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Does zoledronate therapy make mandibular bone susceptible to fracture? A radiographical and biomechanical study in rats

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ABSTRACT

Introduction: The aim of this study was to evaluate the effect of zoledronate therapy on susceptibility of mandibular bone to fracture in rats.
Methods: Fifty rats were randomly allocated to two groups of 25 animals. The rats in group Z received monthly intravenous infusion of 0.06 mg/kg zoledronate for 6 months. The rats in the group C were injected with an equal volume of saline in the same manner. A month after the last zoledronate/saline administration, all 50 rats were euthanized. Using a cone beam computed tomography, the cortical thickness of inferior border of mandible and the mandibular bone mineral density were calculated, and using a three-point bending test, the peak load failure and the ultimate stress of mandibular bone were determined.
Results: The mean mandibular inferior cortical bone thickness and the mean bone mineral density were significantly larger in zoledronate-treated rats (0.30 ± 0.02 mm and 1045.00 ± 185.79, respectively) compared to control rats (0.21 ± 0.01 mm and 878.66 ± 166.53, respectively). The peak load and the ultimate stress were lower in the zoledronate-treated hemimandibles (84.61 ± 33.62 N and 1.76 ± 0.72 MPa, respectively) compared to the control hemimandibles (98.36 ± 16.5 9 N and 2.03 ± 0.44 MPa, respectively).
Conclusion: Zoledronate therapy reduced the mechanical strength of the mandibles, implying an increased risk of mandibular fracture in rats.

Introduction

Bisphosphonates (BPs) are considered one of the main pharmacological agents in the treatment of a large number of diseases in which bone resorption needs to be reduced or controlled [1]. Their primary action is on osteoclasts, where they inhibit osteoclast activity and proliferation and induce apoptosis, leading to suppression of bone turnover and increase in bone mineralization [2,3].

Since the first report of spontaneous peripheral fractures after long-term alendronate therapy, followed by several studies reporting subtrochanteric or diaphyseal femoral insufficiency fractures in patients under BP therapy, concerns have raised about possible association between prolonged BP therapy and spontaneous bone fractures [4–6]. It was hypothesized that long-term BP exposure and oversuppression of bone turnover have a worsening effect on bone quality, making bone brittle and prone to fracture [5,7,8]. There is now abundant evidence demonstrating that patients receiving prolonged oral BPs are at increased risk of several types of fractures including femur, pelvis, fibula, ankle, metatarsals, and long bones such as the humerus and tibia [4,9–15]. Patients with cancer-associated skeletal-related-events may receive doses of intravenous BPs that are up to ten times greater than that administered for management of osteoporosis and represent a highly exposed population [6].

Despite growing body of orthopedic studies evaluating the association between BP therapy and several type of fractures, there is scant information in the literature about susceptibility of

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mandibular bone to fracture after exposure to long-term or high-dose antiresorptive agents. Because the mandible and the appendicular skeleton are derived from distinct embryonic cell lineage and have different rates of bone turnover, they may show different responses to BP therapy [16,17] as is evidenced by the unique localization of medication-related osteonecrosis to the jaw bone. Therefore, site specific evaluation of susceptibility to fracture after BP therapy is required.

The purpose of present study was to evaluate susceptibility of mandibular bone to fracture after exposure to high-dose zoledronic acid in a rat animal model.

Materials and methods

The protocol of this research was reviewed and approved by the Ethics Committee of the University.

Fifty male Wistar rats aged 6 months and weighing about 330 g were obtained from the Animal House of the University and housed in a temperature- and humidity-controlled environment with food and water supplies ad libitum. After a 2-week acclimation period, rats were randomly allocated to two groups of 25 animals. The rats in group Z (Zoledronate-treated) received monthly intravenous infusion of 0.06 mg/kg zoledronate (Zometa, Novartis Pharma, Basel, Switzerland) for 6 months (a total of 7 doses). The 25 rats in the group C (Control) were injected with an equal volume of saline in the same manner. A month after the last zoledronate/saline administration, all 50 rats were euthanized by an overdose of anesthesia. The mandibles were dissected, defleshed, and scanned using a cone beam computed tomography (CBCT) scanner (CRANEX 3D, *SOREDEX, Tuusula, Finland*) with the following parameters: 0.136 mm voxel size and 60 × 60 mm field of view. For radiographic evaluation of each side of the mandible, a coronal slice perpendicular to the inferior border of the mandible, passing through the distobuccal cusp tip of the first molar was produced and the cortical thickness of the inferior mandibular border and the bone mineral density were measured (Fig. 1). To calculate the mandibular bone density, three areas of bone on each coronal section were randomly selected and their grey values were determined using a rectangular ROI of approximately 10 × 10 pixels and the mean grey scale value representing the mean bone density profile of the hemimandible was calculated. All radiographic measurements were performed by two examiners separately and were recorded in a data sheet. The inter-examiner reliability was assessed using the intraclass correlation coefficient (ICC).

