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THE EFFECT OF SYSTEMIC ZOLEDRONIC ACID ON THE HEALING POTENTIAL OF RATS WITH EXPERIMENTAL PERIODONTITIS

SİSTEMİK OLARAK KULLANILAN ZOLEDRONİK ASİTİN, DENEYSEL PERİODONTİTİS OLUŞTURULAN SIÇANLARDA İYİLEŞME POTANSİYELİ ÜZERİNE ETKİSİNİN İNCELENMESİ

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ABSTRACT

Objective: In this study, our goal was to observe the effect of zoledronic acid histopathologically, which is used without removing the periodontitis agent, on the healing potential of the tissue after the experimental periodontitis formation in an animal model.

Material and Method: 30 adult male Sprague-Dawley rats were divided into 2 groups as bisphosphonate users and non-users. At the beginning of the experiment, all rats were put under anesthesia, and 5.0 silk sutures were placed around their right upper first molars. No suture was placed around the left upper first molar teeth. It was waited for 3 weeks after the placement of the sutures. After experimental periodontitis was observed in the animals on the 21st day, 7.5uq/kg zoledronic acid was injected intramuscularly for 6 weeks in the animals in the experimental group. After intramuscular drug administration once a week for 6 weeks, weekly weight monitoring was performed on days 0, 7, 14, 21, 28, and 35 and noted in the experimental group rats.

At the end of 6 weeks, the sutures were removed under the anesthesia from the experimental group animals whose last drug injections were completed and the control group animals that were administered 0.9% saline on the same days. A recovery period of two weeks was expected after which all animals were sacrificed.

Results: Zoledronic acid was used in the histological evaluation results, and experimental inflammation, necrosis, periodontal space and epithelial proliferation in the group with periodontitis statistically significant $p<0.05$ was found to be higher.

Conclusion: When evaluated clinically, positive effects on wound healing were observed in rats treated with bisphosphonate-derived drugs by treating existing periodontitis prior to drug administration.

Key words: Zoledronic acid, rat, experimental periodontitis, osteonecrosis, histology

ÖZ

Amaç: Bu çalışmada amacımız hayvan modelinde deneysel periodontitis oluşumundan sonra, periodontitis etkeni kaldırılmadan kullandırılan zoledronik asidin, periodontitis etkeni ortadan kaldırıldıktan sonra dokunun iyileşme potansiyeli üzerindeki etkisini histopatolojik olarak gözlemlemeyi hedeflemektir.

Gereç ve Yöntem: Sprague-Dawley cinsi 30 adet yetişkin erkek sıçan bifosfanat kullanan ve kullanmayan olarak 2 gruba ayrılmıştır. Deney başlangıcında tüm sıçanlara, anestezi altında, sağ üst 1.molar dişlerinin etrafına 5.0 ipek dikiş yerleştirilmiştir. Sol üst 1. molar dişlere ise herhangi bir uygulama yapılmamıştır. Dikişlerin yerleştirilmesinden sonra 3 hafta beklenilmiştir. 21. günde hayvanlarda deneysel periodontitis gözlendikten sonra deney grubundaki hayvanlara kas içi 6 hafta boyunca 7,5uq/kg zoledronik asit enjekte edilmiştir. 0., 7., 14., 21., 28., ve 35.günlerde; 6 hafta boyunca, haftada bir, kas içi ilaç verilmesinden sonra deney grubu sıçanlarında, haftalık ağırlık takibi yapılmıştır ve not edilmiştir. Son ilaç enjeksiyonları tamamlanan deney grubu hayvanlarının ve aynı günlerde %0,9 serum fizyolojik uygulanan kontrol grubu hayvanlarının 6 hafta sonunda, anestezi altında yerleştirilen dikişleri kaldırılmıştır. İki haftalık iyileşme süresi için beklenilmiş ve bu iki hafta içinde de deney grubundaki hayvanlara zoledronik asit enjekte edilmiştir.

Bulgular: Histolojik değerlendirme sonuçlarında zoledronik asit kullanılmış ve deneysel periodontitis oluşturulan grupta iltihap, nekroz, periodontal aralık ve epitel proliferasyonu istatiksel anlamlı $p<0,05$ olarak daha fazla bulunmuştur

Sonuç: Klinik açıdan değerlendirildiğinde, bifosfanat türev ilaç kullandırılmış sıçanlarda, ilaç kullanımı öncesinde, var olan periodontitisin tedavi edilmesinin yara iyileşmesi üzerinde olumlu etkileri gözlenmiştir.

