Research Article

Skeletal Site-Specific Response of Jawbones and Long Bones to Surgical Interventions in Rats Treated with Zoledronic Acid

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Bisphosphonates (BPs) have been extensively used for management of bone diseases with pathologically high resorption. Despite the great clinical benefits, a severe complication known as medication-related osteonecrosis of the jaw (MRONJ) has been reported. It is found that most of the reported MRONJ cases were limited in the jawbones/craniofacial bones instead of long bones. The present study aims to investigate the differential bone response to surgical procedures between jawbones and long bones exposed to BPs. Forty-eight skeletal mature Sprague Dawley female rats were administered oncologic dose of zoledronic acid (ZA) or normal saline for 4 weeks and then subjected to tooth extraction on the mandible and maxilla, and a bone defect creation on the femur. After surgical procedures, ZA or saline treatment were continued until sacrifice at week 2, week 4, and week 8, post-operatively. The samples were subjected to micro-computerized tomography (micro-CT) and histological assessment. Osteonecrosis was only found in jawbones in ZA-treated rats. ZA-treated rats showed significantly higher bone mineral density with greater bone volume in all surgical sites than that in the controls. The length of exposure of ZA did not seem to affect trabecular microstructure, and it only showed higher bone volume and BMD with longer healing time which is expected in the healing process.

1. Introduction

Firstly being reported in 2003 (Marx, 2003), medication-related osteonecrosis of the jaws (MRONJ) is one of the potential adverse effects after BPs treatment and has received greater attention more than any other side effects of BPs. One of the most significant characters of MRONJ is the site-specific effect, which is the osteonecrosis tends to occur specifically in maxillofacial bones, the mechanism of which is still unclarified. MRONJ affects the mandible and maxilla with preference for the former, as in mandibles osteonecrosis were found twofold more than that in maxillae [1]. Around half of the MRONJ cases were associated with surgical intervention in the oral cavity [2] or dental diseases [3]. To date, no specific etiological mechanism has been proved to be associated with the pathogenic process in MRONJ. Various assumption and hypotheses have been proposed regarding the etiology of MRONJ, including inhibition of osteoclast activity and over-suppression of bone turnover, suppression of angiogenesis, oral infection, and cell toxicity [4, 5]. However, these hypotheses could not thoroughly explain the exclusive site of occurrence of ONJ. Some studies showed that the high bone turnover rate of jaw bones, together with the increased bone remodeling due to dental surgical operations, resulted in the development of osteonecrosis in this specific site [6]. However, others found that turnover rate of the jawbones was not evidently changed in patients with zoledronic acid or denosumab treatment [7–9].

A minimal number of cases reported atypical fractures of the femur which are related to long-term treatment of
bisphosphonates or denosumab [10, 11]. These studies indicated a fracture distinct from the common osteoporosis induced subtrochanteric or femoral shaft fracture, while researchers deduced that the possible mechanisms might be microdamage accumulation, increased mineralization, reduced mineralization heterogeneity, variations in bone turnover rates and reduced vascularity and anti-angiogenic effect related to BPs treatment [12, 13]. Chen et al. [14] demonstrated in a clinical study that long-term use of BPs does not generate a generalized increase in subtrochanteric femoral cortical thickening—which often observed radio-
graphically in patients on long-term bisphosphonates with atypical femur fractures. Except for the above reports, studies on atypical femur fracture and its possible differences or sim-
ilarity with MRONJ are still lacking. Till now, the skeletal site-specific reactions to BPs treatment associated with trauma have not been fully understood yet. Former studies regarding the comparison of different skeletal site's reaction under surgical intervention toward BPs treatment were in the minority. Ristow et al. [7] investigated the bone turnover rate of femur and jawbones in 90 female cancer patients using scintigraphy with or without BPs treatment. They found that the bone turnover rate was not significantly suppressed by BPs in femur and jawbones. Mandible showed similar bone turnover rate as femur, while the rate in maxilla was signifi-
cantly higher. Given the fact that clinically peripheral bone seldom went through surgical intervention than that in oral cavity, we aim to investigate the differential bone response to surgical procedures between jawbones and long bones exposed to BPs.

