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Original article

Combined treatment with minodronate and vitamin C increases bone mineral density and strength in vitamin C-deficient rats

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Abstract

Objectives: Reduced bone quality caused by vitamin C deficiency in older persons may lead to incidental fragility fractures during bisphosphonate treatment, although bisphosphonate increases bone mineral density (BMD). This study aimed to evaluate the effects of minodronate and ascorbic acid (Aa) on BMD, bone quality, and bone strength in Aa-deficient osteogenic disorder Shionogi (ODS) rats.
Methods: Six-month-old ODS rats were divided into four groups (n = 20 per group): (1) Aa supplementation (Aa^+); (2) Aa-deficient (Aa^-); (3) Aa supplementation and minodronate administration (Aa^+ + Mino); and (4) Aa-deficient and minodronate administration (Aa^- + Mino). BMD, bone strength, bone histomorphometry, and bone quality determined using Fourier transform infrared spectroscopy imaging (FTIRI) were evaluated after 4 and 8 weeks.
Results: BMD was significantly higher in the Aa^+ + Mino group than in the Aa^- group ($p < 0.05$). Bone strength was significantly higher in the Aa^+ and Aa^+ + Mino groups than in the Aa^- group ($p < 0.05$). Furthermore, bone strength was significantly higher in the Aa^+ + Mino group than in the Aa^- + Mino group ($p < 0.05$). Minodronate treatment irrespective of Aa supplementation significantly decreased bone resorption compared with the Aa^+ and Aa^- groups ($p < 0.05$). No significant differences in the parameters evaluated by FTIRI were observed between the groups.
Conclusions: Aa supplementation improved bone strength in ODS rats. Combined treatment with minodronate and Aa, but not minodronate alone, improved bone strength and increased BMD. Aa is required for bone health because it is essential for osteoblast differentiation.

Keywords: Bone mineral density; Bone strength; Minodronate; Vitamin C

1. Introduction

Fragility fractures in older people are related to the impairment of their quality of life or reduced life expectancy and comprise an important clinical issue in aging societies. Prevention of fragility fractures is a very important clinical topic. Regarding the prevention of fragility fractures, oral administration of nitrogen-containing bisphosphonates reduced the relative risk of vertebral fractures from 36% to 49%, and that of nonvertebral fractures from 16% to 40% by increasing bone mineral density (BMD) in postmenopausal women [1–5]. Among nitrogen-containing bisphosphonates, minodronate was at least 10 times more potent than alendronate in inhibiting bone resorption by preventing the function of osteoclasts in vitro [6,7] and by increasing BMD in ovariectomized rats in vivo [8]. Minodronate clinically reduced the vertebral fracture risk by 59% in postmenopausal women over 2 years [9]. However, although BMD is increased with minodronate therapy, minodronate cannot completely prevent new vertebral or nonvertebral fractures, particularly in aged patients [9].

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One possible cause of fragility fractures associated with bisphosphonate treatment is the impairment of bone quality. Bone quality is a broad term encompassing the factors affecting the structural and material properties of bone. Material properties are explained by the mineral/matrix ratio, crystallite maturity and size, collagen cross-links, and microdamage.

Ascorbic acid (Aa; vitamin C) has been gaining attention as a candidate for determining the material properties. Previous in vitro studies have shown that Aa is essential for osteoblast differentiation in animals and humans [10,11]. Aa is an indispensable coenzyme for the hydroxylation of lysine and proline residues that are necessary for collagen triple-helix formation, and the presence of collagen triple helices is essential for the promotion of osteoblast differentiation [12–15]. Aa also modulates the expression of transcription factors such as osterix, which are critical for osteoblast differentiation [16]. However, the in vivo effects of Aa on bone quality, such as the mineral/matrix ratio or crystallite maturity and size, remain unknown.

The blood level of Aa is lower in older people because of decreased absorption from the intestine and reabsorption from the kidney [17–20]. Thus, we speculated that a reason for incidental fragility fractures during treatment with bisphosphonates could be impairment of bone quality caused by Aa deficiency in older people [17]. Therefore, we hypothesized that treatment with minodronate and Aa may exert additive effects on bone strength via increased BMD and improved bone quality under Aa-deficient conditions in vivo.

