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# Evaluation of mandibular fracture healing in rats under zoledronate therapy: A histologic study

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ABSTRACT

*Introduction:* To evaluate fracture healing in mandible of rats under zoledronate therapy.
*Methods:* A total of 135 Wistar rats were randomly allocated into 3 groups. Group L received two intravenous infusion of 0.06 mg/kg zoledronate 6 weeks apart. Group H received the same dose of zoledronate as group L once a week for 6 weeks and group C were treated with normal saline. Seven days after the last infusion, rats underwent unilateral mandibular osteotomy to replicate a fracture. Fifteen rats from each group were sacrificed 2, 4, and 6 weeks after surgery. Fracture calluses were examined and scored using a histological grading system (1 to 10).
*Results:* After 2 weeks, substantial woven bone and some lamellar bone were seen in control and L groups. In group H, healing was delayed and consisted of fibrous and cartilaginous tissue and some woven bone. After 4 weeks, most of woven bone in control group was replaced with lamellar bone but in group L, comparatively less bone remodeling occurred. In group H, healing process was nearly the same as that at 2 weeks. After 6 weeks, complete bone remodeling was seen in control group. In group L, bone remodeling was under way and in group H, histological findings were nearly the same as those at 2 and 4 weeks. Except for L and control groups at 2 weeks, healing score was significantly different between all corresponding groups.
*Conclusion:* Zoledronate therapy delayed healing process of mandibular fracture in rats in a dose-dependent manner.



## Introduction

Bisphosphonates (BPs) are a group of pharmacological agents widely used in a variety of bone diseases with high bone resorption, such as osteoporosis, multiple myeloma, Paget's disease, and hypercalcemia of malignancy [1]. Their main mechanism of action includes apoptosis and inhibition of activity of osteoclasts, thereby decreasing bone resorption and increasing bone mineralization [2,3]. BPs exhibit high affinity for bone mineral and in the body, they concentrate in the skeleton at sites of active bone remodeling [4]. They reside in the body long after treatment cessation and were calculated to have a half-life of elimination from the skeleton of nearly 10 years [5]. Long retention of BPs in skeleton might result in prolonged impairment of bone remodeling and harmful effects in bone repair [6].

Fracture healing is a complex process involving a large number of factors at both molecular and cellular levels acting in conjunction with physiological and biomechanical principles, of which the end goal is the return of the damaged bone to a functional and biomechanically sound state [4,7]. Disruption of the underlying cellular activities with the use of antiresorptive agents could disturb the normal fracture healing process [5].

Although many studies have shown the beneficial effects of BPs in reducing the risk of new fractures in patients with osteoporosis, few studies have evaluated the healing process in patients already on BP therapy who sustain a fracture [4,7]. In a study by Edwards et al. (2013), it was found that up to 26% of published cases of fractures in patients under long-term treatment with BPs exhibited delayed healing or non-healing [8].

Most of previous investigations on fracture healing during BP therapy have been on long bones. There is scant information in the

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published literature about mandibular fracture healing following a period of BP therapy. The mandibular bone and appendicular skeleton are derived from distinct cell lineages during embryonic development, which may lead to differences in their regenerative capacities and susceptibility to bisphosphonate treatment [9,10]. Because of an increased bone turnover in the alveolar region, the jawbones might be more affected by BPs than other skeletal area [11]. So, site specific assessment of the effect of BPs on fracture healing is required.

The aim of present investigation was to evaluate mandibular fracture healing in rats under Zoledonic acid therapy.

## Methods

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

A total of 135 male Wistar rats (age: 6 months; weight: 300 ± 20 g) were obtained from the Animal House of the University and acclimatized for 10 days prior to the experiment. Animals were housed in a temperature- and humidity-controlled environment, with food and water supplies ad libitum.

The rats were randomly allocated into three groups (45 rats per group). The group L (Low-cumulative dose) received two intravenous (IV) infusion of 0.06 mg/kg zoledronate (Zometa, Novartis Pharma, Basel, Switzerland) 6 weeks apart. The rats in group H (High-cumulative dose) were administered IV infusion of 0.06 mg/kg zoledronate once a week for 6 weeks (a total of 7 doses). The group C (Control) received the same volume of normal saline as L and H groups once a week for 6 weeks. In all three groups, the first injection was done at the starting day of study. Seven days after the last Saline/zoledronate infusion, all of the rats underwent unilateral mandibular osteotomy to replicate a mandibular fracture. Surgery was performed under intraperitoneal general anesthesia using 75 mg/kg of Ketamine hydrochloride (Rotexmedica, Trittau, Germany) and 7.5 mg/kg of midazolam (Midazolex, Exir, Iran). After placing the animal in a supine position and prepping and draping, a submandibular incision was made unilaterally. After exposure of the mandibular bone, a bicortical vertical osteotomy, 5 mm posterior to the third molar tooth, was done with a 0.5-mm diameter fissured burr under saline irrigation. Then the bone segments were repositioned and fixed with a stainless steel wire which was placed at the inferior border of the mandible. The soft tissue and skin were closed using resorbable sutures. Postoperatively, all of the rats received 25 mg/kg cefazolin (Ancef; Kefzol, 1gr, Razi, Iran) for 7 days, analgesics (1 mg/kg tramadol, subcutaneously), and appropriate basic care, such as soft diet and hygiene. Of the 45 rats in each group, 15 rats were sacrificed 2, 4, and 6 weeks after surgery using an intraperitoneal injection of 200 mg/kg sodium pentobarbital.