2. Materials and Methods

2.1. Animal Care and Grouping. A total of forty-eight Sprague Dawley (SD) rats were obtained from the Laboratory Animal Unit (LAU) of Li Ka Shing Faculty of Medicine, the University of Hong Kong. The SD rats were 12-week-old females and weighed from 270 g to 300 g. All animals were kept in a dedicated animal holding facility under the supervision of veterinary. All animals were housed in an indoor environment at a temperature of 20°C± 5°C in a 12 : 12-h light–dark circle with free access to water and standard rodent diet (Irradiated, PMI, USA). The animal study was approved by the Committee on Use Live Animal for Teaching and Research, the University of Hong Kong (CULATR 3775-15).

Forty-eight rats were randomly assigned into two groups with twenty-four rats in each. Animals in Group ZA received intraperitoneal injection (i.p.) of zoledronic acid (Zometa, Novartis, Switzerland. 0.066 mg/kg) dissolved in 0.2 ml sterile saline three times per week. This dosage scheme corresponds to 4 mg/60 kg drug dosages monthly for cancer patients with skeletal complications [15]. However, Group C (control group) received an equivalent amount of normal saline i.p. three times per week. The administration of ZA or saline continued from baseline until the sacrifice of the animals.

After four weeks (which roughly equivalent to 2.5 human years of administration [16]) of ZA/saline administration, all animals received surgical intervention. These two groups of animals were further divided into six subgroups which were subjected to different length of exposure (LoE) with the treat-
ment of ZA/saline sustained until sacrifice of the animals. The rats in Group ZAs (short-term experiment group, n = 8) and Group Cs (short-term control group, n = 8) were sacrificed at the second week after surgery. Likewise, animals of Group ZAm (medium-term experiment group, n = 8) and Group Cm (medium-term control group, n = 8) were sacrificed at the fourth week and Group ZAl (long-term experiment group, n = 8) and Group Cl (long-term control group, n = 8) were sacrificed at the eighth week after surgery (Figure 1).

2.2. Surgery. All the animals received right femur defect creation and right lower and upper first molar extraction. Using a Ø2.0 trephine bur (Trehpine Drill 2 mm (3 mm OD) × 10 mm Barrel, ForeverGreen, Hong Kong) in a low-
speed motor handpiece, a round defect with a diameter of 3.0 mm in the lateral aspect of the right femur was created through the cortical bone. The whole thickness of the cortical bone was removed until narrow cavity was reached without further destroying the opposite side of the cortical bone.

After the operation on the right femur, the right maxillary and mandibular first molars were extracted using a standard protocol. A dental explorer was used as a gingival separator to disconnect the surrounding gingiva of the molar. The right mandibular and maxillary first molars were removed using a children’s extracting forceps. The extraction sites were left open, and a small cotton wool roll was pressed onto the extrac-
tion socket until bleeding stops.

2.2.1. Post-Operative Care. Post-operatively, the rats were given Enrofloxacin (Baytril 5%-enrofloxacin 250 ml, Bayer, Leverkusen, Germany) adding in drinking water, with 2 ml in 500 ml water in q72 hours. Mobic (Meloxicam, 0.6 mg/ kg) and Temgesic (Buprenorphine, Reckitt Benckiser Health, Slough, UK, 0.05 mg/kg) were administered subcutaneously (s/c) for pain relief. Animals were closely monitored until alert and drinking. Gel diets were given q72 hours post-operatively. The animal’s clinical condition, weight, and food consumption were carefully monitored. Sutures were removed at the seventh
day after the operation.