To confirm this hypothesis, we used congenitally Aa-deficient osteogenic disorder Shionogi (ODS) rats that are unable to produce Aa. These rats cannot survive without Aa supplementation, and their Aa levels are controlled by Aa supplementation [21]. The purpose of this study was to evaluate the effects of minodronate and Aa on BMD, bone quality, and bone strength in Aa-deficient ODS rats. Bone quality was evaluated by Fourier transform infrared spectroscopy imaging (FTIRI), which provides a way to explore bone quality at multiple bone hierarchical levels [22].

2. Materials and methods

2.1. Animals

Four-month-old female ODS rats (mutant Wistar rats; Clea Japan, Tokyo, Japan) were used [23]. Normal rats can synthesize Aa; however, ODS rats have a hereditary defect in ability to synthesize Aa because they lack L-gulono-γ-lactone oxidase [24,25]. When ODS rats are fed an Aa-free diet, their polypeptide hydroxyproline levels, which are known to be related to collagen synthesis [26], decrease below those in normal rats after 1 week and are about one-third of the normal level after 2 weeks [24,25]. When Aa is added to drinking water, the scorbutic symptoms of these rats resolve in a few days. The rats were housed in a controlled environment at 22 °C with a 12-hour/12-hour light/dark cycle. They were pair-fed and allowed ad libitum access to water as well as standard Aa-free food (CE-2; Clea Japan) containing 1.14% calcium, 1.06% phosphorus, and 250 IU vitamin D3 per 100 g of food as previously described [27,28]. The rats received 2 mg/ml Aa (Iwaki Pharma, Tokyo, Japan) in their drinking water for 4 months. At the age of 8 months, they received 0.5 mg/ml Aa in their drinking water [21] for 4 weeks to create Aa-deficient conditions.

2.2. Experimental design

After a 4-week experimental period of feeding with Aa-deficient (0.5 mg/ml) drinking water, the rats were divided into the following four groups (n = 20 per group): (1) Aa supplementation (Aa+) group, which received 2 mg/ml Aa-supplemented water; (2) Aa-deficient (Aa−) group, which received 0.5 mg/ml Aa-deficient water; (3) Aa supplementation and minodronate (Aa+ + Mino) group, which received Aa-supplemented water and minodronate; (4) Aa-deficient and minodronate (Aa− − Mino) group, which received Aa-deficient water and minodronate. In addition to these four groups, Wistar rats of the same age (n = 20) were included as a control group. Minodronate (Astellas Pharma, Tokyo, Japan) was dissolved in 0.1 M sodium acetate buffer (pH 7.2) containing 0.1% bovine serum albumin. This was administered subcutaneously once a week for 4 or 8 weeks (0.15 mg/kg body weight) [8].

The animals were euthanized under anesthesia with an intra-abdominal injection of ketamine (Sankyo, Tokyo, Japan) and xylazine (Zenoaq, Fukushima, Japan), and the bilateral femurs and tibias were harvested. Our animal experimental protocols were approved by the Animal Committee of our institution, and all animal experiments conformed to the "Guidelines for Animal Experimentation" of our institution.

2.3. BMD and sample preparation

The BMD of the right femur was measured in 2.5-mm proximal regions of the femur by dual energy X-ray absorptiometry (QDR-4500; Hologic Inc., Waltham, MA, USA). Thereafter, the right femur and bilateral tibias were fixed in neutral-buffered formalin until preparation for histological examination and FTIRI. The left femurs were dissected of soft tissue, wrapped in gauze moistened with saline, and frozen to −80 °C until biomechanical testing.

2.4. Bone histomorphometry

The left proximal halves of the tibias were decalcified in neutral-buffered 10% ethylene diamine tetra-acetic acid for 3 weeks and embedded in paraffin. Next, 3-μm-thick mid-frontal sections were prepared for hematoxylin and eosin (H & E) staining for cancellous bone histomorphometry.

Bone histomorphometric analyses of H & E-stained sections of the left tibia were performed using a semiautomatic graphic system (Histometry RT Camera; System Supply, Nagano, Japan). Measurements were obtained in regions that were 390 μm from the lowest point of the growth