Cumhuriyet Dental Journal, 25(2): 103-110, 2022
DOI: https://doi.org/10.7126/cumudj.932462

Cumhuriyet Dental Journal

| cdj.cumhuriyet.edu.tr | Founded: 1998 Available online, ISSN: 1302-5805 Publisher: Sivas Cumhuriyet Üniversitesi

The Potential of Bisphosphonate Risedronate Hydrogel in Preventing Relapse Movement

Tita Ratya Utari[1,a,*], Pinandi Sri Pudyani[2,b], Ika Dewi Ana[3,c], Widya Asmara[4,d]

[1]*School of Dentistry, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Indonesia*
[2]*Department of Orthodontics, Faculty of Dentistry, Gadjah Mada University, Yogyakarta, Indonesia*
[3]*Department of Dental Biomedical Sciences, Faculty of Dentistry, Gadjah Mada University, Yogyakarta, Indonesia*
[4]*Department of Microbiology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia*
**Corresponding author*

Research Article

History

Received: 10/12/2021
Accepted: 12/01/2022

ABSTRACT

Objectives: To analyze the effect of risedronate hydrogel on enzyme alkaline phosphatase (ALP) and osteoclast/osteoblast ratio during tooth relapse movement

Materials and methods: The research design is experimental with time series. The lower incisors of 75 guinea pigs are distally moved using open coil spring. The guinea pigs were divided into three groups: without risedronate (group A; n = 25); given 250 µmol/L of risedronate hydrogel (group B; n = 25), and given 500 µmol/L of risedronate hydrogel (group C; n = 25). Risedronate were applied intrasulcularly in the mesial part of the gingival sulcus every 3 days. After 14 days of stabilization, the open coil spring was removed (bisphosphonate administration was continued). The relapsed teeth and ALP levels on days 0, 3, 7, 14, and 21 were measured. The osteoclast/osteoblast ratio was measured by hematoxylin and eosin staining. ANOVA test was used to determine the difference in the three groups and their interactions with concentration and time.

Results: There was a significant difference in osteoclast/osteoblast ratio on day 3 and 14 the ratio was higher in group A than in groups B and C on day 3, and the ratio was higher in group C than in groups A and B on day 14. ALP levels were significantly different on day 14 and 21.

Conclusions: The intrasulcular application of bisphosphonate risedronate affected the osteoclast/osteoblast ratio and increased ALP levels.

Keywords: Alkaline Phosphatase, Orthodontics, Osteoclasts, Risedronic Acid, Tooth Movement.

Ortodontik Nüks Hareketi Esnasında Bisfosfanat Risedronat Hidrojel'in Alkalin Fosfataz ve Osteoklastlar Üzerine Etkisi

Süreç

Geliş: 10/12/2021
Kabul: 12/01/2022

ÖZ

Amaç: Dişin relaps hareketi sırasında risedronat hidrojelin enzim alkalin fosfataz (ALP) ve osteoklast/osteoblast oranı üzerindeki etkisini analiz etmek.

Gereç ve Yöntem: Araştırma tasarımı zaman serileri ile deneyseldir. 75 kobayın alt kesici dişleri, açık helezon yay kullanılarak distale doğru hareket ettirilir. Kobaylar, risedronat uygulanmayan (grup A; n = 25); 250 µmol/L risedronat hidrojel uygulanan (grup B; n = 25) ve 500 µmol/L risedronat hidrojel uygulanan (grup C; n = 25) olmak üzere 3 gruba ayrıldı. Risedronat gingival sulkusun mezial kısmına 3 günde bir intrasulküler olarak uygulandı. 14 günlük stabilizasyondan sonra, açık helezon yay çıkarıldı (bifosfonat uygulamasına devam edildi). 0, 3, 7, 14 ve 21. günlerde nüks eden dişler ve ALP seviyeleri ölçüldü. Osteoklast/osteoblast oranı hematoksilen ve eozin boyama ile ölçüldü. Üç grup arasındaki farkı ve bunların konsantrasyon ve zamanla etkileşimlerini belirlemek için ANOVA testi kullanıldı.

Bulgular: Osteoklast/osteoblast oranında 3. günde ve 14. günde anlamlı bir fark vardı: 3. günde grup A'da grup B ve C'den daha yüksekti ve 14. günde grup C'de grup A ve B'ye göre daha yüksekti. ALP seviyeleri 14. günde ve 21. günde önemli ölçüde farklıydı.

Sonuçlar: Bifosfonat risedronat intrasulkuler uygulaması osteoklast/osteoblast oranını etkilemiş ve ALP düzeylerini yükseltmiştir.