2.2.2. Micro-Computerized Tomography. All collected specimens were scanned with a micro-computerized tomography (micro-CT) system (SkyScan1076; Bruker, Kontich, Belgium) according to the manufacturer’s instructions in Department of Orthopaedics and Traumatology, Li Ka Shing Faculty of Medicine, the University of Hong Kong. The samples were scanned at an energy of at 88 kV and 100 µA intensity with a resolution of 8.665 µm pixel with a filter of 1.0 mm-thickness aluminum. Reconstruction was done using the SkyScan NRecon program (Version, 1.7.3.0, SkyScan, Kontich, Belgium). The data sets obtained after reconstruction were loaded into the SkyScan CT-analyzer software (CTan, version 1.12.0, SkyScan, Kontich, Belgium) for further assessment and three-dimensional images obtained by the CTvox software (Version 3.3, SkyScan, Kontich, Belgium).
2.2.3. Region of Interest. The whole specimens were carefully observed for any bone sequestra or periosteal reaction before selection of Volume of interest (VOI). A cylindrical region in the trabecular bone of the first molar (M1) socket was selected to be the region of interest in maxilla and mandible. Sagittally, we chose 80 layers at the coronal plane starting from the mesial surface of the second molar crown (M2) to the alveolar socket of M1 and set for the bottom layer of the VOI and then continued selection for 140 layers and set for the top layer of the VOI. Coronally, a round region of interest (ROI) of 3.00 mm in diameter was set among the trabecular bone in these 140 layers (Figure 2). For femur defect, ROI was defined as 140 layers from the level of cortical bone with a 3.0 mm diameter circle covering the defect size (Figure 2).

The trabecular microstructure was assessed using the parameters as described in Table 1.

2.2.4. Histopathology Assessments. After completion of micro-CT scanning, the specimens were trimmed to appropriate size and rinsed in tap water for two hours. After rinsing, samples were decalcified in 12.5% ethylenediaminetetraacetic acid (EDTA) (pH = 7.2) at room temperature for three months. Once decalcified, all specimens were embedded and sliced in a thickness of 5 µm using semi-automated microtome (Leica Biosystems, Wetzlar, Germany). Goldner trichrome staining was performed using a standard protocol [17].

Microscope sections were viewed under Eclipse LV 100POL (Nikon Corporation, Japan), and images were taken using DS-Ri1 high-resolution microscope camera (Nikon Corporation, Japan). All measurements were standardized and carried out by two well-trained examiners in Centralized Research Laboratory, Faculty of Dentistry, the University of Hong Kong. Osteonecrosis was determined as contiguous empty osteocytic lacunae (continued empty lacunae up to 5 in a row) in trabecular bone together with the loss of osteocytes. Semiquantitative analysis was carried out using ImageJ software (version 1.51 s, National Institutes of Health, Bethesda, USA). Three high power fields were randomly selected, and empty/viable lacunae were calculated.

2.2.5. Statistical Analysis. The data were presented as mean ± SD and were analyzed using IBM SPSS statistic software (version 24.0, IBM Crop, Armonk: NY, USA). Two-way ANOVA test was conducted to examine the effect of zoledronic acid on bone mineral density (BMD) in surgically treated jawbones and long bones in different time points. Where applicable, further assessment of Bonferroni’s multiple comparison post hoc or independent-samples t-test were used. Kruskal–Wallis test and Mann–Whitney test were used for non parametric data. The significance level was set at \( p < 0.05 \).

3. Results

3.1. Clinical Observation. All the rats completed the experiment uneventfully. Among all groups, six rats were observed with femur fracture, with two in Group Cm, one in Group Cs, one in Group ZAm, and two in Group ZAs. Three cases were observed with bone exposure (3/24; one in maxilla, two in mandible) in ZA-treated rats. Only one case in control group presented with exposed bone (1/24; in maxilla). Soft tissue fenestrations were observed in four cases (4/24) in the maxilla, and four (4/24) in the mandible in ZA-treated group, while two cases (2/24) in the mandible in control group. There were no soft tissue fenestrations or bone exposure observed in femur region in both ZA-treated and control groups.

3.2. Micro-CT Examination. Micro-CT analysis was used to better identify bone sequestrum and provide a three-dimension analysis for changes of bone density and microarchitecture (Figure 3). Periosteal reaction and bone sequestrum formation were observed.
comparison of ZA and control groups, only a marginal significance was found between long-term ZA-treated group and long-term control group ($\bar{F} = 0.049$).

