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Histological evaluation of the healing process of autografted mandibular bone defects in rats under treatment with zoledronate

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ABSTRACT

Objective: This study aims to evaluate the healing process of autografted mandibular bone defects in rats treated with zoledronate (ZOL).

Subjects and methods: A total of 180 Wistar rats were divided into four groups: group L received intravenous infusion of two doses of 0.06 mg/kg ZOL, nine weeks apart; group H received 0.06 mg/kg ZOL, while groups C and NC received normal saline at three-week intervals for nine weeks. Three weeks following the last infusion, a unilateral mandibular bone defect (5 mm) was created. Except in the NC group, all defects were repaired with autologous iliac bone graft. Fifteen animals from each group were sacrificed on postoperative Day 20, Day 40, and Day 60. Graft healing was scored using a histological grading system (ranging from 1 to 6).

Results: Histological evaluations performed on postoperative Day 60 showed that the mandibular defects were mainly repaired with fibrous tissue in the NC and H groups (93.00% ± 7.51% and 82.67% ± 13.08%, respectively) and with bone in the C and L groups (75.33% ± 14.20% and 92.67% ± 8.84%, respectively). The percentage of fibrous tissue and bone as well as the healing score of the NC and H groups were significantly different ($P = 0.001$) from those of the C and L groups. However, these were not different between neither the NC and H groups nor the C and L groups.

Conclusion: Based on the results of the present study the hypothesis can be established that there also might be a dose-dependent effect of ZOL on the healing of bone grafts in humans. This hypothesis has to be verified or rejected in clinical trials.

1. Introduction

Reconstruction of large maxillofacial bone defects, which may result from a variety of causes such as trauma, tumor ablation, and infections, remains as a significant challenge for surgeons. Among various graft materials, autologous bone depicts the best osteogenic, osteoinductive, and osteoconductive characteristics. Thus, it is still considered as the gold standard in bone replacement procedures (Sakkas et al., 2017). Healing of bone graft is a complex process that involves different cells, growth factors, cytokines, chemokines, as well as various proteins and enzymes. The biological events that occur during graft healing at the graft-tissue interface and the graft material itself include hematoma formation, inflammation, vascularization, resorption, regeneration, and remodeling. Furthermore, the natural process of bone healing may be affected by various factors such as age, medical comorbidities, smoking, radiation, and the use of medications (Jayakumar and Di Silvio, 2010).

Bisphosphonates (BPs) are the most widely used antiresorptive agents for the treatment of osteoporosis. These agents increase the bone density and reduce the risk of fractures in osteoporotic patients. Moreover, they are used for the treatment of calcium metabolism disorders, such as multiple myeloma, Paget's disease,

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and hypercalcemia of malignancy (McClung et al., 2013; Imai et al., 2015). Patients currently or previously treated with BPs, similar to individuals unexposed to BPs, could develop a mandibular bone defect and require autograft bone reconstruction. BPs are unique given that they exhibit a high affinity for bone minerals and can remain there for several years (McClung et al., 2013). It is possible that the BP incorporated into the bone could be released at the time of reconstructive surgery and interfere with the bone graft healing by inhibiting osteoclast differentiation and activity, increasing osteoclast apoptosis, interfering with proliferation and maturation of osteoblasts, and impairing angiogenesis and vasculogenesis (Landesberg et al., 2008; Tatli et al., 2011; Ziebart et al., 2011; Ruggiero et al., 2014).

Although several studies have evaluated the effect of post-operative administration of BPs on graft healing in craniofacial, vertebral, and appendicular reconstructive surgeries (Xue et al., 2005; Park et al., 2014; Ayranci et al., 2015; Koparal et al., 2016), there is a paucity of information in the literature published regarding the graft healing process in patients currently or previously on BP therapy, especially those who require mandibular bone defect reconstruction. Since the mandibular bone is derived from distinct cell lineage during embryonic development, and its reparative and regenerative capacities and turnover is different from that of other skeletal bones, the effects of BPs on graft healing in mandibular defects need to be exclusively investigated (Marx et al., 2007; Zandi et al., 2017). Impairment of mandibular fracture healing in rats under ZOL therapy has been demonstrated in previous studies (Rozental et al., 2009; Zandi et al., 2017). The aim of the present preclinical study was to evaluate the healing process of autografted mandibular bone defect in rats under treatment with ZOL.

2. Material and methods

The protocol of this study was reviewed and approved (IR.UMSHA.REC.1396.548) by the Ethics Committee of the Hamadan University of Medical Sciences, Iran.

