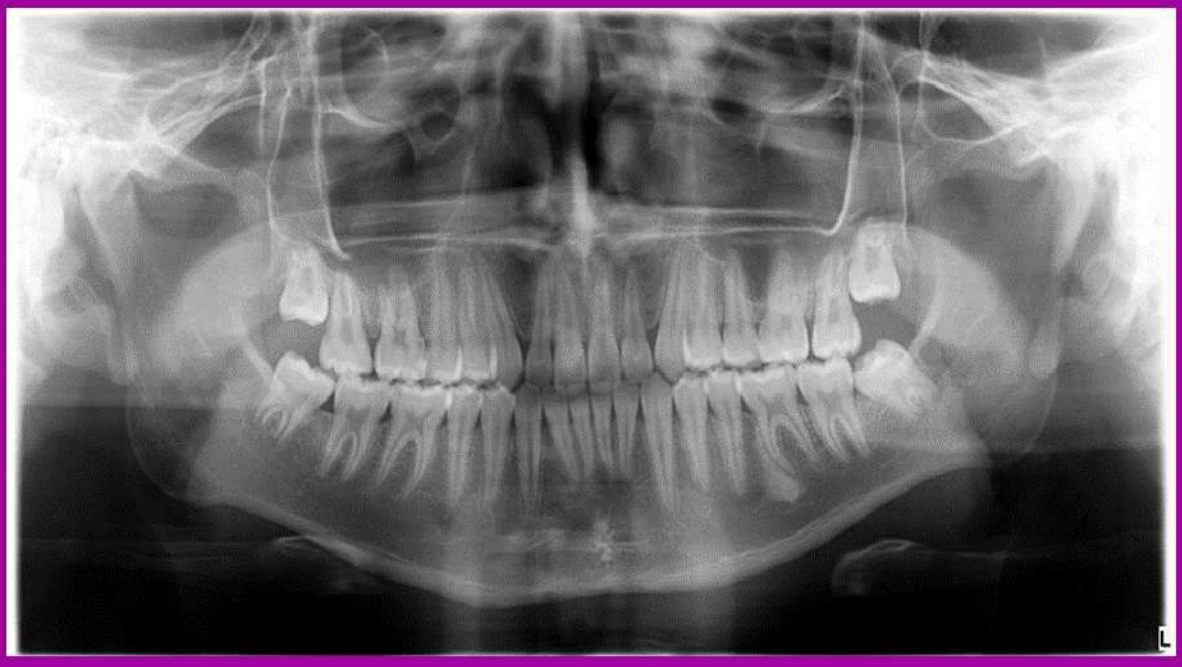
E-ISSN: 22502-0412

JDS

Journal of Syiah Kuala Dentistry Society

Vol 9 No. 2, December 2024



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JDS Journal of Syiah Kuala Dentistry Society
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E-ISSN: 22502-0412
JDS (Journal of Syiah Kuala Dentistry Society) Phone: +62 651-7555183 Email: jds.fkg@usk.ac.id

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Vol 9, No 2 (2024)

December 2024

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JDS Journal of Syiah Kuala Dentistry Society

https://jurnal.usk.ac.id/JDS ORIGINAL RESEARCH
E-ISSN: 2502-0412
Vol. 9 (2) 169–176. December 2024
DOI Prefix: Prefix 10.24815

The Number of Alveolar Bone Cells Exposed to Zoledronate in the Post-Tooth Extraction Socket

Septina Sabilla Lubis[1], Anggraeny Putri Sekar Palupi[2], Komariah[3], Benny Syariefsjah Latief[4], Bambang Pontjo Priosoeryanto[5], Dewi Fatma Suniarti[6]

[1] Under graduate, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia
[2]Departement of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia
[3]Departement of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jakarta Indonesia
[4]Departement of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia
[5]Departement of Veterinary Pathology, Faculty of Veterinary Medicine, IPB University, Jawa Barat, Indonesia
[6]Departement of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia

Corresponding Author: anggraeny@trisakti.ac.id

ARTICLE INFO : Received, May 11, 2024; Revised, August 13, 2024; Accepted, September 26, 2024; Published, December 16, 2024
ORCID : Lubis SS (0009-0001-6853-4231); Palupi APS (0000-0002-5128-9604);Komariah (0000-0001-8945-716X);Latief BS (0000-0003-1708-0628); Priosoeryanto BP (0000-0001-5942-7402); Suniarti DF (0000-0001-5064-7361)
DOI : 10.24815/jds.v9i2.41502

ABSTRACT

Background: Zoledronate is an intravenous bisphosphonate that effectively treats osteoporosis by inhibiting osteoclast resorption, thereby reducing the risk of fractures. However, its use in post-extraction patients may increase the risk of *bisphosphonate-related osteonecrosis of the jaws* (BRONJ). **Objective:** This study aims to analyze the effects of zoledronate on alveolar bone structure in Sprague Dawley rats, focusing on changes in osteoblast, osteocyte, and osteoclast counts. **Methods:** This cross-sectional study employed an observational analytical approach. Samples were categorized based on zoledronate exposure. Osteoblast, osteocyte, and osteoclast counts were measured using ImageJ software at 40x magnification. Data were analyzed using SPSS, with one-way ANOVA employed for statistical analysis. Normality and homogeneity tests were conducted to ensure data validity. **Results:** Zoledronate exposure resulted in a decrease in osteoblast and osteoclast counts, while osteocyte counts increased. Statistical analysis showed no significant differences between the zoledronate-exposed group and the control group. **Conclusion:** Zoledronate reduces osteoclasts and increases osteocytes, potentially leading to cellular apoptosis in osteoporosis patients. The risk of BRONJ can be minimized by administering zoledronate at appropriate doses and maintaining optimal oral hygiene to prevent bacterial activity.