Anahtar Kelimeler: Zoledronik asit, sıçan, deneysel periodontitis, osteonekroz, histoloji

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INTRODUCTION

Periodontitis is a chronic infectious disease characterized by inflammation of the tissues supporting the teeth, resulting in loss of periodontal support tissues; loss of attachment and significant bone loss due to the accumulation of chronic plaque and calculus in its pathogenesis (1-3). Bisphosphonates are chemical compounds characterized by phosphorus-carbon-phosphorus structure (4). Nitrogenous bisphosphonates show their antiresorptive effects via the mevalonate pathway. Inhibition of farnesyl pyrophosphate synthesis leads to inhibition of the mevalonate pathway end product, geranylgeranyl pyrophosphate (5,6). Although the effect of bisphosphonates on bone tissue has been clearly demonstrated in scientific studies today, studies on the effect on the oral mucosa are limited (7,8). The limited number of studies conducted are not sufficient to determine the safety, efficacy and usage doses of bisphosphonates. It has been shown in studies that this drug, which acts on the bone tissue, also affects the jaw bones and periodontal tissues. Today, there are studies showing that the use of bisphosphonates increases the risk of osteonecrosis in the jaw bones (9,10). However, studies on the effects of bisphosphonates on periodontal tissue are insufficient. It is clear that more work needs to be done on this subject.

Zoledronic acid is a strong, new generation nitrogen-containing imidazole cyclic side-chain heterocyclic nitrogen-containing bisphosphonate. The difference from other bisphosphonates is the presence of the second nitrogen atom in the ring structure. Zoledronic acid (ZA) has the highest binding ability to bone among bisphosphonates; it has antitumor activity that affects apoptosis, tumor cell growth, adhesion, invasion and angiogenesis (11). Although there is knowledge about the effect of bisphosphonates on healthy bone tissue in scientific studies today, its possible effects on periodontal tissues in the presence of chronic periodontal inflammation and when this inflammation is eliminated constitute a new field of study (12,13).

Therefore, the aim of this study is to histopathologically evaluate the effect of zoledronic acid on the healing potential of periodontal tissues after removal of the periodontitis agent in an experimental periodontitis model.

MATERIAL and METHOD

Ethics committee approval

For the animal subjects to be used in the experiments, T.C. Istanbul University, Aziz Sancar Experimental Medicine Research Institute, Animal Experiments Local Ethics Committee's Presidency approval was obtained on 25.09.2020 with the number 2020/26.

The sample number of the study was calculated with the program named G*Power 3.1.9.2. Based on the results of Vaycan's study and considering the distribution of histological findings, the sample size for each group was made with a minimum of n=15 animals, a total of 60 cases, and the power of the test was 98.8% according to the PostHoc power analysis result (14).

Procedures

In our study, a total of 30 male rats, Sprague-Dawley, 15 in control and 15 in experimental groups, were used. Periodontitis was induced by placing suture on the right molar teeth of the rats,but the left side was never touched. Therefore, 2 different groups were created in each rat, and there are 4 different groups in total. According to the condition of the periodontium in the right upper jaw and left upper jaw of the 15 rats in the control group; a control group of 15 rats with healthy periodontium and a control group of 15 rats with periodontitis were formed. The same situation is valid for rats in the experimental group.

Experimental animals are housed in polycarbonate transparent cages at room temperature of 21 degrees Celsius, humidity of 60%, with 2 rats in each cage. Each cage contains libitum pellets and fresh, clean tap water.

At the beginning of the experiment, all rats were ligated with 5.0 silk sutures around the right upper first molars under general anesthesia. Clinical signs of periodontitis in animals were evaluated by measuring bleeding on probing on day 21 after placement of the sutures. Animals in the experimental group were injected intramuscularly with 7.5uq/kg zoledronic acid for 6 weeks. The animals in the control group were injected with 0.9% saline for 6 weeks.

Following the administration of drugs and saline for 6 weeks, the sutures around the first molar teeth were removed under anesthesia, and it was waited for 2 weeks for healing. Zoledronic acid continued to be given to the experimental group for the expected time for this improvement. The control group continued to be given physiological saline.

All animals were then sacrificed by cervical dislocation. In the cervical dislocation method, the spinal cord is severed, and the connection between the brain and vital organs is lost.