2.1. Animals

In the present investigation, a total of 180 naive male Wistar albino rats aged five months with an average weight of 275 g, bred at the Hamadan University of Medical Sciences, were involved. Ten days prior to the start of the study, the animals were housed in pairs in plastic cages in a temperature- and humidity-controlled environment. Moreover, they were provided food and water ad libitum.

2.2. Experimental design

The schematic diagram of the experimental timeline has been presented in Fig. 1. The rats were randomly divided into four equal groups of forty five. The rats in the group L (low cumulative dose) received intravenous infusion of two doses of 0.06 mg/kg ZOL (Zometa, Novartis Pharma, Basel, Switzerland), nine weeks apart, whereas the rats in the group H (high cumulative dose) received intravenous infusion of 0.06 mg/kg ZOL at three-week intervals for four treatments (administered for nine weeks). The rats in the groups C (Control) and NC (Negative Control) were administered the same volume of normal saline as group H every three weeks for four treatments (provided for nine weeks).

Three weeks after the last saline/ZOL infusion, a critical-sized defect with a diameter of 5 mm was created in all 180 rats on one side of the mandible, and a bone graft was harvested from the iliac crest. The mandibular defects in the groups C, L, and H (but not in group NC) were repaired with the harvested autograft (Fig. 2). Using an intraperitoneal injection with 200 mg/kg of sodium pentobarbital, 15 animals from each group were randomly sacrificed on Day 20, Day 40, and Day 60 after surgery.

In the present study, all randomizations were performed by means of a lottery (http://www.graphpad.com/quickcalcs/randomize1.cfm).

2.3. Surgical procedure

After induction of general anesthesia using an intraperitoneal injection with 75 mg/kg of ketamine hydrochloride (Rotexmedica, Trittau, Germany) and 7.5 mg/kg of midazolam (Midazolex, Exir, Iran), the donor and recipient areas were shaved, prepped, and draped. A 1.5 cm-long linear incision was made on the skin along the inferior border of the mandible on one (randomly selected) side. Following dissection of the soft tissue, the periosteum was gently elevated, and the mandibular bone was exposed. Using a slow-speed surgical handpiece and trephine, a full-thickness defect measuring 5 mm was created in the body of the mandible just distal

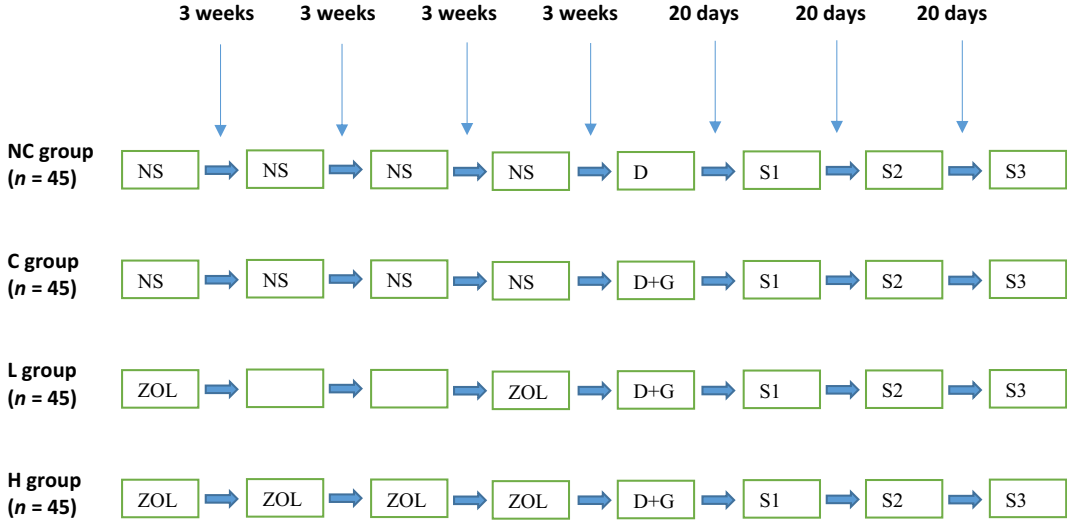

Fig. 1. Schematic diagram of the experimental timeline. NS: normal saline; ZOL: zoledronate; D: defect; G: graft; S: sacrifice.

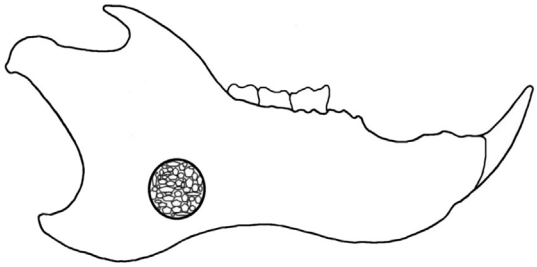

Fig. 2. Design of the full-thickness bone defect in the body of the mandible.

to the roots of the last molar tooth. The ostectomy was performed under copious irrigation with sterile saline and did not the interrupt the continuity of the mandible's inferior border.