Following euthanasia, the mandible was harvested and split at the midline. The 135 fractured hemimandibles were fixed in 10% formalin solution, decalcified with EDTA, and embedded in paraffin. Serial 4-μm thick sagittal sections were cut at the fracture site and stained with haematoxylin and eosin (H&E).

The stained sections were evaluated by a pathologist who was blind to the treatment groups and the fracture healing was scored using a grading system as proposed by Perry et al. (2003), which was modified by the authors of present study as follows: Grade 1, fibrous union; Grade 2, predominantly fibrous tissue with some cartilage; Grade 3, comparable amounts of fibrous and cartilaginous tissues; Grade 4, predominantly cartilage with some fibrous tissue; Grade 5, predominantly cartilaginous tissue with some woven bone; Grade 6, comparable amounts of cartilage and woven bone; Grade 7, predominantly woven bone with some lamellar bone; Grade 8, comparable amounts of woven and lamellar bones; Grade 9, predominantly lamellar bone with some woven bone; Grade 10, entirely lamellar bone [12].

For statistical analyses, SPSS 16.0 (SPSS Inc., Chicago, IL) statistics software was used. Data were expressed as the mean ± standard deviation. After applying the Kolmogorov-Smirnov test to check the normality assumption, non-parametric Kruskal-Wallis and its corresponding multiple comparison tests were used to assess pairwise differences.

## Results

The rats tolerated the experiment very well and developed no complication.

Histological evaluation of the 2 weeks post-fracture callus in the control group showed an extensive new bone formation. The

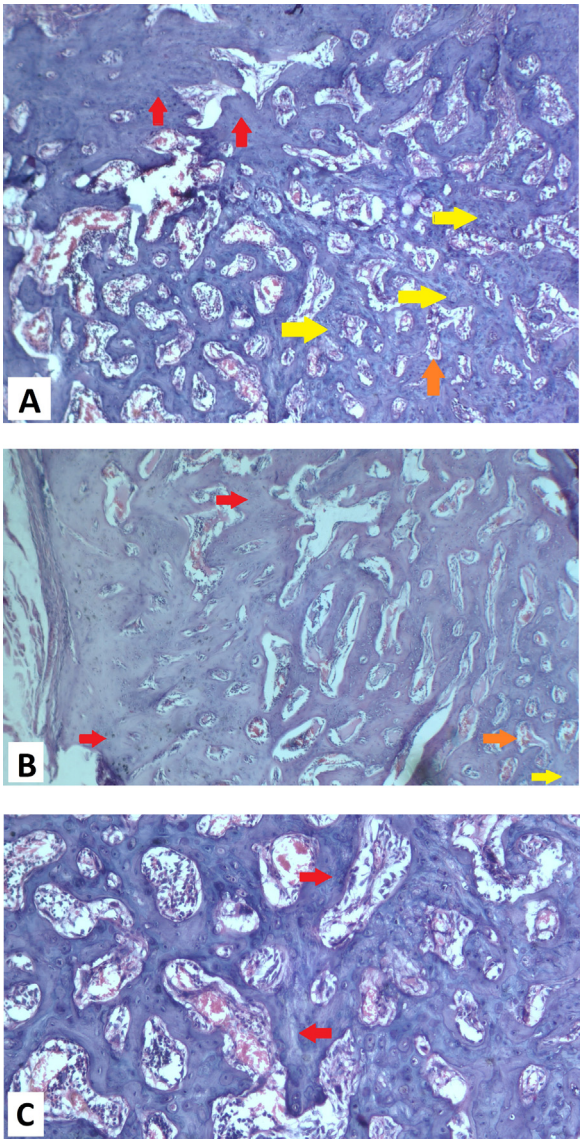


**Fig. 1.** Histological findings of control rats. A, two weeks after mandibular fracture, extensive new bone formation (yellow arrow), neovascularization (orange arrow) and some replacement of woven bone with mature bone (red arrow) were seen in the fracture gap. B, at 4 weeks post-fracture, most of the woven bone in the fracture callus was replaced with lamellar bone. C, after 6 weeks, callus was filled entirely with mature bone. Sections were stained with haematoxylin and eosin, and images A, B, and C were acquired at × 100, × 100, and × 250 magnification, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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