Anahtar Kelimeler: Alkalen Fosfataz, Ortodonti, Osteoklastlar, Risedronik Asit, Diş Hareketi.

[a] *tita.ratya@umy.ac.id* *https://orcid.org/0000-0001-6579-5260*
[b] *pinandi_fkg@ugm.ac.id* *https://orcid.org/0000-0001-6111-1538*
[c] *ikadewiana@ugm.ac.id* *https://orcid.org/0000-0001-9957-4247*
[d] *wied_as@ugm.ac.id* *https://orcid.org/0000-0001-6386-8892*

How to Cite: Utari TR, Pudyani PS, Ana ID, Asmara W. (2022) The Potential of Bisphosphonate Risedronate Hydrogel In Preventing Relapse Movement, Cumhuriyet Dental Journal, 25(2): 103-110.

Introduction

Orthodontic treatment aims to achieve occlusal balance and correction of stable teeth, but there remains an important problem in orthodontic treatment, that is, relapse.[1] Research conducted by Vaida *et al.*[2] shows that, from 771 samples of patients who returned to control after 6 months of post-orthodontic treatment with the use of a retainer, 72 (10.13%) patients experienced relapses. There were 41 (5.77%) patients who experienced relapses 12 months after using the retainer and 19 (2.67%) patients who experienced relapses 24 months after using the retainer.

Relapse is a condition in which the teeth alignment is returned to pre-orthodontic treatment. Relapse can be due to patient noncompliance with the retainer and occlusion of incomplete treatment results. The tooth began to relapse into its original position as soon as the orthodontic appliance was removed. Orthodontic relapse and orthodontic tooth movement undergo the same process, namely, an increase in osteoclast differentiation in the stress area and a decrease in the stress area;[3] therefore, post-orthodontic relapse involves tooth movement within the alveolar bone and a bone modeling process that requires osteoblast homeostasis in the stretch area and osteoclasts homeostasis in stress area.[4]

Bisphosphonates have been reported to inhibit bone resorptive function by osteoclasts and reduce relapse. Simvastatin can prevent relapse by inhibiting osteoclast bone resorption activity and stimulating bone formation. Bone morphogenetic protein has also been used to inhibit relapse, and the results have shown that it can promote bone and cementum formation. The results of the study of Zhao *et al.*[5] using osteoprotegerin (OPG) showed significant inhibition of relapse and decreased number of osteoclasts. These data suggest that relapse can be inhibited by manipulating alveolar bone remodeling.

Bisphosphonates are synthetic analogs of pyrophosphate that are powerful inhibitors of bone resorption and are usually used as drugs for the prevention and therapy of osteoporosis and osteopenia, as well as for the treatment of tumors. The use of bisphosphonates can cause side effects in dental care, such as interfering with bone healing, inhibiting tooth movement, and causing osteonecrosis of the jaw[6] or bisphosphonate-related necrosis of the jaw (BRONJ). BRONJ is a condition in which the bone necrotizes; it does not heal for 8 weeks in the oral cavity due to bisphosphonate exposure. This can be due to the duration, dose, and route of intravenous and oral administration of bisphosphonates, which have a systemic effect. Also, the risk of BRONJ is increased with invasive dental procedures or exposure to high doses of bisphosphonates via the intravenous route. Invasive dental procedures such as tooth extraction will cause thrombus formation (blood clot) and create granulation tissue, and mineralization of the bone occurs. Bisphosphonates that have been bound to the bone will be slowly resorbed while the bone in the area that is removed still contains bacteria and cannot be resorbed. As a result, wound healing will take longer, increasing the risk of bacterial invasion that will cause chronic osteomyelitis.[6-8] Relapse occurs when the orthodontic treatment has been complete, the teeth are well arranged and oral hygiene is good, and does not require invasive action such as extraction, therefore, it is very unlikely that BRONJ will occur.

Bisphosphonates can prevent bone resorption by inhibiting osteoclast differentiation and activity, as well as breaking the attachment of mature osteoclasts to the bone, triggering apoptosis so that it can inhibit bone resorption[9]. Bisphosphonates are of two types: nitrogenous and nonnitrogenous.[10] One of the nitrogenous bisphosphonates that are often used is the risedronate type, which is known to have an effect through binding to hydroxyapatite in bone tissue, inhibiting osteoclastic activity, and inducing osteoclast apoptosis. Risedronate also induces apoptosis of macrophages at relatively low concentrations when compared to other bisphosphonates such as alendronate and pamidronate *in vitro.*[11] Several authors reported that bisphosphonates also influence osteoblast proliferation and differentiation, partly via the macrophage-activated protein kinase pathway and through different enzyme regulations.[12]