To harvest the iliac bone graft, a skin incision measuring about 20 mm in length was made on a randomly selected side of the lower back. After soft tissue dissection, a corticocancellous bone from the iliac crest was harvested using a rongeur and tightly placed into the mandibular defect, without using any fixation device to avoid disturbing the normal process of graft healing. The soft tissues at both the donor and recipient sites were subsequently repositioned and sutured in layers using absorbable sutures. Postoperatively, all rats were administered an intramuscular injection with 25 mg/kg of cefazolin (Ancef; Kefzol, 1gr, Razi, Iran) for seven days, in addition to analgesics and appropriate basic care.

2.4. Histologic processing and examination

Following surgical dissection, the mandibles were split from the midline between the incisor teeth. In all 180 rats, the hemimandible with the bone defect was fixed in 10% formalin solution, decalcified with EDTA, and embedded in paraffin. Moreover, serial sagittal sections were made with 4 μm thickness and stained with hematoxylin and eosin (H&E). From each sample, two representative sections, including the maximum length of the fracture and a lack of artefact tissues, were selected for histological assessments conducted under light microscopy by a pathologist, who was unaware of the treatment assignments.

In each section, the percentage of connective tissue and regenerated bone and/or remaining graft (RBRG) areas within the confines of the total surgical defect area was measured using a 10 mm × 10 mm eyepiece grid reticule at 400× magnification. Subsequently, the healing process in the defect was scored using a histological grading system (Table 1), as proposed by Zandi et al. (2018).

2.5. Statistical methods

Based on a power analysis, for a power of 90% along with a two-sided significance level of 0.05, and assuming a dropout rate of 5%, the sample size required for each group was 45 rats.

Table 1
Histological grading for healing process in autografted mandibular bone defects.

Grade	Description
1	No healing or healing with connective tissue
2	Mostly connective tissue with some RBRG
3	Comparable amounts of connective tissue and RBRG
4	Predominantly RBRG with some connective tissue
5	Entirely RBRG — defect distinguishable from surrounding uninjured bone
6	Complete repair — defect indistinguishable from surrounding uninjured bone

RBRG: regenerated bone and/or remaining graft.

In the current study, data analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL) version 20.0. The data were expressed as means and standard deviations. Since the assumption of normality was not met using the Shapiro–Wilk test, the mean values between the groups at each time points were compared by conducting a non-parametric Kruskal–Wallis test. Subsequently, a pairwise multiple comparison test was performed to determine the significant differences between each pair of means. Significance values were adjusted via the Bonferroni correction for multiple tests. Values of $P < 0.05$ were considered statistically significant.

3. Results

Healing score and the percentage of various tissues in the mandibular bone defect among the groups on Day 20, Day 40, and Day 60 after surgery have been presented in Table 2.

Histological evaluations of the mandibular bone defects in both the NC and H groups revealed an impaired bone healing on the aforementioned postoperative days. As shown in Fig. 3, even on Day 60 after surgery, scarce new bone formation occurred in the mandibular defect of the NC and H groups, and the bone defects were primarily filled with fibrous tissue. Furthermore, in the H group, a large number of empty lacunae (with no osteocyte) were found in the mandibular bone adjacent to the defect area.

Histological examination of the sections from the C and L groups on Day 20 following surgery showed that 42.33% ± 10.15% and 53.67% ± 16.09%, respectively, of the defect area were filled with RBRG, and the remaining part was repaired with fibrous tissue (Fig. 4). On the postoperative Day 40, 58.00% ± 11.62% and 79.00% ± 21.15% and on the postoperative Day 60, 75.33% ± 14.20% and 92.67% ± 8.84% of the mandibular defect area of the C and L groups were filled with RBRG, respectively (Figs. 5 and 6). Although a higher portion of RBRG was observed in the L group than in the C group on the three aforementioned postoperative days, the differences were not statistically significant ($P = 0.779$ for Day 20, $P = 0.801$ for Day 40, and $P = 0.678$ for Day 60).