In mandible tooth extraction site, an increase in bone mineral density after surgery in control group was also found. However, in ZA-treated group, the bone mineral density slightly decreased at 4 weeks after surgery compared with 2 weeks after surgery and increased to the highest in the 8 weeks group. Both two independent variables (ZA treatment and length of exposure) showed significant difference, yet the interaction effect was not significant. The main effect for treatment yielded an $F$ ratio of $\bar{F}(1, 42) = 7.858, p < 0.01$, indicating a significant difference between ZA treatment ($M = 0.856, SD = 0.09$) and control group ($M = 0.798, SD = 0.07$). The

3.2.1. Bone Mineral Density. The average bone mineral density (BMD) of mandibular and maxillary extraction sockets was higher than that in femur defect area compared between inter-groups and intra-groups (Table 2).

In femur defect healing site, a steady increase in bone mineral density was noted in ZA-treated group and control group. Inter-group comparison of different time points showed higher BMD with longer length of exposure (LoE) ($\bar{F}(2, 36) = 47.774, p < 0.001$). However, no significant difference was found between ZA-treatment group and control group in BMD. The interaction effect of ZA treatment and LoE was not significant as well. Within treatment groups and control groups, significant difference of BMD was found as longer LoE with higher BMD ($p < 0.001$); however intra-group comparison of ZA and control groups, only a marginal significance was found between long-term ZA-treated group and long-term control group ($p = 0.049$).

In mandible tooth extraction site, an increase in bone mineral density after surgery in control group was also found. However, in ZA-treated group, the bone mineral density slightly decreased at 4 weeks after surgery compared with 2 weeks after surgery and increased to the highest in the 8 weeks group. Both two independent variables (ZA treatment and length of exposure) showed significant difference, yet the interaction effect was not significant. The main effect for treatment yielded an $F$ ratio of $\bar{F}(1, 42) = 7.858, p < 0.01$, indicating a significant difference between ZA treatment ($M = 0.856, SD = 0.09$) and control group ($M = 0.798, SD = 0.07$). The
Table 1: Output parameters for micro-CT examination of trabecular bone.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Abbreviation (unit)</th>
<th>Meaningfulness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone volume/tissue volume</td>
<td>BV/TV (%)</td>
<td>The percentage of trabecular bone volume in the selected volume of interest</td>
</tr>
<tr>
<td>Bone surface/bone volume (specific bone surface)</td>
<td>BS/BV (mm⁻¹)</td>
<td>The ratio of solid surface to bone volume which characterizing the thickness and complexity of trabecular structures</td>
</tr>
<tr>
<td>Bone surface/tissue volume</td>
<td>BS/TV (mm⁻¹)</td>
<td>The ratio of solid surface in the selected volume of interest</td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>Tb.Th (mm)</td>
<td>The thickness of trabecular structures</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>Tb.N (mm⁻¹)</td>
<td>The number of traversals across a trabecular structure per unit in selected VOI</td>
</tr>
<tr>
<td>Trabecular separation</td>
<td>Tb.Sp (mm)</td>
<td>The thickness of space in selected VOI, in which higher value indicates reduced connectivity</td>
</tr>
<tr>
<td>Bone mineral density</td>
<td>BMD (g/cm³)</td>
<td>The mass of bone mineral per volume of bone</td>
</tr>
</tbody>
</table>

Figure 3: Micro-CT 3D and 2D cross-sections of maxilla (a1-f1), mandible (a2-f2), and femur defect areas (a3-f3). 3D VOI, transverse and coronal plane cross-sectional images of control groups and ZA-treated group at week 2, week 4, and week 8. Red arrow points to periosteal reaction. Green and yellow arrows indicate bone sequestrum. 3D cylindrical sub-volume covered the defect area is the VOI for assessment of BMD and trabecular indices.

The effect of LoE was also found significant, which yielded an $F$ ratio of $F(2, 42) = 10.251, p < 0.001$. In inter-group comparison between ZA and control groups, significant difference of BMD was found in long-term groups between ZA treatment and control $(p < 0.01)$, with ZA-treated group exhibiting higher bone mineral density. Intra-group comparison showed significant difference comparing long-term ZA group with medium-term and short-term $(p < 0.01)$, while in control groups, significance was found only between long-term group and short-term group $(p < 0.01)$.