Keywords: Zoledronate, Osteoporosis, BRONJ, Osteoblasts, Osteocytes, Osteoclasts

ABSTRAK

Latar Belakang: Zoledronate adalah bisfosfonat intravena yang efektif dalam mengobati osteoporosis dengan menghambat resorpsi osteoklas, sehingga mengurangi risiko patah tulang. Namun, penggunaannya pada pasien pasca-ekstraksi gigi dapat meningkatkan risiko *bisphosphonate-related osteonecrosis of the jaws* (BRONJ). **Tujuan:** Penelitian ini bertujuan untuk menganalisis efek zoledronate terhadap struktur tulang alveolar pada tikus Sprague Dawley dengan fokus pada perubahan jumlah osteoblas, osteosit, dan osteoklas. **Metode:** Studi ini menggunakan desain *cross-sectional* dengan pendekatan observasional analitik. Sampel dibagi berdasarkan perlakuan zoledronate. Jumlah sel osteoblas, osteosit, dan osteoklas dihitung menggunakan perangkat lunak ImageJ pada perbesaran 40x. Data dianalisis menggunakan SPSS dengan uji *one-way ANOVA*, serta uji normalitas dan homogenitas untuk validasi. **Hasil:** Kelompok yang terpapar zoledronate mengalami penurunan jumlah osteoblas dan osteoklas, sementara jumlah osteosit meningkat. Uji statistik menunjukkan tidak ada perbedaan signifikan antara kelompok yang terpapar zoledronate dan yang tidak. **Kesimpulan:** Zoledronate mengurangi osteoklas dan meningkatkan osteosit, yang dapat menyebabkan apoptosis seluler pada pasien osteoporosis. BRONJ dapat dicegah dengan pemberian dosis zoledronate yang tepat serta menjaga kebersihan mulut untuk menghambat aktivitas bakteri.

Kata kunci: Zoledronate, Osteoporosis, BRONJ, Osteoblas, Osteosit, Osteoklas

1. Introduction

Osteoporosis is a skeletal disorder characterized by reduced bone mineral density (BMD) and deterioration of bone structure, making bones fragile and prone to fractures.[1] The prevalence of

osteoporosis is influenced by factors such as age, gender, and ethnicity, with older populations being at higher risk. Osteoporosis is diagnosed when the T-score is equal to or less than -2.5, indicating a bone density that is 2.5 standard deviations below the average for healthy young adults.[2] It is estimated that over 200 million people worldwide suffer from osteoporosis, with a prevalence of 10.3% in Indonesia.[3] Primary osteoporosis typically occurs in individuals over 70 years old and postmenopausal women, while secondary osteoporosis results from underlying diseases, medication use, or idiopathic causes. Lifestyle modifications, including increased physical activity, smoking cessation, alcohol reduction, fall prevention, and adequate calcium and vitamin D intake, play a crucial role in improving bone health.[4]

To counteract bone resorption in osteoporosis, anti-resorptive agents such as bisphosphonates are widely used. Bisphosphonates are classified into two generations: non-nitrogen-containing bisphosphonates (clodronate, etidronate, and tiludronate) and nitrogen-containing bisphosphonates (alendronate, risedronate, ibandronate, and zoledronate).[5] While effective, bisphosphonates are associated with complications such as *bisphosphonate-related osteonecrosis of the jaws* (BRONJ), a condition that can develop following tooth extraction or invasive dental procedures. BRONJ risk factors include poor oral hygiene, pre-existing dental disease, cancer, chemotherapy, and glucocorticoid therapy.[6] Since Marx first reported BRONJ cases in 2003, the condition has been increasingly recognized worldwide, including in Japan.[7]

Tooth extraction is a common dental procedure in which a tooth is removed from the alveolar socket. The primary reasons for tooth extractions include periodontal disease (41.8%) and dental caries (32.7%).[8] Other indications include pulp disease, retained roots, orthodontic treatment, malpositioned or impacted teeth, supernumerary teeth, pathological abnormalities, fractures, aesthetic considerations, and economic factors.[9] Bone is a highly dynamic tissue that undergoes continuous remodeling, a process involving osteoblasts, osteocytes, and osteoclasts to regulate bone formation and resorption. Osteoblasts are crucial for bone development as they produce and deposit osteoid, the organic matrix of bone. Osteocytes, which are osteoblasts embedded within the bone matrix, function as mechanosensors regulating bone remodeling.[10] Meanwhile, osteoclasts are found on bone surfaces, playing a role in bone resorption, regeneration, and repair.[11]

This study investigated the effects of zoledronate on alveolar bone healing using Sprague Dawley rats, which are commonly used in bone metabolism and calcium regulation research due to their physiological similarities to humans. The study involved four groups of rats: (1) those that underwent ovariectomy (OVX) and received a single zoledronate injection, (2) OVX rats that received six zoledronate injections, (3) OVX rats treated with saline solution, and (4) a control group that underwent sham surgery (without ovariectomy) and received intravenous saline injections. The analysis was performed using Hematoxylin and Eosin (H&E) staining, a widely applied technique for examining tissue and cellular structures. The primary objective was to assess the impact of zoledronate on osteoblast, osteocyte, and osteoclast populations in alveolar bone healing following tooth extraction. Understanding how zoledronate affects post-extraction bone healing is critical for optimizing osteoporosis management while minimizing BRONJ risks, ultimately contributing to safer therapeutic strategies for osteoporosis patients requiring dental extractions or oral surgical procedures.

2. Material and Methods

The research samples consisted of histological sections from the alveolar bone of the lower jaw of Sprague Dawley rats that had undergone four different treatments in previous studies. Histological images revealed osteoblasts, osteocytes, and osteoclasts in rats that had undergone ovariectomy (OVX) and received either a single zoledronate injection or six injections, as well as those treated with saline solution. Additionally, a control group of rats that underwent sham surgery (surgery without ovariectomy) and received intravenous saline injections was included. Ethical exemption for this research was granted by the Health Research Ethics Commission, Faculty of Dentistry, Trisakti University, under approval number 645/S1/KEPK/FKG/7/2023.

2.1. Tooth Extraction Methods

Adherence to correct extraction techniques ensures a successful and atraumatic tooth removal. Three essential principles must be met: adequate access and visualization, an unobstructed extraction pathway, and controlled force application during luxation and extraction. Tooth extraction

methods are categorized into two types: the closed method (simple or forceps technique) and the open method (surgical or flap technique). The closed method is the most commonly used, involving forceps and an elevator to remove the tooth or remaining root from the socket. [14] This method consists of two stages: first, the tooth is loosened from the surrounding soft tissue using an elevator, and then it is extracted with forceps or an elevator. In contrast, the open (surgical) method is employed in specific cases, such as when the tooth has a fragile crown, retained roots, divergent roots, root proximity to the sinus, or root hypercementosis.[12]

2.2. Wound Healing After Tooth Extraction

Wound healing after tooth extraction is observed to occur more rapidly in the oral cavity than on the skin due to the presence of saliva, which contains essential proteins for the healing process. The healing process, known as socket healing, is categorized into three phases: inflammatory (24–48 hours), proliferative (48 hours–5 days), and remodeling (5 days–several months). In the inflammatory phase, blood clot formation is initiated, and inflammatory cells are migrated to the extraction site, where granulation tissue is gradually formed and later replaced by connective tissue containing collagen fibers to facilitate bone regeneration. During the proliferative phase, tissue formation occurs rapidly, capillary ingrowth from surrounding blood vessels is promoted, and fibroblast activity increases, leading to wound contraction and the development of scar tissue. In the final remodeling phase, woven bone is progressively replaced by lamellar bone and bone marrow through osteon formation, and the healing process is considered complete once bone marrow renewal has occurred.[13]