Comparison of the healing score between the four groups at each time-point showed that on the postoperative Day 20, the healing score of the C, L, and H groups (2.53 ± 0.64, 3.20 ± 0.77, and 1.93 ± 0.70, respectively) was significantly higher ($P = 0.001$, $P = 0.001$, and $P = 0.038$, respectively) than that of the NC group (1.20 ± 0.41). On Day 40 after surgery, the healing score of both the C and L groups (3.47 ± 0.64 and 4.33 ± 0.90, respectively) was significantly higher ($P = 0.001$; between the C and H groups, $P = 0.008$) than that of the NC and H groups (1.33 ± 0.49 and 1.87 ± 0.52, respectively). Moreover, on the postoperative Day 60, the healing score of both the C and L groups (4.13 ± 0.53 and 5.20 ± 0.86, respectively) was significantly higher ($P = 0.001$) than that of the NC and H groups (1.47 ± 0.52 and 1.67 ± 0.49).

A comparison of the healing process at the mandibular defect site during the 60-day study period between the four groups has been presented in Fig. 7.

4. Discussion

Maxillofacial bone defects occur owing to a variety of causes, including trauma, ablation of pathological bone lesions, infections, and congenital problems. Osteoporotic and cancer patients currently or previously exposed to BPs, similar to BP-naïve patients, could develop a mandibular bone defect and require autograft bone reconstruction. As BPs bind to the hydroxyapatite in the skeleton and remain there for several years (McClung et al., 2013), they may affect the normal process of graft healing after bone reconstruction.

Table 2
Healing scores and percentages of various tissues in mandibular bone defects in the four groups at 20, 40, and 60 days postoperatively.

Subgroup	No.	Fibrous tissue (mean ± SD)	Regenerated bone and/or remaining graft (mean ± SD)	Healing score (mean ± SD)
NC20	15	98.67 ± 2.97[b,c] ($p = 0.001$), d ($p = 0.019$)	1.33 ± 2.97[b,c] ($p = 0.001$), d ($p = 0.019$)	1.20 ± 0.41[b,c] ($p = 0.001$), d ($p = 0.038$)
C20	15	57.67 ± 10.15[a] ($p = 0.001$)	42.33 ± 10.15[a] ($p = 0.001$)	2.53 ± 0.64[a] ($p = 0.001$)
L20	15	46.33 ± 16.09[a] ($p = 0.001$)	53.67 ± 16.09[a] ($p = 0.001$)	3.20 ± 0.77[a] ($p = 0.001$)
H20	15	71.00 ± 18.44[a] ($p = 0.019$)	29.00 ± 18.44[a] ($p = 0.019$)	1.93 ± 0.70[a] ($p = 0.038$)
NC40	15	95.33 ± 4.42[b,c] ($p = 0.001$)	4.67 ± 4.42[b,c] ($p = 0.001$)	1.33 ± 0.49[b,c] ($p = 0.001$)
C40	15	42.00 ± 11.62[a] ($p = 0.001$), d ($p = 0.017$)	58.00 ± 11.62[a] ($p = 0.001$), d ($p = 0.017$)	3.47 ± 0.64[a] ($p = 0.001$), d ($p = 0.008$)
L40	15	21.00 ± 21.15[a,d] ($p = 0.001$)	79.00 ± 21.15[a,d] ($p = 0.001$)	4.33 ± 0.90[a,d] ($p = 0.001$)
H40	15	79.67 ± 14.45[b] ($p = 0.017$), c ($p = 0.001$)	20.33 ± 14.45[b] ($p = 0.017$), c ($p = 0.001$)	1.87 ± 0.52[b] ($p = 0.008$), c ($p = 0.001$)
NC60	15	93.00 ± 7.51[b,c] ($p = 0.001$)	7.00 ± 7.51[b,c] ($p = 0.001$)	1.47 ± 0.52[b,c] ($p = 0.001$)
C60	15	24.67 ± 14.20[a,d] ($p = 0.001$)	75.33 ± 14.20[a,d] ($p = 0.001$)	4.13 ± 0.53[a,d] ($p = 0.001$)
L60	15	7.33 ± 8.84[a,d] ($p = 0.001$)	92.67 ± 8.84[a,d] ($p = 0.001$)	5.20 ± 0.86[a,d] ($p = 0.001$)
H60	15	82.67 ± 13.08[b,c] ($p = 0.001$)	17.33 ± 13.08[b,c] ($p = 0.001$)	1.67 ± 0.49[b,c] ($p = 0.001$)

[a] Statistically significant difference compared with NC group at the same time point.
[b] Statistically significant difference compared with C group at the same time point.
[c] Statistically significant difference compared with L group at the same time point.
[d] Statistically significant difference compared with H group at the same time point.