This sacrification method was preferred due to the fact that it does not cause chemical substance residues in the tissues and forms rapid death, and with the approval of the ethics committee.

After sacrification, the upper jaws were resected, and the removed parts were kept in 10% buffered formalin solution for 2 weeks. After fixation, it was decalcified in 20% sodium citrate and 50% formic acid solution.

After this process, the hard palate was removed from the vestibule surfaces of the molar teeth from all jaws. Sagittal sections passing through the middle were taken, and dissection of the parts was made. Vestibule faces 3 microns obtained from paraffin blocks prepared by laying on the cross-sectional

Table 1: The flow followed during the experiment

Day	Experiment	Control
1	Silk stitch binding upper right 1.molar	Silk stitch binding (upper right 1.molar)
21	ZA injection	SF injection
28	ZA injection	SF injection
35	ZA injection	SF injection
42	ZA injection	SF injection
56	ZA injection	SF injection
63	ZA injection Removal of stitches	SF injection Removal of stitches
70	ZA injection	SF injection
77	ZA injection	SF injection
84	Sacrification	Sacrification

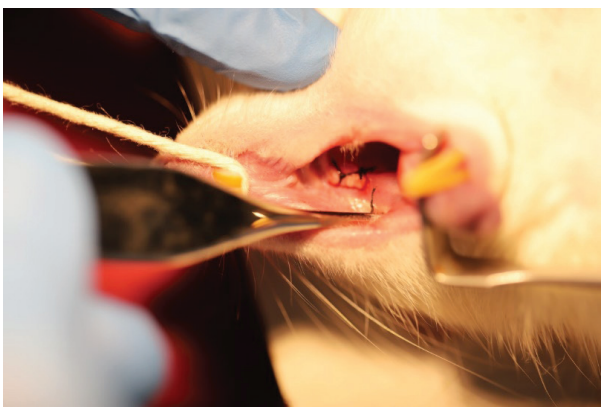

Figure 1: Placement of sutures in the interproximal areas of the upper jaw of rats

Statistical analysis

Data were analyzed with IBM SPSS V23. Conformity to the normal distribution was examined using the Shapiro-Wilk Test. In the data that did not confirm to the normal distribution, comparisons between groups were made with the Kruskal Wallis Test, and multiple comparisons were examined with the Dunn Test.

In categorical data, comparisons between groups were made with the Pearson Chi-Square test, and multiple comparisons were examined with the Bonferroni-corrected Z test. For categorical data, results were presented as frequency (percentage). Significance level was accepted as $p<0.050$.

RESULTS

Histological evaluation

Histological images and statistical results obtained from the experimental animals are below. When the results were evaluated histologically, inflammation, necrosis increase in periodontal space and epithelial proliferation were observed more in the groups with experimental periodontitis where zoledronic acid was used, and it was statistically significant ($p<0.05$) (Figures 2, 3, 4, 5).

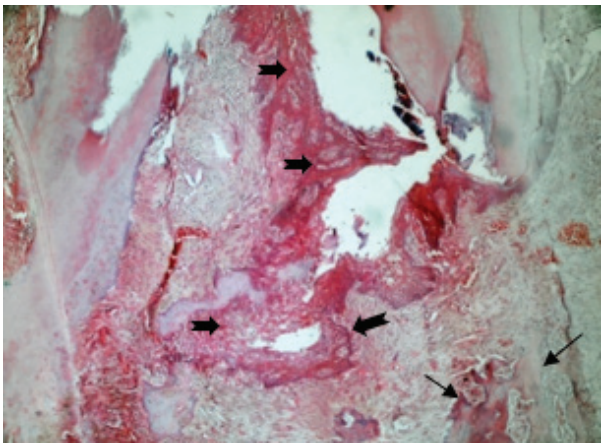

Figure 2: Necrotic bone fragments are seen in the histopathological image of the EEP (experimental group in experimental periodontitis) group (H&EX100).
Thick arrows: stratified squamous epithelium, thin arrows: necrotic bone

In the experimental animals, necrotic bone was found in one or more areas in 46.7 percent of the first molar teeth that were given zoledronic acid and ligated (Table 3). In the presence of experimental periodontitis, epithelial proliferation in subjects using zoledronic acid was found to be statistically significant compared to healthy periodontal groups (Table 2).