2.3. Hematoxylin and Eosin (HE) Staining

Histological staining techniques are classified into two categories: general staining using hematoxylin-eosin and specialized histochemical or immunohistochemical staining. Histochemical staining highlights biological structures and identifies various tissue components, employing stains such as Periodic Acid Schiff (PAS), Toluidine Blue, and Giemsa. Immunohistochemical staining, on the other hand, relies on antigen-antibody interactions combined with enzymatic reactions to identify specific tissue structures. In this study, hematoxylin and eosin (HE) staining was employed due to its ability to differentiate nuclear and cytoplasmic structures effectively. Hematoxylin stains cell nuclei blue, while eosin imparts a pink hue to the cytoplasm and extracellular matrix. Bone tissue comprises four primary cell types: osteoblasts, bone lining cells, osteocytes, and osteoclasts. Osteoblasts, which are initially larger, eventually differentiate into osteocytes, which are smaller, elongated cells encased in lacunae. Histological preparations of the alveolar bone sockets of Sprague Dawley rats were stained with HE to enhance microscopic observation, facilitating the identification of cellular structures and tissue differentiation.[14]

3. Result and Discussion

The study analyzed 16 histological bone preparations of Sprague Dawley rats, with each treatment group consisting of 4 preparations. Once all relevant data were collected, the samples were categorized based on treatment type, and the number of osteoblasts, osteocytes, and osteoclasts was determined using the ImageJ application at 40x magnification. The collected data were then statistically processed using the SPSS application to assess variations in cell counts across different treatment groups.

Figure 1 illustrates the profile of bone cells observed in the histological sections. Image **(A)** highlights the differences in size and location between osteoblasts and osteocytes, where osteoblasts are found along the bone surface, while osteocytes are embedded within the bone matrix. Image **(B)** presents osteoblasts and osteoclasts, showing osteoclasts as larger, multinucleated cells responsible for bone resorption, whereas osteoblasts are smaller and involved in bone formation. The histological staining with Hematoxylin and Eosin (HE) facilitated clear differentiation of these cell types, allowing for precise identification and quantification of bone cells across different experimental conditions.

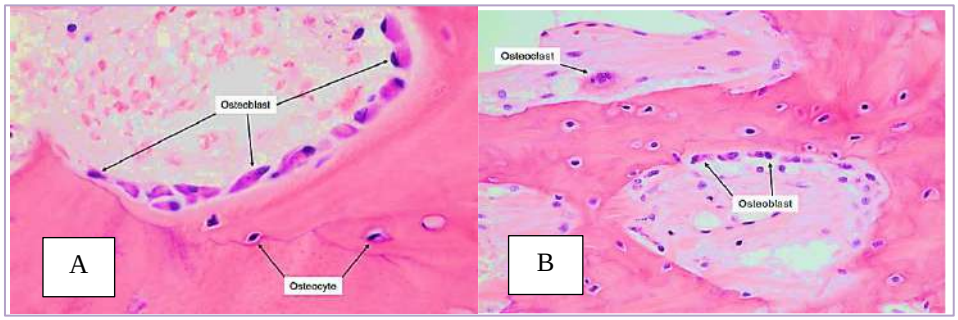

Figure 1. Profil of Bone Cells. (A) Differences in size and location between osteoblasts and osteocytes. (B) Osteoblasts and osteoclasts.

Table 1 presents the means and standard deviations of osteoblast, osteocyte, and osteoclast counts across four experimental groups, reflecting the effects of different treatments on bone cell populations. Group A consisted of rats that underwent ovariectomy (OVX) and received a single zoledronate injection, while Group B received six zoledronate injections after OVX. Group C underwent OVX but was treated with saline water instead of zoledronate, serving as a non-bisphosphonate-treated OVX group. Group D, as the control group, underwent sham surgery without OVX and received saline water.

The results indicate that Group C exhibited the highest osteoblast count (8.25 ± 5.439), while Group B showed the lowest (2.00 ± 1.414). Group A and Group D displayed intermediate osteoblast counts, with means of 3.50 ± 3.109 and 5.50 ± 6.191, respectively. Regarding osteocytes, Group B recorded the highest mean count (46.50 ± 21.440), whereas Group C had the lowest (26.75 ± 16.500). Groups A and D reported osteocyte counts of 30.50 ± 3.873 and 39.25 ± 9.287, respectively. For osteoclasts, Group A exhibited the highest mean count (1.00 ± 2.000), while no osteoclasts were detected in Group B (0.00). Groups C and D demonstrated relatively low osteoclast counts, with means of 0.75 ± 0.957 and 0.50 ± 1.000, respectively.

These findings suggest that zoledronate administration influences bone cell activity, particularly osteoblast and osteocyte populations. Multiple doses of zoledronate (Group B) appear to be associated with a decrease in osteoblast counts and an increase in osteocytes, possibly indicating an effect on bone remodeling processes. Meanwhile, osteoclast counts remained consistently low across all groups, further supporting the inhibitory role of zoledronate in bone resorption.

Table 1. Means and Standar Deviations

Variabel	Group	Average	Standard Deviation
osteoblast	Group A	3.50	3.109
	Group B	2.00	1.414
	Group C	8.25	5.439
	Group D	5.50	6.191
osteocyte	Group A	30.50	3.873
	Group B	46.50	21.440
	Group C	26.75	16.500
	Group D	39.25	9.287
osteoclasts	Group A	1.00	2.000
	Group B	0.00	-
	Group C	0.75	0.957
	Group D	0.50	1.000

Table 2 presents the results of statistical analyses using the ANOVA and Kruskal-Wallis tests to evaluate differences in osteoblast, osteocyte, and osteoclast counts among the four experimental groups (A, B, C, and D). The ANOVA test for osteoblasts resulted in a p-value of 0.272, while the

Kruskal-Wallis test showed a p-value of 0.558, indicating no statistically significant differences in osteoblast counts among the groups. This suggests that variations in treatment did not substantially affect osteoblast populations. For osteocytes, the ANOVA test yielded a p-value of 0.261, suggesting that no significant differences were observed among the groups, and the Kruskal-Wallis test was not applied, implying that the data met the assumptions for parametric testing. Regarding osteoclasts, the ANOVA test resulted in a p-value of 0.501, showing no statistically significant differences across the groups, and similar to osteocytes, the Kruskal-Wallis test was not used, indicating the data were suitable for ANOVA analysis. Overall, these statistical results suggest that the administration of zoledronate and other experimental treatments did not lead to significant variations in osteoblast, osteocyte, or osteoclast counts. The absence of statistical significance implies that the differences in cell counts observed among the groups may not be biologically meaningful, highlighting the need for further investigation into the effects of zoledronate on bone cell activity.