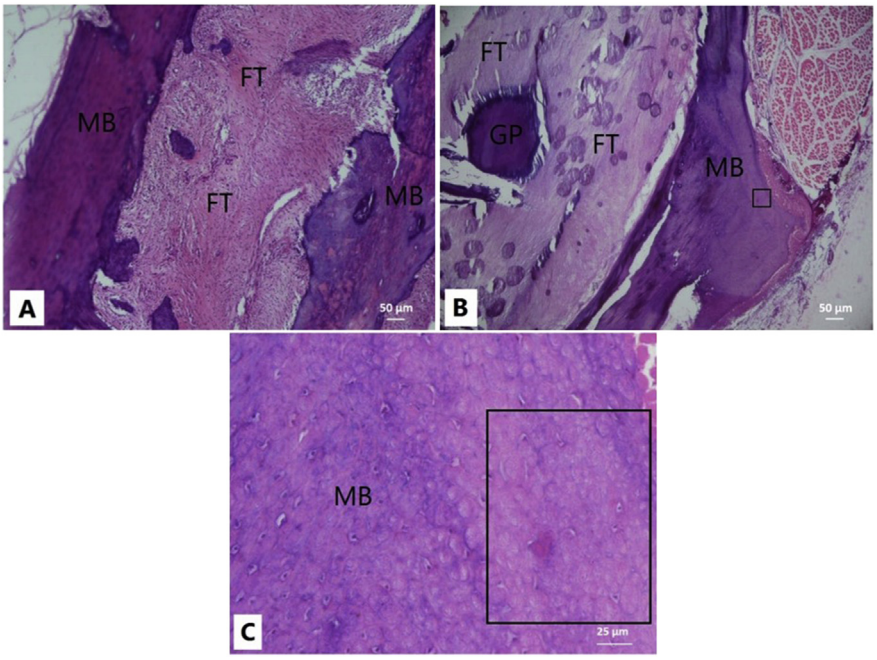

Fig. 3. Histological findings of the bone defect in the NC and H groups on Day 60 after surgery. A: In the NC group, the defect of the mandibular bone (MB), is primarily repaired with fibrous tissue (FT). B: In the H group, a large amount of FT and the remnant of graft particles (GP) can be seen in the defect of the MB. C: Higher magnification of the MB adjacent to the defect in image B and a large accumulation of empty lacunae with no osteocyte (black rectangle) can be observed. The histological slides were stained with hematoxylin and eosin dye.

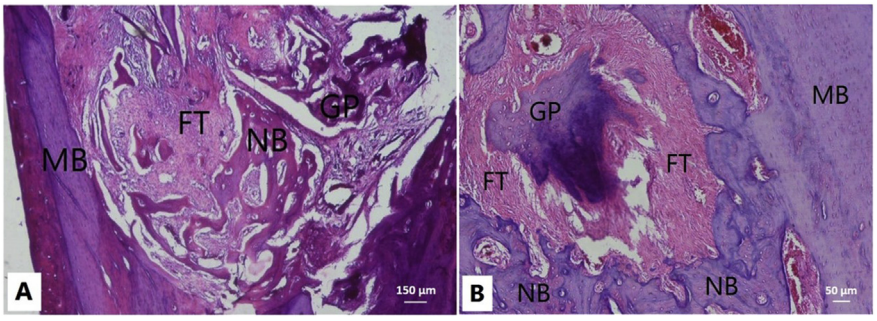

Fig. 4. Histological findings of the bone defect in the C and L groups (images A and B, respectively) on Day 20 after surgery. A comparable amount of fibrous tissues (FT) and new trabecular bone (NB), which surround the remnants of autologous bone graft particles (GP), can be observed in the mandibular bone (MB) defect. The histological slides were stained with hematoxylin and eosin dye.

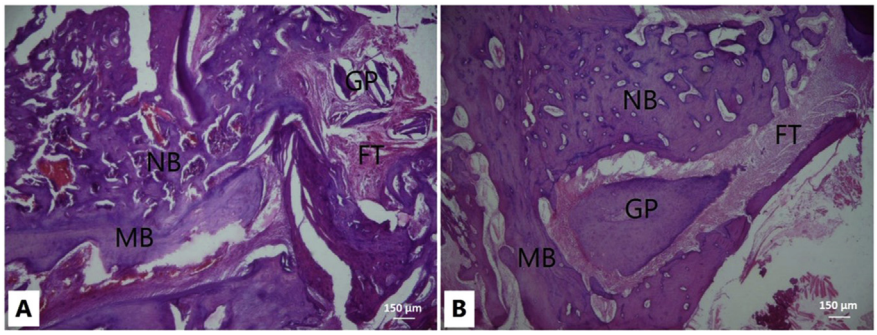

Fig. 5. Histological findings of the bone defect in the C and L groups (images A and B, respectively) on Day 40 after surgery. A large portion of the mandibular bone (MB) defect is repaired with new trabecular bone (NB). The remnants of autologous bone graft particles (GP) and a small amount of fibrous tissues (FT) can also be observed in the defect area. The histological slides were stained with hematoxylin and eosin dye.