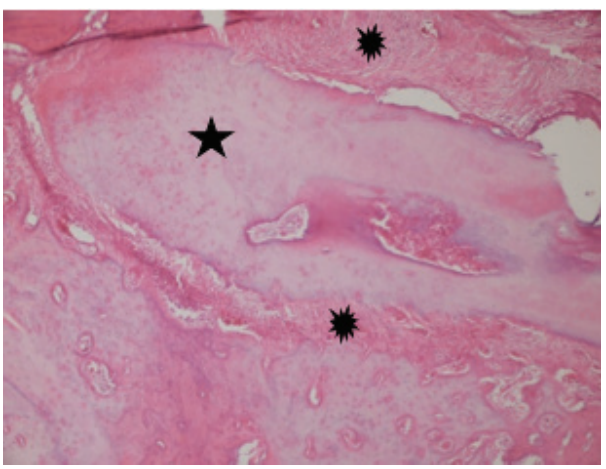

Figure 3: In the histopathological image of the EHP (experimental group in healthy periodontium group), tooth roots and periodontal tissues with normal physiological appearance are seen (H&EX100. Star: tooth's root, asterisk: periodontal space).
No necrotic bone was found around the first molar roots of the subjects in this group. No epithelial proliferation was observed around the roots of the first molar tooth.
Star: Tooth's root, thick arrows: Alveolar bone, asterisk: Periodontal space

No necrotic bone was found in any of the subjects in this group. There is no inflammatory infiltration in this group (Tables 1, 3).

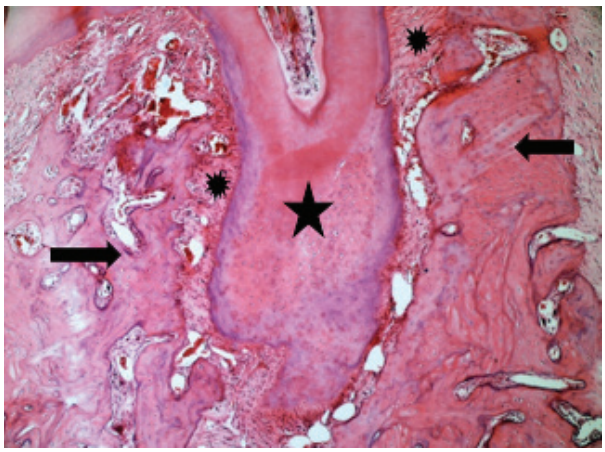

Figure 4: In the histopathological image of the CHP (control group healthy periodontium) group, natural tooth roots and healthy periodontal tissues are seen around them(H&EX100). Thin arrows: cell epithelium, star: Tooth's root

The presence of necrotic bone was not detected in the experimental animals in this group. In this group, inflammatory infiltration is 60 percent. This shows that periodontal inflammation developed significantly in ligatured teeth (Table 2).

Statistics

A statistically significant difference was found between the distributions of inflammation between the groups (p<0.001).

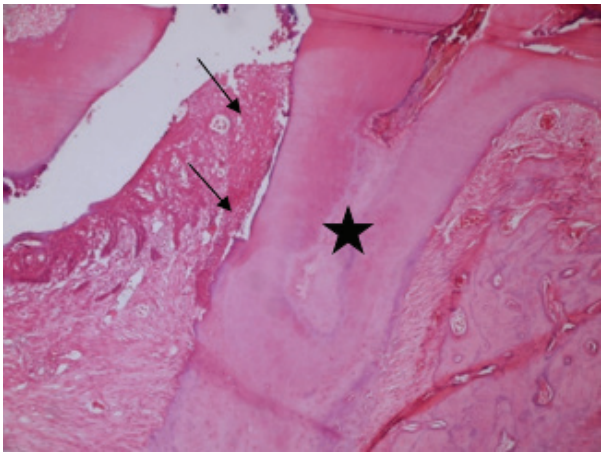

Figure 5: Histopathological image of CDP (control group experimental periodontitis) group (H&EX100)

Epithelial proliferation distributions between the groups showed statistically significant differences (p<0.001). A statistically significant difference was found between the distributions of necrosis status between the groups (p=0.001). No necrosis was found in the CDP, CHP, and EHP groups (Table 4). The median value of the periodontal space in the CHP group was lower than the median value of the EEP group (Table 5). A statistically significant difference was found between the median value of the periodontal space in the CHP group and the median value of the periodontal space in the EEP group (p<0.001).