Table 2. ANOVA and Kruskal-Wallis Test

Variabel	Group	ANOVA	*Kruskal-Wallis*
osteoblast	Group A Group B Group C Group D	$p = 0.272$	$p = 0.558$
osteocyte	Group A Group B Group C Group D	$p = 0.261$	
osteoclasts	Group A Group B Group C Group D	$p = 0.501$	

Figure 2 illustrates the average number of osteoblasts, osteocytes, and osteoclasts across the four experimental groups (A, B, C, and D). The first graph represents the osteoblast count, showing that Group C exhibited the highest number (8.25), while Group B had the lowest (2.00). Groups A and D displayed intermediate values of 3.50 and 5.50, respectively. The second graph presents the osteocyte count, where Group B recorded the highest value (46.50), whereas Group C had the lowest (26.75). Groups A and D showed counts of 30.50 and 39.25, respectively. The third graph displays the osteoclast count, revealing that Group A had the highest value (1.00), while Group B had no detectable osteoclasts (0.00). Groups C and D showed relatively low osteoclast counts of 0.75 and 0.50, respectively. These visual representations support the numerical data in Table 1, highlighting variations in bone cell populations across different treatment groups.

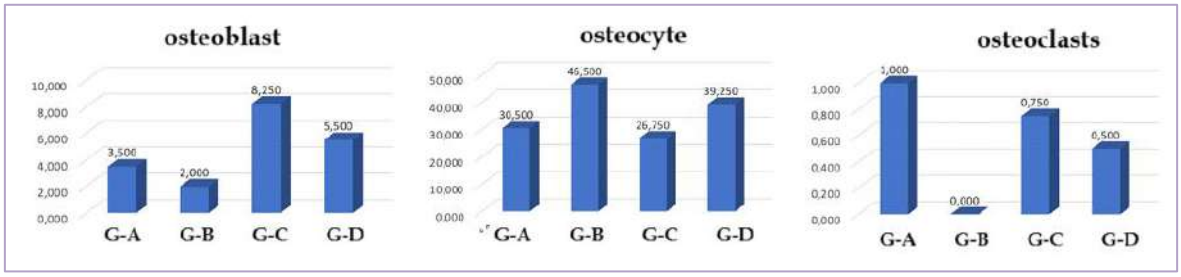

Figure 2. Bar graph of the average number of bone cells.

Osteoporosis is a condition characterized by decreased bone mineral density, leading to weakened bone strength and an increased risk of fractures.[15] Women are more susceptible to osteoporosis due to the role of estrogen in bone remodeling, which regulates osteoclast apoptosis. A reduction in estrogen levels, particularly after menopause, disrupts bone remodeling by increasing osteoclast activity, leading to excessive bone resorption and decreased bone formation.[16] This study utilized an ovariectomy (OVX) model in Sprague Dawley rats to mimic estrogen deficiency-induced osteoporosis, a method validated by previous studies showing significant reductions in bone density and altered trabecular microarchitecture in OVX rats.[17]

Bisphosphonates, including zoledronate, are widely used for osteoporosis treatment due to their strong antiresorptive effects. By inhibiting osteoclast activity, bisphosphonates reduce bone resorption, thereby lowering the risk of fractures. However, their impact on osteocytes and osteoblasts remains debated. Zoledronate has been shown to prevent osteocyte apoptosis, which may disrupt osteoblast formation and contribute to bone necrosis.[18] This study examined the effects of zoledronate on alveolar bone remodeling in OVX rats, comparing different dosages and treatment groups. The experimental groups included rats that received either a single or six intravenous zoledronate injections, OVX rats treated with saline, and a control group that underwent sham surgery and received saline injections.

The results showed a decrease in osteoblast counts and an increase in osteocytes in the zoledronate-exposed groups compared to the OVX group treated with saline. These findings align with prior research suggesting that high doses of bisphosphonates suppress bone remodeling by limiting osteoblast activity.[19] Furthermore, von Kossa staining quantification revealed a 33% reduction in bone formation in bisphosphonate-exposed groups compared to the control group.[20] However, some studies have contradicted these results, reporting that bisphosphonates can enhance osteoblast differentiation and maintain bone remodeling by reducing osteoclast activity.[21] For example, Plotkin et al. (2020) found that bisphosphonates prolong osteocyte lifespan and improve bone integrity by preventing excessive osteoclast-mediated resorption.[22] Nevertheless, other researchers support the findings of this study, suggesting that zoledronate significantly reduces osteoblast numbers, leading to impaired synthesis and mineralization of the bone matrix in a dose-dependent manner ($p > 0.05$).[23]

In addition, a decrease in osteoclast counts was observed when zoledronate was administered six times, supporting previous studies demonstrating the inhibitory effect of zoledronate on osteoclast activity in osteoporosis.[24] Research has shown that zoledronate disrupts the mevalonate pathway by inhibiting farnesyl pyrophosphate synthase, preventing the formation of geranylgeranyl pyrophosphate (GGPP).[25] This disruption inhibits the prenylation of small GTPase proteins such as Ras and Rho, which are essential for osteoclast cytoskeletal integrity and intracellular signaling. As a result, osteoclast apoptosis increases, leading to a decline in osteoclast numbers and reduced bone resorption activity.[26]

Statistical analysis using one-way ANOVA revealed no significant differences in osteoblast and osteoclast counts among the groups. However, descriptive analysis of graphical data indicated that the zoledronate-exposed groups had the lowest osteoblast and osteoclast counts. This supports previous findings that prolonged bisphosphonate therapy may lead to alterations in bone remodeling dynamics. Consequently, dentists must exercise caution when performing tooth extractions in osteoporosis patients receiving zoledronate therapy, as prolonged bisphosphonate use has been linked to post-extraction osteonecrosis.[27]

Despite these findings, this study had several limitations. The small sample size of alveolar bone preparations made it challenging to accurately count osteoblasts, osteocytes, and osteoclasts at 40x magnification. Furthermore, histological preparation quality was suboptimal, with potential issues in tissue sectioning and imaging affecting accuracy. Prior studies, including those by Kostenuik P (2021), have highlighted variability in bone turnover rates in bisphosphonate-treated models, making precise assessment challenging.[28] Given these limitations, further research using larger sample sizes, enhanced histological techniques, and additional molecular markers is necessary to gain a more comprehensive understanding of zoledronate's effects on alveolar bone remodeling.