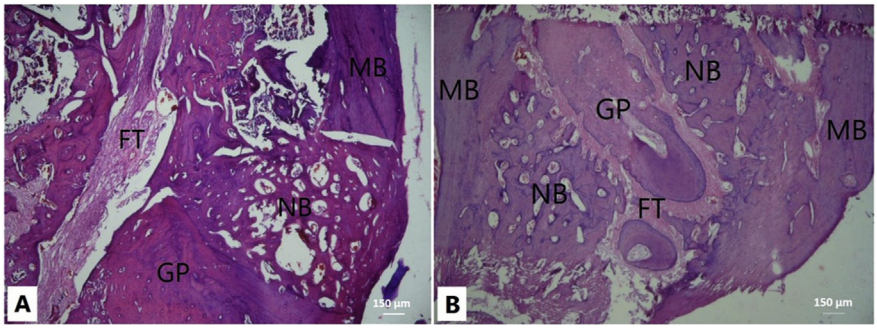

Fig. 6. Histological findings of the bone defect in the C and L groups (images A and B, respectively) on Day 60 after surgery. Nearly the entire mandibular bone (MB) defect is filled with new trabecular bone (NB) and graft particles (GP), and a small amount of fibrous tissues (FT) can also be seen. The histological slides were stained with hematoxylin and eosin dye.

In the current research, the healing process of autografted mandibular bone defect in rats under two different protocols of ZOL therapy was evaluated. The rats in the H group received 0.06 mg/kg ZOL at three-week intervals for four treatments in order to nearly correspond to the total dose of ZOL administered to a cancer patient following four consecutive months of treatment. The rats in the L group received two doses of 0.06 mg/kg ZOL, nine weeks apart, to produce a low cumulative dose of ZOL compared to that of the H group. The use of ZOL for the treatment of patients with multiple myeloma and bone metastases has been approved by the Food and Drug Administration. The said drug is administered as a 4-mg infusion at a typical interval of every three to four weeks (Saad

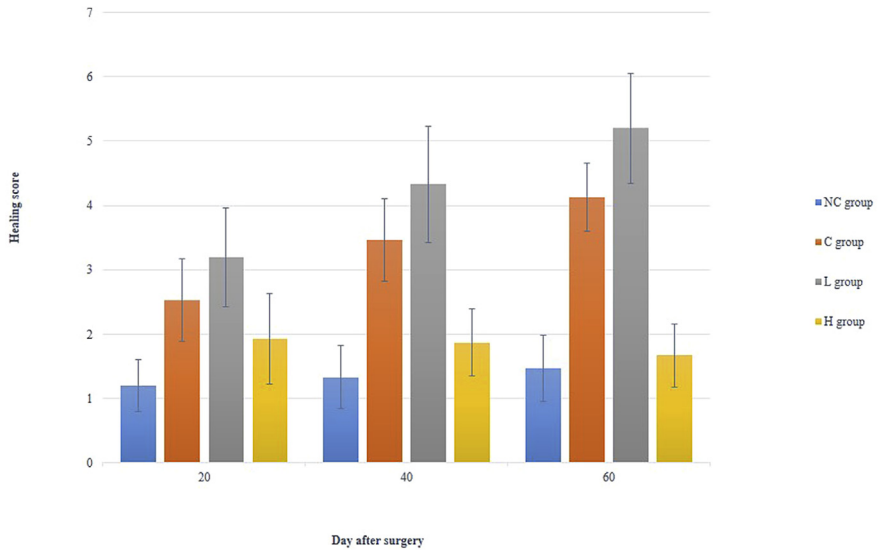

Fig. 7. Comparison of the graft healing process in the mandibular bone (MB) defect among the groups during the 60 days following surgery. On postoperative Day 20, the healing score of the C, L, and H groups was significantly higher ($P = 0.001$, $P = 0.001$, and $P = 0.038$, respectively) compared to the NC group. On postoperative Day 40 and Day 60, the healing score of both the C and L groups was significantly higher than that of the NC and H groups ($P = 0.001$; between C and H groups on Day 40, $P = 0.008$).