Table 2: Histopathology results- inflammation

	CEP	CHP	EEP	EHP	Total	p
Inflammation						
None	6 (40)a	15 (100)b	5 (33.3)a	15 (100)b	41 (68.3)	
Mild	9 (60)a	0 (0)b	8 (53.3)a	0 (0)b	17 (28.3)	**<0.001***
Moderate	0 (0)a	0 (0)a	2 (13.3)a	0 (0)a	2 (3.3)	

*Pearson Chi-Square Test; a-b: No difference between groups with the same letter in each line, frequency (percent) EEP: Experimental group in experimental periodontitis group, EHP: Experimental group in healthy periodontium group, CHP: Control group healthy periodontium, CDP: Control group experimental periodontitis

Table 3: Histopathology results- epithelial cell proliferation

	CEP	CHP	EEP	EHP	Total	p
Epithelial proliferation						
None	11 (73.3)abc	15 (100)c	5 (33.3)b	15 (100)ac	46 (76.7)	
Mild	4 (26.7)a	0 (0)a	5 (33.3)a	0 (0)a	9 (15)	**<0.001***
Moderate	0 (0)a	0 (0)a	2 (13.3)a	0 (0)a	2 (3.3)	
Severe	0 (0)a	0 (0)a	3 (20)a	0 (0)a	3 (5)	

*Pearson Chi-Square Test, a-c: There is no difference between the distributions of groups with the same letter, frequency (percentage)
EEP: Experimental group in experimental periodontitis group, EHP: Experimental group in healthy periodontium group, CHP: Control group healthy periodontium, CDP: Control group experimental periodontitis

Table 4: Histopathology results- Osteonecrosis

	CEP	CHP	EEP	EHP	Total	p
Osteonecrosis						
None	15 (100)a	15 (100)a	8 (53.3)b	15 (100)a	53 (88.3)	**0.001***
Mild	0 (0)a	0 (0)a	6 (40)b	0 (0)a	6 (10)	
Moderate	0 (0)a	0 (0)a	1 (6.7)a	0 (0)a	1 (1.7)	

* Pearson Chi-Square Test, a-b: No difference between groups with the same letter in each line, frequency (percent); EEP: Experimental group in experimental periodontitis) group, EHP: Experimental group in healthy periodontium group, CHP: control group healthy periodontium, CDP: Control group experimental periodontitis

Table 5: Histopathology results- periodontal space group

	n	Average	Standard deviation	Median	Minimum	Maximum	p
CEP	15	0.1227	0.0212	0.13	0.09	0.16	**<0. 001***
CHP	15	0.07	0.0169	0.07	0.04	0.1	
EEP	15	1.0227	3.31348	0.17	0.14	13	
EHP	15	0.0953	0.1125	0.07	0.05	0.5	
Total	60	0.3277	1.66508	0.095	0.04	13	

*Kruskal Wallis Test, EEP: Experimental group in experimental periodontitis group, EHP: Experimental group in healthy periodontium group, CHP: Control group healthy periodontium, CDP: Control group experimental periodontitis

DISCUSSION

The evaluation of the findings is from a histopathological point of view. With this method, we aimed to examine the effect of using zoledronic acid on the healing of periodontal tissues when the factor causing the periodontal problem is removed.

In other words, the medical situation applied in our study is to evaluate the effect of ZA treatment on the periodontal tissue of the patients whose periodontal health is not in good condition, but who received zoledronic acid due to medical need, when they regain their periodontal health in this process. In this way, we aimed to obtain new strategies for the prevention and treatment of the condition that causes alveolar bone loss in periodontal diseases.

The most important effect of bisphosphonate is to prevent bone resorption by inhibiting osteoclast activity. The decrease in osteoclast activity causes a change in the osteoclast-osteoblast interaction. Considering this feature of bisphosphonates, the extent to which it will control alveolar bone destruction due to periodontal diseases can form the basis of a new field of study (15).

Dental trauma procedures, such as tooth extraction, are serious risk factors for drug-induced osteonecrosis. More than fifty percent of patients with osteonecrosis of their jaws have a history of tooth extraction. It is known that tooth extraction increases the risk of developing osteonecrosis 33 times (16,17). However, it can be thought that the spontaneous development of drug-induced jaw necrosis is related to the presence of periodontal or periapical infection in the mouth.

The most well-known side effect of bisphosphonates is necrosis of the jaws. Reported bisphosphonate-related chin necrosis emerged after the use of infused, nitrogen-containing drugs such as zoledronic acid or pamidronate (18). Bacterial infection and inflammation are present in the lesions revealed in the bronchi.