4. Conclusion

This study examined the effects of zoledronate on alveolar bone remodeling in Sprague Dawley rats after ovariectomy (OVX), revealing a decrease in osteoblasts, an increase in osteocytes, and a reduction in osteoclasts, particularly with repeated zoledronate administration. While statistical analysis showed no significant differences, descriptive data indicated lower osteoblast and osteoclast counts in zoledronate-treated groups. These findings highlight zoledronate's inhibitory effect on bone resorption but also suggest potential disruptions in bone formation. Clinically, caution is advised when performing tooth extractions in osteoporosis patients receiving zoledronate to minimize the risk of bisphosphonate-related osteonecrosis of the jaw. Further research with larger samples and improved histological techniques is needed to clarify zoledronate's long-term impact on bone remodeling.

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Authors Contribution

Contribution	Lubis SS,	Palupi APS,	Komariah	Latief BS,	Priosoeryanto BP	Suniarti DF.
Concepts or ideas		√				
Design		√	√	√	√	√
Definition of intellectual content	√	√	√	√	√	√
Literature search	√	√	√	√	√	√
Experimental studies	√	√	√	√	√	
Data acquisition	√	√	√	√	√	√
Data analysis	√	√	√	√	√	√
Statistical analysis	√	√				
Manuscript preparation	√	√	√	√		
Manuscript editing	√	√	√	√	√	√
Manuscript review	√	√	√	√	√	√

Citation Format: Lubis SS, Palupi APS, Komariah, Latief BS, Priosoeryanto BP, Suniarti DF. The number of alveolar bone cells exposed to zoledronate in the post-tooth extraction socket. J Syiah Kuala Dent Soc. 2024; 9(2): 169–176.

The Number of Alveolar Bone Cells Exposed to Zoledronate in the Post-Tooth Extraction Socket

by Anggraeny Putri Sekar Palupi FKG

Submission date: 22-Jul-2025 09:23AM (UTC+0700)
Submission ID: 2718787575
File name: 41502-153396-3-PB_anggraeny_Putri.pdf (332.81K)
Word count: 4621
Character count: 27860

JDS
Journal of Syiah Kuala Dentistry Society

https://jurnal.usk.ac.id/JDS

ORIGINAL RESEARCH
E-ISSN: 2502-0412
Vol. 9 (2) 169-176. December 2024
DOI Prefix: Prefix 10.24815

The Number of Alveolar Bone Cells Exposed to Zoledronate in the Post-Tooth Extraction Socket

Septina Sabilla Lubis[1], Anggraeny Putri Sekar Palupi[2], Komariah[3], Benny Syariefsjah Latief[4], Bambang Pontjo Priosoeryanto[5], Dewi Fatma Suniarti[6]

[1] Under graduate, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia
[2]Departement of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia
[3]Departement of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jakarta Indonesia
[4]Departement of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia
[5]Departement of Veterinary Pathology, Faculty of Veterinary Medicine, IPB University, Jawa Barat, Indonesia
[6]Departement of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia

Corresponding Author: anggraeny@trisakti.ac.id

ARTICLE INFO : Received, May 11, 2024; Revised, August 13, 2024; Accepted, September 26, 2024; Published, December 16, 2024
ORCID : Lubis SS (0009-0001-6853-4231); Palupi APS (0000-0002-5128-9604);Komariah (0000-0001-8945-716X);Latief BS (0000-0003-1708-0628); Priosoeryanto BP (0000-0001-5942-7402); Suniarti DF (0000-0001-5064-7361)
DOI : 10.24815/jds.v9i2.41502

ABSTRACT

Background: Zoledronate is an intravenous bisphosphonate that effectively treats osteoporosis by inhibiting osteoclast resorption, thereby reducing the risk of fractures. However, its use in post-extraction patients may increase the risk of *bisphosphonate-related osteonecrosis of the jaws* (BRONJ). **Objective:** This study aims to analyze the effects of zoledronate on alveolar bone structure in Sprague Dawley rats, focusing on changes in osteoblast, osteocyte, and osteoclast counts. **Methods:** This cross-sectional study employed an observational analytical approach. Samples were categorized based on zoledronate exposure. Osteoblast, osteocyte, and osteoclast counts were measured using ImageJ software at 40x magnification. Data were analyzed using SPSS, with one-way ANOVA employed for statistical analysis. Normality and homogeneity tests were conducted to ensure data validity. **Results:** Zoledronate exposure resulted in a decrease in osteoblast and osteoclast counts, while osteocyte counts increased. Statistical analysis showed no significant differences between the zoledronate-exposed group and the control group. **Conclusion:** Zoledronate reduces osteoclasts and increases osteocytes, potentially leading to cellular apoptosis in osteoporosis patients. The risk of BRONJ can be minimized by administering zoledronate at appropriate doses and maintaining optimal oral hygiene to prevent bacterial activity.

Keywords: Zoledronate, Osteoporosis, BRONJ, Osteoblasts, Osteocytes, Osteoclasts

ABSTRAK

Latar Belakang: Zoledronate adalah bisfosfonat intravena yang efektif dalam mengobati osteoporosis dengan menghambat resorpsi osteoklas, sehingga mengurangi risiko patah tulang. Namun, penggunaannya pada pasien pasca-ekstraksi gigi dapat meningkatkan risiko *bisphosphonate-related osteonecrosis of the jaws* (BRONJ). **Tujuan:** Penelitian ini bertujuan untuk menganalisis efek zoledronate terhadap struktur tulang alveolar pada tikus Sprague Dawley dengan fokus pada perubahan jumlah osteoblas, osteosit, dan osteoklas. **Metode:** Studi ini menggunakan desain *cross-sectional* dengan pendekatan observasional analitik. Sampel dibagi berdasarkan perlakuan zoledronate. Jumlah sel osteoblas, osteosit, dan osteoklas dihitung menggunakan perangkat lunak ImageJ pada perbesaran 40x. Data dianalisis menggunakan SPSS dengan uji *one-way ANOVA*, serta uji normalitas dan homogenitas untuk validasi. **Hasil:** Kelompok yang terpapar zoledronate mengalami penurunan jumlah osteoblas dan osteoklas, sementara jumlah osteosit meningkat. Uji statistik menunjukkan tidak ada perbedaan signifikan antara kelompok yang terpapar zoledronate dan yang tidak. **Kesimpulan:** Zoledronate mengurangi osteoklas dan meningkatkan osteosit, yang dapat menyebabkan apoptosis seluler pada pasien osteoporosis. BRONJ dapat dicegah dengan pemberian dosis zoledronate yang tepat serta menjaga kebersihan mulut untuk menghambat aktivitas bakteri.