et al., 2002; Vogel et al., 2004; Kohno et al., 2005; Tanvetyanon and Stiff, 2006; Himelstein et al., 2017). In a randomized, placebo-controlled trial performed by Rosen et al. (2004), long-term efficacy and safety of a 21-month treatment with ZOL, at a dose of 4 mg every three weeks, were reported. ZOL has also been successfully administered for the prevention and treatment of osteoporosis. The approved dose of intravenous ZOL for the treatment of osteoporosis is 5 mg administered annually (Grey et al., 2014; Tu et al., 2018). It has been shown that BP embedded within the skeleton matrix has lasting biological effects. Studies with C14-labeled ZOL have measured the actual concentrations of ZOL in the skeleton at various time points after starting or stopping treatment in animals. After multiple intravenous doses of 0.15 mg/kg ZOL (daily on 16 consecutive days) to rats, no indication of saturation of available binding capacity in bones was found, and the concentration of ZOL in bone remained high during the entire 240-day observation period. Although, direct measurements of BP concentration in human bone biopsies are not available, studies with adequate data collection over at least one year and use of sufficiently sensitive assays have confirmed the specific property of BPs to be very slowly released from the skeleton (Allen, 2008; Weiss et al., 2008; Cremers and Papapoulos, 2011).

Histological examination of the autografted mandibular bone defects conducted on Day 20, Day 40, and Day 60 after reconstruction showed that healing in the L group was not significantly different from that of the saline-treated group. However, in the H group, the majority of the defect was repaired with fibrous tissue, and the healing was similar to the non-grafted defects observed in the rats of the NC group. These findings indicated that a low cumulative dose of ZOL does not affect the healing process of autografted mandibular defects, whereas high cumulative doses of ZOL severely impair bone graft healing.

Healing of bone grafts is a complex biological process and is unique among connective tissues. In the early phase of autograft transplantation, hematoma and inflammation are formed. Furthermore, the recruited mesenchymal stem cells lay down fibrous granulation tissue, and the transplanted cells in the graft proliferate and produce new osteoid. In the second phase, angiogenesis and fibroblastic proliferation from the graft bed and osteogenesis from the host connective tissues begin. Fibroblasts and other mesenchymal cells differentiate into osteoblasts and form the new bone (Axhausen, 1956; Burwell, 1964; Gray and Elves, 1979; Goldberg and Akhavan, 2005; Khan et al., 2005; Wang and Yeung, 2017). It has been shown that BPs interfere with bone healing by affecting different cell types and biological processes in a dose-dependent manner. These antiresorptive agents inhibit osteoclasts and fibroblasts, restrict vasculogenesis and angiogenesis, reduce the viability of oral keratinocytes, resulting in impaired mucosal wound healing, and reduce the production of extracellular matrix protein (Lam et al., 2007; Landesberg et al., 2008; Ziebart et al., 2011). Moreover, the effect of ZOL on osteoblast proliferations depends on the dosage. While lower drug concentrations (10^{-10} M to 10^{-8} M) have no effect, concentrations higher than 10^{-7} M significantly reduce cell proliferation. Osteoblast apoptosis is enhanced after treatment with the highest ZOL concentrations (Corrado et al., 2010). In the same line of research, an in vitro study conducted by Fliefel et al. (2019) demonstrated that the exposure of bone cells to different concentrations of ZOL resulted in variable effects. The lowest dose of ZOL (0.1 μM) did not significantly affect bone cells' viability; instead, it slightly stimulated cell growth in osteoblasts. However, high concentrations of ZOL (25 and 100 μM) had cytotoxic effects on osteoblasts and osteoclasts. The dose-dependent effects of BPs on the healing process may explain normal bone graft healing in the mandibular defects of rats in the L group and impaired healing in the bone defects of the H group, which was observed in the present study. In addition, the high dose of ZOL in the H group may inhibit angiogenesis and neovascularization, reduce osteoblast activity, and promote the apoptosis of this cell, resulting in bone graft necrosis and reduced new bone formation. Furthermore, the decreased osteoclast activity following BP exposure causes decreased osteoblast signaling, which by itself further reduces osteoblast activity and bone formation (Lehman et al., 2004).