Oral mucosa has a unique and complex structure in the human body. Unlike other parts of the body, it is very close to the bone underneath. Fat and muscle tissues in the mouth serve as an insulator between the cells of the oral mucosa and the bisphosphonate-rich bone that is treated with bisphosphonates. Areas with a tendency to BRONJ in the mouth are areas with thin oral mucosa, such as the crests of the torus, maxilla, and mandible. These anatomical areas are formations specific to the oral environment. The occurrence of these necrosis, which is a side effect of bisphosphonates, especially in the oral mucosa, shows that the cells here have an important place in explaining the formation of BRONJ. It is a matter of debate whether BRONJ originates from the cells of the oral mucosa or from the underlying bone (19, 20).

Unlike the results of our study, in the study of Li et al., in 2 of 15 animals in the periodontally healthy group, in which zoledronic acid was used, and silk sutures were not placed; the presence of necrotic bone, characterized by large empty lacunae and loss of mineral density in the osteocytes, was observed (21). This difference is remarkable and can be thought to be related to technical sensitivity and sample size.

According to the results of our study, in the group with periodontal destruction and ZA (EEP), bone necrosis was detected at a rate of 46.7% around the alveolar bone and root. Bone necrosis was not observed in the experimental (EHP) and control groups (CHP), which were periodontally healthy, and in the control groups (CEP) with periodontal destruction.

The presence of necrotic bone was significantly higher in the experimental group given this ZA and periodontal destruction compared to the other groups.

When the experimental group experimental periodontitis (EEP) and the experimental group healthy periodontium (EHP) groups were compared, the presence of inflammation was not present in EHP. According to the findings of our study, no inflammation was found in either group as a result of the histopathological results obtained from the healthy periodontium group (CHP) that did not use ZA and the healthy periodontium group (EHP) that used ZA. As a result of these histopathological findings, it can be thought that ZA alone does not potentiate the inflammatory effect. By inhibiting the mevalonate pathway ZA, which is one of the nitrogenous bisphosphonates, they disrupt the cytoskeletal structure of the osteoclast, thereby inhibiting bone resorption.

In the study of Aghaloo et al., the role of progressive periodontal disease due to the stimulation of osteonecrosis of the jaw due to bisphosphonate use was examined by microtomography and histopathological method on ligatured rats (22).

Aghaloo et al.'s drug-induced inflammation hypothesis of osteonecrosis of the jaw parallels with the presence of inflammation and necrosis in the EEP group in our study.

According to the results of the study by Aghaloo et al., out of 19 animals with experimental periodontitis given ZA, 4 had exposed bone surfaces. Unlike our study, experimental periodontitis was created, and necrotic areas characterized by empty lacunae were found in 1 of 19 animals in the control group that did not receive ZA.

CONCLUSION

In our study, zoledronic acid was used in subjects with existing periodontitis, and its effects on alveolar bone destruction and necrosis were investigated. In the histopathological findings in our study; in the presence of periodontal disease, necrotic bone was present when we gave zoledronic acid, but in the absence of periodontal disease, no necrotic bone finding was found despite giving zoledronic acid.

These findings show that there is bone necrosis due to bisphosphonate use in the presence of periodontal disease, which is the precursor of periodontal destruction. More studies are needed to evaluate the possibility of bone necrosis caused by systemic use of bisphosphonates in cases where periodontal health is preserved. In addition, new immunohistochemical studies are needed to explain the effect of zoledronic acid on proliferation and differentiation in the bone formation mechanism. The healing potential of zoledronic acid on the tissue can be evaluated more effectively by changing the dose, the frequency of administration, the period of use, and by creating more study groups.

Ethics Committee Approval: This study was approved by Istanbul University, Aziz Sancar Experimental Medicine Research Institute, Animal Experiments Local Ethics Committee's Presidency (Date: 25.09.2020, No: 2020/26).