Kata kunci: Zoledronate, Osteoporosis, BRONJ, Osteoblas, Osteosit, Osteoklas

1. Introduction

Osteoporosis is a skeletal disorder characterized by reduced bone mineral density (BMD) and deterioration of bone structure, making bones fragile and prone to fractures.[1] The prevalence of

osteoporosis is influenced by factors such as age, gender, and ethnicity, with older populations being at higher risk. Osteoporosis is diagnosed when the T-score is equal to or less than -2.5, indicating a bone density that is 2.5 standard deviations below the average for healthy young adults.[2] It is estimated that over 200 million people worldwide suffer from osteoporosis, with a prevalence of 10.3% in Indonesia.[3] Primary osteoporosis typically occurs in individuals over 70 years old and postmenopausal women, while secondary osteoporosis results from underlying diseases, medication use, or idiopathic causes. Lifestyle modifications, including increased physical activity, smoking cessation, alcohol reduction, fall prevention, and adequate calcium and vitamin D intake, play a crucial role in improving bone health.[4]

To counteract bone resorption in osteoporosis, anti-resorptive agents such as bisphosphonates are widely used. Bisphosphonates are classified into two generations: non-nitrogen-containing bisphosphonates (clodronate, etidronate, and tiludronate) and nitrogen-containing bisphosphonates (alendronate, risedronate, ibandronate, and zoledronate).[5] While effective, bisphosphonates are associated with complications such as *bisphosphonate-related osteonecrosis of the jaws* (BRONJ), a condition that can develop following tooth extraction or invasive dental procedures. BRONJ risk factors include poor oral hygiene, pre-existing dental disease, cancer, chemotherapy, and glucocorticoid therapy.[6] Since Marx first reported BRONJ cases in 2003, the condition has been increasingly recognized worldwide, including in Japan.[7]

Tooth extraction is a common dental procedure in which a tooth is removed from the alveolar socket. The primary reasons for tooth extractions include periodontal disease (41.8%) and dental caries (32.7%).[8] Other indications include pulp disease, retained roots, orthodontic treatment, malpositioned or impacted teeth, supernumerary teeth, pathological abnormalities, fractures, aesthetic considerations, and economic factors.[9] Bone is a highly dynamic tissue that undergoes continuous remodeling, a process involving osteoblasts, osteocytes, and osteoclasts to regulate bone formation and resorption. Osteoblasts are crucial for bone development as they produce and deposit osteoid, the organic matrix of bone. Osteocytes, which are osteoblasts embedded within the bone matrix, function as mechanosensors regulating bone remodeling.[10] Meanwhile, osteoclasts are found on bone surfaces, playing a role in bone resorption, regeneration, and repair.[11]

This study investigated the effects of zoledronate on alveolar bone healing using Sprague Dawley rats, which are commonly used in bone metabolism and calcium regulation research due to their physiological similarities to humans. The study involved four groups of rats: (1) those that underwent ovariectomy (OVX) and received a single zoledronate injection, (2) OVX rats that received six zoledronate injections, (3) OVX rats treated with saline solution, and (4) a control group that underwent sham surgery (without ovariectomy) and received intravenous saline injections. The analysis was performed using Hematoxylin and Eosin (H&E) staining, a widely applied technique for examining tissue and cellular structures. The primary objective was to assess the impact of zoledronate on osteoblast, osteocyte, and osteoclast populations in alveolar bone healing following tooth extraction. Understanding how zoledronate affects post-extraction bone healing is critical for optimizing osteoporosis management while minimizing BRONJ risks, ultimately contributing to safer therapeutic strategies for osteoporosis patients requiring dental extractions or oral surgical procedures.

2. Material and Methods

The research samples consisted of histological sections from the alveolar bone of the lower jaw of Sprague Dawley rats that had undergone four different treatments in previous studies. Histological images revealed osteoblasts, osteocytes, and osteoclasts in rats that had undergone ovariectomy (OVX) and received either a single zoledronate injection or six injections, as well as those treated with saline solution. Additionally, a control group of rats that underwent sham surgery (surgery without ovariectomy) and received intravenous saline injections was included. Ethical exemption for this research was granted by the Health Research Ethics Commission, Faculty of Dentistry, Trisakti University, under approval number 645/S1/KEPK/FKG/7/2023.

2.1. Tooth Extraction Methods

Adherence to correct extraction techniques ensures a successful and atraumatic tooth removal. Three essential principles must be met: adequate access and visualization, an unobstructed extraction pathway, and controlled force application during luxation and extraction. Tooth extraction

methods are categorized into two types: the closed method (simple or forceps technique) and the open method (surgical or flap technique). The closed method is the most commonly used, involving forceps and an elevator to remove the tooth or remaining root from the socket. [14] This method consists of two stages: first, the tooth is loosened from the surrounding soft tissue using an elevator, and then it is extracted with forceps or an elevator. In contrast, the open (surgical) method is employed in specific cases, such as when the tooth has a fragile crown, retained roots, divergent roots, root proximity to the sinus, or root hypercementosis.[12]

2.2. *Wound Healing After Tooth Extraction*

Wound healing after tooth extraction is observed to occur more rapidly in the oral cavity than on the skin due to the presence of saliva, which contains essential proteins for the healing process. The healing process, known as socket healing, is categorized into three phases: inflammatory (24–48 hours), proliferative (48 hours–5 days), and remodeling (5 days–several months). In the inflammatory phase, blood clot formation is initiated, and inflammatory cells are migrated to the extraction site, where granulation tissue is gradually formed and later replaced by connective tissue containing collagen fibers to facilitate bone regeneration. During the proliferative phase, tissue formation occurs rapidly, capillary ingrowth from surrounding blood vessels is promoted, and fibroblast activity increases, leading to wound contraction and the development of scar tissue. In the final remodeling phase, woven bone is progressively replaced by lamellar bone and bone marrow through osteon formation, and the healing process is considered complete once bone marrow renewal has occurred.[13]