Limited information is available in the published literature pertaining to the effects of BPs on the graft healing of mandibular bone defects; most of our current knowledge is based on the findings of spinal fusion studies. In the study conducted by Takahata et al. (2008), the effect of antiresorptive therapies on bone graft healing in an ovariectomized rat spinal arthrodesis model was investigated. It was ascertained that BPs not only suppressed bone remodeling but also inhibited endochondral ossification during the bone graft healing process. Similarly, a study performed by Huang et al. (2005) revealed that alendronate inhibited spine fusion in rats. Moreover, quantitative histomorphometry confirmed that alendronate inhibited bone graft resorption and incorporation. Thus, it was recommended that patients undergoing spine arthrodesis should not take alendronate until the achievement of fusion. Accordingly, an animal study by Lehman et al. (2004) depicted an inhibited or delayed spinal fusion in rabbits treated with alendronate sodium, which might be due to the uncoupling of balanced osteoclastic and osteoblastic activity inherent to bone healing. Therefore, they suggested that a discontinuance of alendronate sodium postoperatively during the acute fusion period might be warranted. In contrast, studies conducted by Xue et al. (2005) and Babat et al. (2005) showed no significant adverse effect of BP on the fusion outcome. The controversial results of the previous spinal fusion studies could have been acquired owing to factors such as differences in sample size, animal model, as well as the type, dosage, and schedule of BP therapy; and the method of assessing the outcomes.

In most of the previous studies evaluating the effects of BPs on bone graft healing, BP therapy was started just after bone replacement surgery. Thus, the autograft harvested from the iliac bone was devoid of any BP. Moreover, revascularization and the initial phases of bone healing had occurred before postoperatively administered drugs could accumulate in the graft (Babat et al., 2005). However, a more probable clinical scenario in this regard is that osteoporotic and cancer patients are already on a BP regimen when they undergo reconstructive surgery of the mandibular defect. Therefore, in the present study, the effects of BP already deposited in the skeleton, prior to the bone graft surgery, were assessed. BPs remain in the bone long after treatment cessation, and if their adverse effects are associated with skeletal persistence of these drugs, it may take a long time for these to resolve (McClung et al., 2013).

In the current research, among the BPs, ZOL was administered to the rats. ZOL is a potent agent that quickly accumulates in large amounts in the bone, and its intravenous route of administration avoids the issue of variation in bioavailability due to the absorption noted with orally administered drugs.

The authors of the present investigation hypothesize that during the reconstruction of the mandibular defect with the iliac bone graft, the bone injury resulted from surgical trauma causes a substantial amount of BPs, which is incorporated into the bone, to be released and interfere with healing process of bone graft. Owing to the dose-dependent effects of BPs, evaluation of graft healing is more crucial for patients with skeletal malignancy, who are administered doses of intravenous BPs up to ten times greater than those used for osteoporosis. The current study demonstrated an impaired autograft healing in rats receiving a high cumulative dose

of ZOL. This finding suggests that the chance of graft failure in mandibular reconstruction is expected to be higher for cancer patients than for osteoporotic patients who are administered BPs. However, bone formation and remodeling in rats occur faster and more vigorously than in primates, and such differences among species should be considered before applying these findings to clinical surgery (Takahata et al., 2008).

On the other hand, previous studies have shown that discontinuation of BP therapy results in an increase in bone turnover and the improvement of bone healing, due to the recovery of osteoclastic function (Zandi et al., 2015). Therefore, discontinuation of BP therapy after careful evaluation of its benefits and risks as well as consultation with the physician in charge of treatment may have a beneficial effect on the graft healing process. This possibility needs to be investigated further.

The primary limitations of the present study were the use of an animal model with different physiology and bone turnover compared to humans, in addition to the relatively short-term follow up of the bone healing process prior to the sacrifice of the animals. The current investigation was also limited by the use of a simplified histological grading system (Zandi et al., 2018) for the evaluation of the graft healing process regarding the mandibular defect of rats. Till date, several histological grading systems for the evaluation of bone fracture and graft healing in different animal models have been introduced in the literature (Bosch et al., 1995; Perry et al., 2003; de Almeida et al., 2013). Since the pattern of bone graft healing differs from fracture healing, and it varies among species and at different sites in the skeleton (Eames and Helms, 2004; Noden and Trainor, 2005; Zandi et al., 2017), a species- and site-specific grading system for graft healing assessment is required. Furthermore, the current research had several advantages, including the use of a relatively large sample size, concurrent assessment of two different schedules of BP therapy, and the evaluation of the outcomes at three time-points.

Further experimental and clinical research works to evaluate the effects of different BP agents, treatment protocols on autograft bone healing, and long-term follow up of the relevant outcomes are recommended.

5. Conclusion

Based on the results of the present study the hypothesis can be established that there also might be a dose-dependent effect of ZOL on the healing of bone grafts in humans. This hypothesis has to be verified or rejected in clinical trials.

Ethical approval

Hamadan University of Medical Sciences Ethics Committee (approval number IR.UMSHA.REC.1396.548).

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Conflicts of interest

None declared.

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