In maxilla tooth extraction site, the BMD value experienced a steady increase with longer LoE in both ZA-treated group and control group. Only LoE factor showed significant difference with an $F$ ratio of $F(2, 42) = 27.919, p < 0.001$. No
Table 2: Effect of zoledronic acid on bone mineral density (mean ± SD) in femur defect, and mandibular/maxillary extraction socket.

<table>
<thead>
<tr>
<th></th>
<th>2 wk</th>
<th>4 wk</th>
<th>8 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Femur</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZA</td>
<td>0.32 ± 0.09</td>
<td>0.57 ± 0.18</td>
<td>0.79 ± 0.06*</td>
</tr>
<tr>
<td>Control</td>
<td>0.35 ± 0.16</td>
<td>0.54 ± 0.06</td>
<td>0.68 ± 0.06</td>
</tr>
<tr>
<td><strong>Mandible</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZA</td>
<td>0.84 ± 0.05</td>
<td>0.83 ± 0.09</td>
<td>0.93 ± 0.05**</td>
</tr>
<tr>
<td>Control</td>
<td>0.72 ± 0.07</td>
<td>0.81 ± 0.07</td>
<td>0.85 ± 0.03</td>
</tr>
<tr>
<td><strong>Maxilla</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZA</td>
<td>0.75 ± 0.05</td>
<td>0.84 ± 0.03</td>
<td>0.91 ± 0.08</td>
</tr>
<tr>
<td>Control</td>
<td>0.67 ± 0.09</td>
<td>0.84 ± 0.13</td>
<td>0.93 ± 0.07</td>
</tr>
</tbody>
</table>

* Differences were considered significant at p < .05. ** p < 0.01.

3.2.2. Trabecular Microarchitecture. The main effect of ZA treatment and treatment duration, and their interaction in bone microstructure are listed in Table 3.

Intra-group comparison among different time points had no significant differences in femur, mandible, and maxilla. However, the inter-group comparison between ZA-treated group and control revealed that in mandible, Tb.N was significantly higher in medium-term and long-term treatment group, and Tb.Sp were significantly lower in medium-term and long-term treatment group. The fraction of bone volume out of the total volume (BV/TV) in medium-term and long-term ZA-treated mandibular extraction socket both showed significantly higher compared with the corresponding control group. This is correlated with a reduction in the bone marrow space in the mandibular bone and between the roots of the first molar.

Overall, in femur defect, the trabecular thickness and trabecular number per mm² increased with the increasing bone volume fraction, while trabecular separation decreased. This indicated the healing of femur defect manifested as both increasing of trabecular number and thickness. Yet in mandible extraction sites, Tb.Th, Tb.N, and Tb.Sp reached plateau (though slightly increased for Tb.Th and Tb.N and slightly decreased for Tb.Sp in eight weeks group) in week 4 for control group, while for ZA-treated group, the remodeling continued to the eighth week after surgery. In maxilla extraction sites, changes of trabecular thickness through time was not significant in ZA-treated group. With increasing of bone volume in the healing process, the trabecular number increased steadily with the decreasing of trabecular separation. This indicates that the bone healing of maxilla under ZA treatment mainly resulted from the increased number of trabecular bone rather than widening of trabecular thickness, which in control group, the increase of bone mass was from both sides.

3.3. Histopathology Examination. In femur specimens, histological osteonecrosis (which is defined as continued empty lacunae up to 5 in a row) [18] was only observed in one sample in medium-term ZA-treated group and one in medium-term control group. In short-term groups, ZA-treated femur showed more connected bone structures than that in control group. Four weeks after surgery, the medium-term groups both displayed an increased volume of connected bone structures at the defect area, while ZA-treated femur showed more woven bone extending into the medullary cavity (Figure 4). At the eighth week post-operation, a nearly developed cortical bone bridge has been observed on femur defect in most of the control group, while ZA-treated femur showed less developed cortical bone and more woven bone extending into the medullary cavity.