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REFERENCES

1. Page RC, Shroeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. Lab Invest 1976;34(3):235-49.
2. Seymour GJ, Taylor JJ. Shouts and whispers: an introduction to immunoregulation in periodontal disease. J Clin Periodontol 2004;35(3):9-13.
3. Leira Y, Iglesias-Rey R, Gomez-Lado N, Aguiar P , Sobrino T, D'Aiuto F et al. Periodontitis and vascular inflammatory biomarkers: an experimental in vivo study in rats. Odotonlogy 2020;108(2):202-12.
4. Rusell RGG. Bisphonates from bench to bedside. Ann NY Acad Sci 2006;1068:367-401.
5. Fleisch H. Development of bisphosphonates. Breast Cancer Res 2002;4(1):30-4.
6. Russell RGG, Watts NB, Ebetino FH, Rogers MJ. Mechanisms of action of bisphosphonates: Similarities and differences and their potential influence on clinical efficacy. Osteoporos Int 2008;(19):733-59.
7. Cheng A, Mavrokokki T, Carter G, Stein B, Fazzalari N, Wilson D, et al. The dental implications of bisphosphpnates and bone disease. Aust Dent J 2005;50(4 Suppl 2):S4-13.
8. Russell RGG. Bisphosphonates: The first 40 years. Bone 2011;49(1):2-19.
9. Marx RE, Cillo JE, Ulloa JJ. Oral bishosphonate-induced osteonecrosis: risk factors, prediction of risk using serum CTX testing, prevention and treatment. J Oral Maxillofac Surg 2009;(67):2-12.
10. Russell RGG. Bishosphonates: Mode of action and pharmocology. Am Acad J Pediatr 2007;119 Suppl 2:150-62.
11. Chen T, Berenson J, Vescio R, Swift R, Gilchick A, Goodin S et al. Pharmacokinetics and pharmacodynamics of zoledronic acid in cancer patients with bone metastases. J Clin Pharmacol 2002;42(11):1228-36
12. Nobuyuki H, Hiraga T, Williams PJ, Niewolna M, Shimizu N, Mundy GR, et al. The bisphosphonate zoledronic acid inhibits metastases to bone and liver with suppression of osteopontin production in mouse mammary tumor. J Bone Miner Res 2001;16(Suppl 1):S191.
13. Senaratne SG, Mansi JL, Colston KW. The bisphosphonate zoledronic acid impairs membrane localisation and induce cytochrome c release in breast cancer cells. Br J Cancer 2002;86(9):1479-86.
14. Vaycan HS. Sistemik kullanılan zoledronik asitin var olan

deneysel periodontitis üzerine etkisinin histomorfometrik olarak incelenmesi. İstanbul Üniversitesi Sağlık Bilimleri Enstitüsü. Doktora Tezi. İstanbul 2020.

15. Ficarra G, Beninati F, Rubino I, Vannucchi A, Longo, Tonelli P. GOsteonecrosis of the jaws in periodontal patients with a history of bisphosphonates treatment J Clin Periodontol 2005;32(11):1123-8.
16. Saad F, Brown JE, Van Poznak C, Ibrahim T, Stemmer SM, Stopeck AT et al. Incidence, risk factors, and outcomes of osteonecrosis of the jaw: integrated analysis from three blinded active-controlled phase III trials in cancer patients with bone metastases. Ann Oncol 2012;(23):1341-7.
17. Vahtsevanos K, Kyrgidis A, Verrou E, Katodritou E, Triaridis S, Andreadis CG et al. Longitudinal cohort study of risk factors in cancer patients of bisphosphonate-related osteonecrosis of the jaw. J Clin Oncol 2009;(27):5356-62
18. Sonis ST, Watkins BA, Lyng GD, Lerman MA, Anderson KC. Bony changes in the jaws of rats treated with zoledronic acid and dexamethasone before dental extractions mimic bisphosphonate-related osteonecrosis in cancer patients Oral Oncol 2009;45(2):164-72.
19. Kim RH, Lee RS, Williams D, Bae S, Woo J, Lieberman M et.al. Bisphosphonates induce senescence in normal human oral keratinocytes. J Dent Res 2011;90(6):810-6.
20. Landesberg R, Cozin M, Cremers S, Woo V, Kousteni S, Sinha S et al. Inhibition of oral mucosal cell wound healing by bisphosphonates. J Maxillofac Surg 2008;66(5):839-47.
21. Li CL, Lu WW, Seneviratne CJ, Leung WK, Zwahlen RA, Zheng LW et al. Role of periodontal disease in bisphosphonates-related osteonecrosis of the jaws in ovariectomized rats. Clin Oral Implants Res 2016;27(1):1-6.
22. Aghaloo TL, Kang B, Sung EC, Shoff M, Ronconi M, Gotcher JE et al. Periodontal disease and bisphosphonates induce osteonecrosis of the jaws in the rat. J Bone Miner Res 2011;26(8):1871-81