2.3. *Hematoxylin and Eosin (HE) Staining*

Histological staining techniques are classified into two categories: general staining using hematoxylin-eosin and specialized histochemical or immunohistochemical staining. Histochemical staining highlights biological structures and identifies various tissue components, employing stains such as Periodic Acid Schiff (PAS), Toluidine Blue, and Giemsa. Immunohistochemical staining, on the other hand, relies on antigen-antibody interactions combined with enzymatic reactions to identify specific tissue structures. In this study, hematoxylin and eosin (HE) staining was employed due to its ability to differentiate nuclear and cytoplasmic structures effectively. Hematoxylin stains cell nuclei blue, while eosin imparts a pink hue to the cytoplasm and extracellular matrix. Bone tissue comprises four primary cell types: osteoblasts, bone lining cells, osteocytes, and osteoclasts. Osteoblasts, which are initially larger, eventually differentiate into osteocytes, which are smaller, elongated cells encased in lacunae. Histological preparations of the alveolar bone sockets of Sprague Dawley rats were stained with HE to enhance microscopic observation, facilitating the identification of cellular structures and tissue differentiation.[14]

3. Result and Discussion

The study analyzed 16 histological bone preparations of Sprague Dawley rats, with each treatment group consisting of 4 preparations. Once all relevant data were collected, the samples were categorized based on treatment type, and the number of osteoblasts, osteocytes, and osteoclasts was determined using the ImageJ application at 40x magnification. The collected data were then statistically processed using the SPSS application to assess variations in cell counts across different treatment groups.

Figure 1 illustrates the profile of bone cells observed in the histological sections. Image **(A)** highlights the differences in size and location between osteoblasts and osteocytes, where osteoblasts are found along the bone surface, while osteocytes are embedded within the bone matrix. Image **(B)** presents osteoblasts and osteoclasts, showing osteoclasts as larger, multinucleated cells responsible for bone resorption, whereas osteoblasts are smaller and involved in bone formation. The histological staining with Hematoxylin and Eosin (HE) facilitated clear differentiation of these cell types, allowing for precise identification and quantification of bone cells across different experimental conditions.

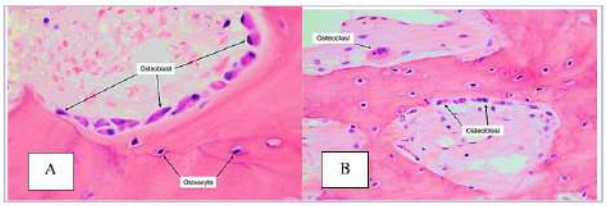

Figure 1. Profil of Bone Cells. (A) Differences in size and location between osteoblasts and osteocytes. (B) Osteoblasts and osteoclasts.

Table 1 presents the means and standard deviations of osteoblast, osteocyte, and osteoclast counts across four experimental groups, reflecting the effects of different treatments on bone cell populations. Group A consisted of rats that underwent ovariectomy (OVX) and received a single zoledronate injection, while Group B received six zoledronate injections after OVX. Group C underwent OVX but was treated with saline water instead of zoledronate, serving as a non-bisphosphonate-treated OVX group. Group D, as the control group, underwent sham surgery without OVX and received saline water.

The results indicate that Group C exhibited the highest osteoblast count (8.25 ± 5.439), while Group B showed the lowest (2.00 ± 1.414). Group A and Group D displayed intermediate osteoblast counts, with means of 3.50 ± 3.109 and 5.50 ± 6.191, respectively. Regarding osteocytes, Group B recorded the highest mean count (46.50 ± 21.440), whereas Group C had the lowest (26.75 ± 16.500). Groups A and D reported osteocyte counts of 30.50 ± 3.873 and 39.25 ± 9.287, respectively. For osteoclasts, Group A exhibited the highest mean count (1.00 ± 2.000), while no osteoclasts were detected in Group B (0.00). Groups C and D demonstrated relatively low osteoclast counts, with means of 0.75 ± 0.957 and 0.50 ± 1.000, respectively.

These findings suggest that zoledronate administration influences bone cell activity, particularly osteoblast and osteocyte populations. Multiple doses of zoledronate (Group B) appear to be associated with a decrease in osteoblast counts and an increase in osteocytes, possibly indicating an effect on bone remodeling processes. Meanwhile, osteoclast counts remained consistently low across all groups, further supporting the inhibitory role of zoledronate in bone resorption.

Table 1. Means and Standar Deviations

Variabel	Group	Average	Standard Deviation
osteoblast	Group A	3.50	3.109
	Group B	2.00	1.414
	Group C	8.25	5.439
	Group D	5.50	6.191
osteocyte	Group A	30.50	3.873
	Group B	46.50	21.440
	Group C	26.75	16.500
	Group D	39.25	9.287
osteoclasts	Group A	1.00	2.000
	Group B	0.00	-
	Group C	0.75	0.957
	Group D	0.50	1.000

Table 2 presents the results of statistical analyses using the ANOVA and Kruskal-Wallis tests to evaluate differences in osteoblast, osteocyte, and osteoclast counts among the four experimental groups (A, B, C, and D). The ANOVA test for osteoblasts resulted in a p-value of 0.272, while the

Kruskal-Wallis test showed a p-value of 0.558, indicating no statistically significant differences in osteoblast counts among the groups. This suggests that variations in treatment did not substantially affect osteoblast populations. For osteocytes, the ANOVA test yielded a p-value of 0.261, suggesting that no significant differences were observed among the groups, and the Kruskal-Wallis test was not applied, implying that the data met the assumptions for parametric testing. Regarding osteoclasts, the ANOVA test resulted in a p-value of 0.501, showing no statistically significant differences across the groups, and similar to osteocytes, the Kruskal-Wallis test was not used, indicating the data were suitable for ANOVA analysis. Overall, these statistical results suggest that the administration of zoledronate and other experimental treatments did not lead to significant variations in osteoblast, osteocyte, or osteoclast counts. The absence of statistical significance implies that the differences in cell counts observed among the groups may not be biologically meaningful, highlighting the need for further investigation into the effects of zoledronate on bone cell activity.

Table 2. ANOVA and Kruskal-Wallis Test

Variabel	Group	ANOVA	*Kruskal-Wallis*
osteoblast	Group A Group B Group C Group D	$p = 0.272$	
osteocyte	Group A Group B Group C Group D	$p = 0.261$	$p = 0.558$
osteoclasts	Group A Group B Group C Group D	$p = 0.501$	

Figure 2 illustrates the average number of osteoblasts, osteocytes, and osteoclasts across the four experimental groups (A, B, C, and D). The first graph represents the osteoblast count, showing that Group C exhibited the highest number (8.25), while Group B had the lowest (2.00). Groups A and D displayed intermediate values of 3.50 and 5.50, respectively. The second graph presents the osteocyte count, where Group B recorded the highest value (46.50), whereas Group C had the lowest (26.75). Groups A and D showed counts of 30.50 and 39.25, respectively. The third graph displays the osteoclast count, revealing that Group A had the highest value (1.00), while Group B had no detectable osteoclasts (0.00). Groups C and D showed relatively low osteoclast counts of 0.75 and 0.50, respectively. These visual representations support the numerical data in Table 1, highlighting variations in bone cell populations across different treatment groups.