In mandibular specimens, two weeks after surgery, the extraction socket of ZA-treated sample, as well as control sample was filled with fibrous tissue with some newly formed viable woven bone. Eight weeks after extraction, the woven bone was almost entirely replaced by lamellar bone or trabecula in the socket in most of the control samples, which showed as normal wound healing and bone remodeling. ZA-treated mandible showed delayed bone remodeling and bone necrosis in some of the cases. Histological osteonecrosis precedes clinical manifestation; there were only 1/6 of histological osteonecrosis found with clinical bone exposure in the maxilla and 2/14 in the mandible. Histological osteonecrosis was observed mostly at the top of the lingual or buccal side of the cortical bone in ZA-treated groups at all three time points, while few short-term control samples showed clustered empty lacunae in cortical area adjacent to extraction socket as well. Randomly diffused non viable osteocytes were also found in mandible samples of control groups.

In maxilla specimens, histological osteonecrosis was also found at the top of lingual or buccal maxillary cortical bone in ZA-treated groups at different time points, while only one long-term control sample was found with osteonecrosis.

The results of histological assessment of fraction of empty lacunae out of viable lacunae are shown in Table 4.

4. Discussion

The prolonged duration of drug administration has been well documented in relation to the development of MRONJ. The drug accumulating effect on different sites of skeletal bones might play a part in the site-specific effect of MRONJ. Studies showed that BPs exerted direct toxic effects on oral epithelial cells and fibroblasts when accumulated at sufficient concentrations in bone tissue [19–22]. This toxic effect further led to delayed soft tissue healing and secondary infection and resulted in necrosis of the underlying bone. However, a number of MRONJ cases occurred prior to soft tissue infection and injury [23]. Thus, the causal relationship between infection and MRONJ is still a matter of debate [24–26].

Tooth extraction is believed to be the most common predisposing event of MRONJ. A previous systematic review reported that around 61.7% of patient with MRONJ had a history of tooth extraction [27], while among cancer patient
reported. In the present study, a femur defect of 3 mm in diameter was created along with tooth extraction to provide the same surgical intervention to mandible, maxilla, and femur. ZA treatment was continued for 2, 4, and 8 weeks after the surgery. In addition to the clinical and macroscopic examination, micro/uniCT examination was used to assess bone sequestration and periosteal reaction as it provides 3D and 2D images in three different planes. Although the MRONJ staging system is based on clinical manifestations without radiological evaluation [36, 37], there are still characteristics that clinical examination could not access. Previous investigations have reported that CT examination of patients with MRONJ showed periosteal reaction, cortical perforation, periosteal bone deposition, mandibular fractures, etc., [38]. A recent study using CT receiving intravenous BPs treatment, the estimates of osteonecrosis after tooth extraction range from 1.6% to 14.8% [27–30]. The pathogenesis of MRONJ are multifactorial yet not fully explained; meanwhile, the bone-specific character of MRONJ has not been well investigated.

Given the fact a large portion of MRONJ cases had a history of tooth extraction/invasive intervention in the oral cavity, it is the oral cavity that takes up the most invasive operation rather than other parts of skeletal sites. We designed an animal model with surgical intervention at jawbones as well as long bones simultaneously. To our knowledge, animal models for MRONJ following trauma/tooth extraction have been established in many studies [31–35], yet a comparison of surgical intervention in jawbones and peripheral bone has seldom been reported. In the present study, a femur defect of 3 mm in diameter was created along with tooth extraction to provide the same surgical intervention to mandible, maxilla, and femur. ZA treatment was continued for 2, 4, and 8 weeks after the surgery. In addition to the clinical and macroscopic examination, micro-CT examination was used to assess bone sequestration and periosteal reaction as it provides 3D and 2D images in three different planes. Although the MRONJ staging system is based on clinical manifestations without radiological evaluation [36, 37], there are still characteristics that clinical examination could not access. Previous investigations have reported that CT examination of patients with MRONJ showed periosteal reaction, cortical perforation, periosteal bone deposition, mandibular fractures, etc., [38]. A recent study using CT

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**Table 3: Micro-CT assessment of morphology of jawbones and femur.**