After obtaining CBCT, the rat mandibles were split at the midline suture and the biomechanical properties of hemimandibles were measured by three-point bending test using a Universal Testing Machine (STM-*20, Santam, Tehran, Iran*). Mechanical properties were determined from each force (p) versus displacement (d) curve. Each hemimandible was placed on the two lower support points (10 mm span) of the machine with the lingual aspect facing down and a constant deformation rate of 5.0 mm/min was generated until fracture. From the load-displacement curve, the maximal load failure (N) and ultimate stress (MPa) were determined and recorded.

The data were statistically analyzed using SPSS version 16.0 (SPSS inc., Chicago, IL, USA). Results were presented as the mean ± standard deviation. Comparisons between parameters were performed by unpaired Student's *t*-test and one way analysis of variance (ANOVA). A *p*-value less than 0.05 was selected as the criterion for statistical significance.

Results

All rats tolerated the experiment well and no complication was observed.

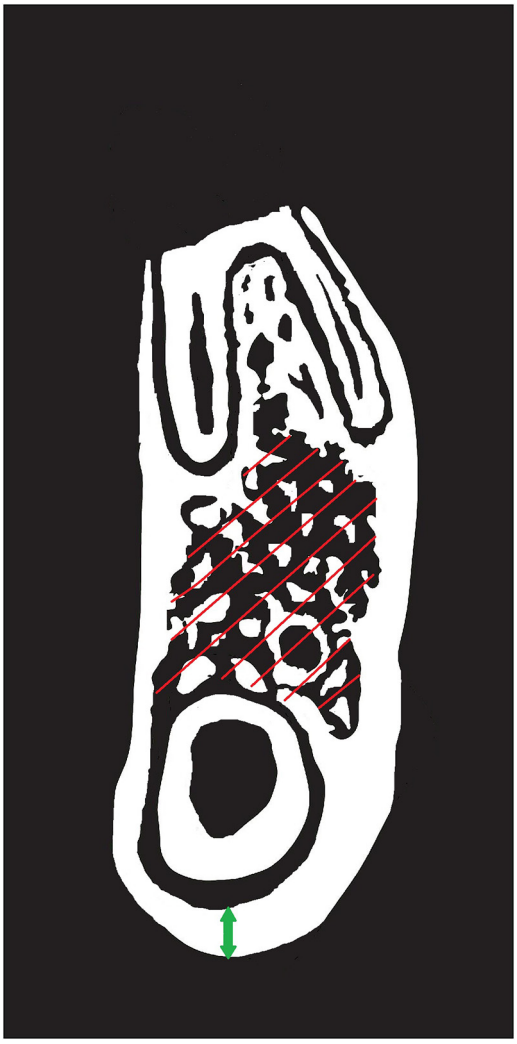

Fig. 1. Red hatched area indicates the area of mandibular bone on each coronal section of CBCT where the grey value measurements are performed. The double-headed green arrow shows the mandibular inferior cortical bone thickness (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

In present study, the mandibular inferior cortical bone thickness, the bone mineral density, the peak load, and the ultimate stress of 50 zoledronate-treated hemimandibles were compared with 50 control hemimandibles. The inter-rater ICC for both mandibular inferior cortical bone thickness and bone density measurements was found to be good (0.86 and 0.81, respectively).

Evaluation of CBCT coronal sections showed that the mean mandibular inferior cortical bone thickness was significantly ($P < 0.001$) larger in rats receiving zoledronate (0.30 ± 0.02 mm) compared to the control rats (0.21 ± 0.01 mm). The mean bone mineral density was also significantly ($P < 0.001$) higher in zoledronate-treated rats (1045.00 ± 185.79) than in the control group (878.66 ± 166.53) (Table 1).

Table 1
Comparison of grey value and cortical thickness of mandible between groups.

Group	Gray value	Inferior border thickness (mm)
	Mean ± SD Mean dif.	Mean ± SD Mean dif.
Study	1045.00 ± 185.79 166.33*	0.30 ± 0.02 0.09*
Control	878.66 ± 166.53	0.21 ± 0.01

* $P < 0.001$, statistically significant difference between groups.

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