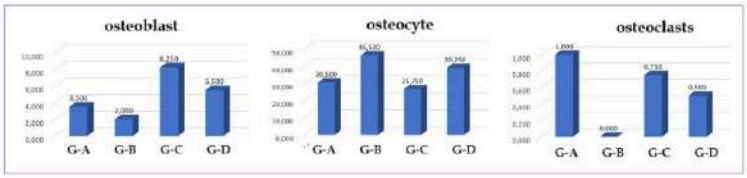

Figure 2. Bar graph of the average number of bone cells.

Osteoporosis is a condition characterized by decreased bone mineral density, leading to weakened bone strength and an increased risk of fractures.[15] Women are more susceptible to osteoporosis due to the role of estrogen in bone remodeling, which regulates osteoclast apoptosis. A reduction in estrogen levels, particularly after menopause, disrupts bone remodeling by increasing osteoclast activity, leading to excessive bone resorption and decreased bone formation.[16] This study utilized an ovariectomy (OVX) model in Sprague Dawley rats to mimic estrogen deficiency-induced osteoporosis, a method validated by previous studies showing significant reductions in bone density and altered trabecular microarchitecture in OVX rats.[17]

Bisphosphonates, including zoledronate, are widely used for osteoporosis treatment due to their strong antiresorptive effects. By inhibiting osteoclast activity, bisphosphonates reduce bone resorption, thereby lowering the risk of fractures. However, their impact on osteocytes and osteoblasts remains debated. Zoledronate has been shown to prevent osteocyte apoptosis, which may disrupt osteoblast formation and contribute to bone necrosis.[18] This study examined the effects of zoledronate on alveolar bone remodeling in OVX rats, comparing different dosages and treatment groups. The experimental groups included rats that received either a single or six intravenous zoledronate injections, OVX rats treated with saline, and a control group that underwent sham surgery and received saline injections.

The results showed a decrease in osteoblast counts and an increase in osteocytes in the zoledronate-exposed groups compared to the OVX group treated with saline. These findings align with prior research suggesting that high doses of bisphosphonates suppress bone remodeling by limiting osteoblast activity.[19] Furthermore, von Kossa staining quantification revealed a 33% reduction in bone formation in bisphosphonate-exposed groups compared to the control group.[20] However, some studies have contradicted these results, reporting that bisphosphonates can enhance osteoblast differentiation and maintain bone remodeling by reducing osteoclast activity.[21] For example, Plotkin et al. (2020) found that bisphosphonates prolong osteocyte lifespan and improve bone integrity by preventing excessive osteoclast-mediated resorption.[22] Nevertheless, other researchers support the findings of this study, suggesting that zoledronate significantly reduces osteoblast numbers, leading to impaired synthesis and mineralization of the bone matrix in a dose-dependent manner ($p > 0.05$).[23]

In addition, a decrease in osteoclast counts was observed when zoledronate was administered six times, supporting previous studies demonstrating the inhibitory effect of zoledronate on osteoclast activity in osteoporosis.[24] Research has shown that zoledronate disrupts the mevalonate pathway by inhibiting farnesyl pyrophosphate synthase, preventing the formation of geranylgeranyl pyrophosphate (GGPP).[25] This disruption inhibits the prenylation of small GTPase proteins such as Ras and Rho, which are essential for osteoclast cytoskeletal integrity and intracellular signaling. As a result, osteoclast apoptosis increases, leading to a decline in osteoclast numbers and reduced bone resorption activity.[26]

Statistical analysis using one-way ANOVA revealed no significant differences in osteoblast and osteoclast counts among the groups. However, descriptive analysis of graphical data indicated that the zoledronate-exposed groups had the lowest osteoblast and osteoclast counts. This supports previous findings that prolonged bisphosphonate therapy may lead to alterations in bone remodeling dynamics. Consequently, dentists must exercise caution when performing tooth extractions in osteoporosis patients receiving zoledronate therapy, as prolonged bisphosphonate use has been linked to post-extraction osteonecrosis.[27]

Despite these findings, this study had several limitations. The small sample size of alveolar bone preparations made it challenging to accurately count osteoblasts, osteocytes, and osteoclasts at 40x magnification. Furthermore, histological preparation quality was suboptimal, with potential issues in tissue sectioning and imaging affecting accuracy. Prior studies, including those by Kostenuik P (2021), have highlighted variability in bone turnover rates in bisphosphonate-treated models, making precise assessment challenging.[28] Given these limitations, further research using larger sample sizes, enhanced histological techniques, and additional molecular markers is necessary to gain a more comprehensive understanding of zoledronate's effects on alveolar bone remodeling.

4. Conclusion

This study examined the effects of zoledronate on alveolar bone remodeling in Sprague Dawley rats after ovariectomy (OVX), revealing a decrease in osteoblasts, an increase in osteocytes, and a reduction in osteoclasts, particularly with repeated zoledronate administration. While statistical analysis showed no significant differences, descriptive data indicated lower osteoblast and osteoclast counts in zoledronate-treated groups. These findings highlight zoledronate's inhibitory effect on bone resorption but also suggest potential disruptions in bone formation. Clinically, caution is advised when performing tooth extractions in osteoporosis patients receiving zoledronate to minimize the risk of bisphosphonate-related osteonecrosis of the jaw. Further research with larger samples and improved histological techniques is needed to clarify zoledronate's long-term impact on bone remodeling.

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Authors Contribution

Contribution	Lubis SS,	Palupi APS,	Komariah	Latief BS,	Priosoeryanto BP	Suniarti DF
Concepts or ideas		√				
Design		√	√	√	√	√
Definition of intellectual content	√	√	√	√	√	√
Literature search	√	√	√	√	√	√
Experimental studies	√	√	√	√	√	
Data acquisition	√	√	√	√	√	√
Data analysis	√	√	√	√	√	√
Statistical analysis	√	√				
Manuscript preparation	√	√	√	√		
Manuscript editing	√	√	√	√	√	√
Manuscript review	√	√	√	√	√	√

Citation Format: Lubis SS, Palupi APS, Komariah, Latief BS, Priosoeryanto BP, Suniarti DF The number of alveolar bone cells exposed to zoledronate in the post-tooth extraction socket. J Syiah Kuala Dent Soc. 2024; 9(2): 169-176.

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