<table>
<thead>
<tr>
<th>p-value*</th>
<th>ZA treatment</th>
<th>LoE†</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mandible</td>
<td>Maxilla</td>
<td>Femur</td>
</tr>
<tr>
<td>BV/TV (%)</td>
<td>0.000</td>
<td>0.069</td>
<td>0.000</td>
</tr>
<tr>
<td>BS/BV (mm⁻¹)</td>
<td>0.000</td>
<td>0.077</td>
<td>0.400</td>
</tr>
<tr>
<td>BS/TB (mm⁻¹)</td>
<td>0.015</td>
<td>0.451</td>
<td>0.011</td>
</tr>
<tr>
<td>Th.Th (mm)</td>
<td>0.102</td>
<td>0.403</td>
<td>0.000</td>
</tr>
<tr>
<td>Th.N (mm⁻¹)</td>
<td>0.040</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td>Th.Sp (mm)</td>
<td>0.003</td>
<td>0.148</td>
<td>0.256</td>
</tr>
</tbody>
</table>

*Significant (p-value) of trabecular indices assessed by two-way ANOVA. Differences were considered significant at p < 0.05. † LoE: length of exposure.

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**Figure 4:** Representative histological findings of osteonecrosis, inflammation and femur defect healing. (a) Bone sequestra found in ZA-treated mandible. (b) Soft tissue inflammation found in ZA-treated mandible. Scale bar = 200 µm. (c) Femur of long-term control group, formation of cortical bridge at defect site. (d) Femur of long-term ZA group, spongy bone without maturation into cortical bone in defect site. Scale bar = 500 µm. NB: necrotic bone; CT: connect tissue; IF: inflammatory infiltration; EP: epithelium; LB: lamellar bone; WB: woven bone.
TABLE 4: Histological assessment of fraction of empty lacunae out of viable lacunae.

<table>
<thead>
<tr>
<th>Empty/viable</th>
<th>Short-term</th>
<th>Medium-term</th>
<th>Long-term</th>
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<tbody>
<tr>
<td>Femur</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZA</td>
<td>0.09 ± 0.08</td>
<td>0.08 ± 0.09</td>
<td>0.14 ± 0.07</td>
</tr>
<tr>
<td>Control</td>
<td>0.18 ± 0.29</td>
<td>0.04 ± 0.03</td>
<td>0.08 ± 0.06</td>
</tr>
<tr>
<td>Mandible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZA</td>
<td>0.52 ± 0.66*</td>
<td>0.22 ± 0.26*</td>
<td>0.30 ± 0.57*</td>
</tr>
<tr>
<td>Control</td>
<td>0.10 ± 0.12</td>
<td>0.04 ± 0.03</td>
<td>0.06 ± 0.06</td>
</tr>
<tr>
<td>Maxilla</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>ZA</td>
<td>0.28 ± 0.38</td>
<td>0.12 ± 0.07***</td>
<td>0.23 ± 0.26***</td>
</tr>
<tr>
<td>Control</td>
<td>0.10 ± 0.11</td>
<td>0.02 ± 0.02</td>
<td>0.03 ± 0.03</td>
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</tbody>
</table>

* Differences were considered significant at \( p < 0.05 \), \( \ast p < 0.05 \), \( \ast\ast\ast p < 0.001 \).
oral diseases, angiogenesis inhibition may play a more crucial role in the site-specific character of MRONJ.

5. Conclusions

The present study showed that zoledronic acid treatment did have site-specific effect on surgically treated jawbones versus long bones, where necrosis only occurred in the jaw. Osteonecrosis occurred more frequently at the site of the mandibles than maxillae. However, there is no noticeable difference concerning the changes in bone tissue healing under ZA treatment between femur and jawbones. The length of exposure of ZA did not seem to affect trabecular microstructure and fraction of empty lacunae/viable lacunae; it only showed higher bone volume and BMD with longer healing time which is expected in the healing process.
This study has the limitation that the femur and jawbones did not go through precisely the same surgical procedure, which might render differences in the bone healing process. However, to better mimic the real situation, we chose tooth extraction procedure rather than creating a same sized defect in jawbones. The latter case will be more challenging to produce and not in accordance with clinical reality.

Data Availability

The datasets used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References


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