N-BPs suppressed remodeling of the callus, resulting in a high content of woven bone with low quantity of lamellar bone, but bone strength was maintained or increased [17]. It was also reported that compared to control, alendronate suppressed the rate of bone formation by 44%, a statistically significant amount, at 4 weeks during stress fracture repair [18]. It was hypothesized that the suppression of bone formation was caused by a decrease in osteoblast activity during BP treatment, as indicated by the lower mineral apposition rate. However, it was not clear whether BP directly affected bone formation.

It was previously demonstrated that exercise can be useful in treating osteoporosis [19,20]. This is due to mechanical stress caused by loading, which activates modeling and suppresses remodeling as a result of promoting bone formation [21]. N-BPs are first-line pharmacologic treatments for osteoporosis, and are often used in patients undergoing exercise therapy. However, the effects of BPs on bone formation during mechanical loading are still unknown. It was clinically demonstrated that compared to BP treatment alone, the combination of BP treatment and exercise led to less of a decrease in BMD in steroid-induced osteoporosis [22]. While a few reports have discussed the ways in which BPs affect bone formation clinically and experimentally, there is still no unified view on these topics [23–25].

In this study, we evaluated the effect of minodronate on the cortical bone response to mechanical loading applied using a 4-point bending device.

Materials and methods

Animals

Six-month female Wistar rats (retired breeder; Shimizu Laboratory Supply, Kyoto, Japan) with body weights (BW) ranging between 255 g and 310 g were used in this experiment. Our procedures were approved by the Committee on Laboratory Animals, Faculty of Medicine, Tottori University. During the experimental period, tap water and commercially available food (CE-2; CLEA Japan, Tokyo, Japan; calcium content 1.18 g/100 g, phosphorus content 1.09 g/100 g, vitamin D3 content 250 IU/100 g) were given ad libitum. The duration of daily light exposure in the breeding room was 12 h (7:00 AM to 7:00 PM), and room temperature was maintained at 24 °C.

After a 7-day acclimation period, rats were randomized into 5 groups based on minodronate dose ($N = 10$ per group), each with the same mean body weight: (1) vehicle administration (VEH), (2) low dose (MIN-L), (3) middle dose (MIN-M), (4) high dose (MIN-H), and (5) very high dose (MIN-VH). Rats were allowed normal cage activity between loading sessions.

Minodronate administration

Minodronate (ONO-5920/YM529; chemical name [1-hydroxy-2-(imidazo [1,2-*a*]pyridin-3-yl)-ethylidene]-bisphosphonic acid monohydrate) was provided by Astellas Pharmaceutical (Tokyo, Japan). This agent was jointly developed by Ono Pharmaceutical (Osaka, Japan) and Astellas Pharmaceutical. We prepared a 0.01–10 mg/kg/5 ml solution of minodronate by dissolving minodronate in a solution of 0.01 N sodium hydroxide and distilled water and diluting it with 2% (weight/volume) methylcellulose. Minodronate or vehicle was administered orally via feeding needle 3 times/week for 3 weeks. The rats received vehicle alone (VEH, controls), or minodronate at doses of 0.01 mg/kg (MIN-L), 0.1 mg/kg (MIN-M), or 1 mg/kg (MIN-H), or 10 mg/kg (MIN-VH). After administration of minodronate or vehicle, tibial mechanical loading was performed on the same day. One rat in the MIN-VH and MIN-M groups and 2 rats in the MIN-L group died during this phase of the experiment.

In vivo external mechanical loading

In vivo mechanical loading involved load application using a 4-point bending device (developed and assembled in the Biomechanics Laboratory, Creighton University) [26,27]. Each rat was anesthetized with ether, and its right lower leg was placed between the pads of the device. The right (loaded) tibia was loaded at 38 N for 36 cycles at 2 Hz, 3 days/week for 3 weeks, for a total of 9 days. The left (non-loaded) tibia was not loaded.

The force applied during loading was monitored by a strain gauge attached to the lever arm as reported previously [26–29]. Before the experiment, the 4-point bending device was calibrated with a load cell. This load cell had previously been calibrated by applying forces ranging from 0 to 70 N using a mechanical testing machine (MTS810; MTS, Minneapolis, MN, USA). The actual applied load during in vivo 4-point bending was calculated based on this calibration [26–29].

Bone histology

Rats received calcein injections (6 mg/kg BW, i.p.) on experimental days 13 and 19. On day 20, rats in all 5 groups were anesthetized with 50 mg/kg BW ketamine hydrochloride and 10 mg/kg BW xylazine, and were sacrificed by exsanguination. Both loaded and non-loaded tibiae were removed, placed in 10% phosphate-buffered formalin for 24 h, and then transferred to 70% ethanol. The tibiae were cut into 3 pieces: (a) the proximal 1 cm; (b) the distal 5 mm; and (c) the remaining central diaphysis. Central regions were stained with Villanueva bone stain for 72 h[27,30]. Specimens were dehydrated with ascending concentrations of ethanol and acetone and then embedded in methyl methacrylate. The region of maximum bending was located in the central diaphysis, 3–13 mm proximal to the tibiofibular junction (TFJ) [26–30]. Two cross-sections were prepared from the region of maximum bending, specifically 4 mm and 4.5 mm proximal to the TFJ. These cross-sections were then ground to a thickness of 60 μm and mounted on glass slides. Histomorphometric data were collected from these 2 sections and mean values were calculated.

Calculation of in vivo strain

The in vivo strain was calculated using the moment of inertia of each central diaphyseal cross-section. The outline of the cortical bone on each slide was traced, and the moment of inertia and section modulus for each cross-section were calculated using Bone Histomorphometry Software (System Supply, Nagano, Japan). The peak compressive strain on the lateral surface was calculated using beam-bending theory [31] as:

$$\mathrm{Ec} = MC/EI \quad (1)$$

where Ec is the calculated peak compressive strain on the lateral periosteal surface, M is the bending moment (N m), E is the longitudinal Young's modulus (estimated as 29×10^9 N/m^2), I is the moment of inertia, and C is the distance from the centroid to the surface.

The in vivo peak compressive strain (Ep) at the lateral periosteal surface was then predicted from Ec using the following formula:

$$\mathrm{Ep} = 0.828 \times \mathrm{Ec} - 127.16[26] \quad (2)$$

Eq. (2) was derived from the in vivo strain gauge measurement [26]. The calculated strain (Ec) derived using beam-bending theory (tibial cross-sectional properties) was highly correlated with the in vivo strain (Ep) measured directly on the lateral surface during 4-point bending ($R^2 = 5\ 0.87$) [26]. Therefore, Eq. (2) accurately predicts the in vivo strain at the lateral (periosteal) surface of the rat tibia.

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