Osteoblasts are bone-forming cells responsible for the mineralization of the bone matrix by secreting type I collagen and releasing calcium, magnesium, and phosphate ions.[13] Increased activity of osteoblasts during bone formation will be accompanied by increased secretion of the enzyme alkaline phosphatase (ALP).[14] ALP has been widely recognized as a biochemical marker of osteoblast activity.[15] ALP is synthesized and secreted by osteoblasts during the bone formation process. ALP expression can reflect the biochemical changes that occur in the supporting tissue after orthodontic force[16], and in several studies, elevated ALP levels have been detected during orthodontic movement at weeks 1–3.[17]

One of the most up-to-date drug carrier materials that have biocompatible and biodegradable properties is hydrogel.[18] Hydrogel can be used to control drug release so that the drug can optimally work in topical applications.[19]. The active substance of the bisphosphonate risedronate carried by the gelatin hydrogel carrier medium and applied intrasulcular is expected to provide a local effect to prevent the occurrence of tooth relapse after orthodontic movement. This study was conducted to obtain an overview of the effect of the active substance bisphosphonate risedronate carried by the gelatin hydrogel carrier and applied intrasulcularly to the gingival crevicular fluid (GCF) ALP, as well as the ratio of the number of osteoclasts and osteoblasts during the movement of tooth relapse so that it is expected to have the potential to inhibit relapse. This study aims to determine and analyze the effect of bisphosphonate risedronate hydrogel on ALP and osteoclast/osteoblast ratio during tooth relapse movement.

Materials and Methods

Ethics committee approval

Research with experimental guinea pigs has received approval from the Research Ethics Commission of the Faculty of Veterinary Medicine, Gadjah Mada University (number 355/KKEP/FKH UGM/EC/2012).

Experimental animals

This study consisted of three groups: control (group A), those administered with risedronate at a concentration of 250 µmol/L (group B), and those administered with risedronate at a concentration of 500 µmol/L (group C). Control is the group that relapsed without receiving any treatment. Each group will be observed on days 0, 3, 7, 14, and 21; therefore, there are 15 groups with five guinea pigs each. Seventy-five male guinea pigs weighing 0.5–0.6 kg were fed and caged (Tomiwa, Japan) at room temperature. During the study, health and weight checks were carried out every day.[20]

Making hydrogel biphosphonate risedronate

The preparation is made using the active substance of the bisphosphonate risedronate, namely, risedronate sodium, which is made using gelatin hydrogel as a carrier so that the drug can have a topical effect. Gelatin (3%) was dissolved in distilled water and then homogenized with a magnetic stirrer for 3 hours at 37°C. Risedronate sodium was added; then, it was stirred for 2 hours, and NaOH was added until a neutral pH (7) was achieved. The mixture is added with a glutaraldehyde solution with a concentration of 25%, washed using glycine and milli-Q three times, and stored in a freezer at -300°C. Lyophilization was carried out afterward using a freeze dryer for 48 hours. The hydrogel will change from semisolid to solid. The hydrogel gelatin block matrix is then processed into microsphere preparations. When used, this preparation is mixed again using distilled water with a ratio of 1:20 (w/w). The final preparation is placed into the injection syringe and is ready to be applied.[21]

Treatment

The guinea pig was anesthetized with ketamine and xylazine by intramuscular injection in the thigh; then, a bonding cleat was placed on the lower incisors. A round stainless steel wire with a diameter of 0.016 and open coil spring with a length of 1.5 times the inter cleat distance (measured using a sliding caliper) were installed between the cleats. After the teeth moved and the open coil spring was no longer active, the open coil spring was replaced according to the new inter-clear distance until an inter-incisor distance of ±3 mm was obtained. A distance of ±3 mm was maintained for 14 days as a stabilization period. In the treatment group, bisphosphonate risedronate was administered in the form of a hydrogel dosage by intraligament injection every 3 days (Figure 1). After stabilization for 14 days, the wire and open coil were removed. The treatment group was still given topical bisphosphonate risedronate, and the relapse of the teeth on days 0, 3, 7, 14, and 21 was observed.

Alkaline phosphatase measurement

Sampling was carried out on days 0, 3, 7, 14, and 21. The area around the teeth was cleaned with cotton pellets to maintain the purity of the GCF so as not to be contaminated. Paper points are inserted approximately 1 mm into the gingival sulcus for 30 seconds with 90-second intervals to increase the volume of GCF taken per side (Figure 2). The Eppendorf tube was centrifuged for 5 minutes at a rate of 2000 g to elute the complete GCF components. Paper points were taken, and the supernatant solution was stored at -80°C until it was analyzed for a maximum of 1 week. ALP activity was determined using a spectrophotometer (model 6330 Jenway UK) at a wavelength of 405 nm.[22]

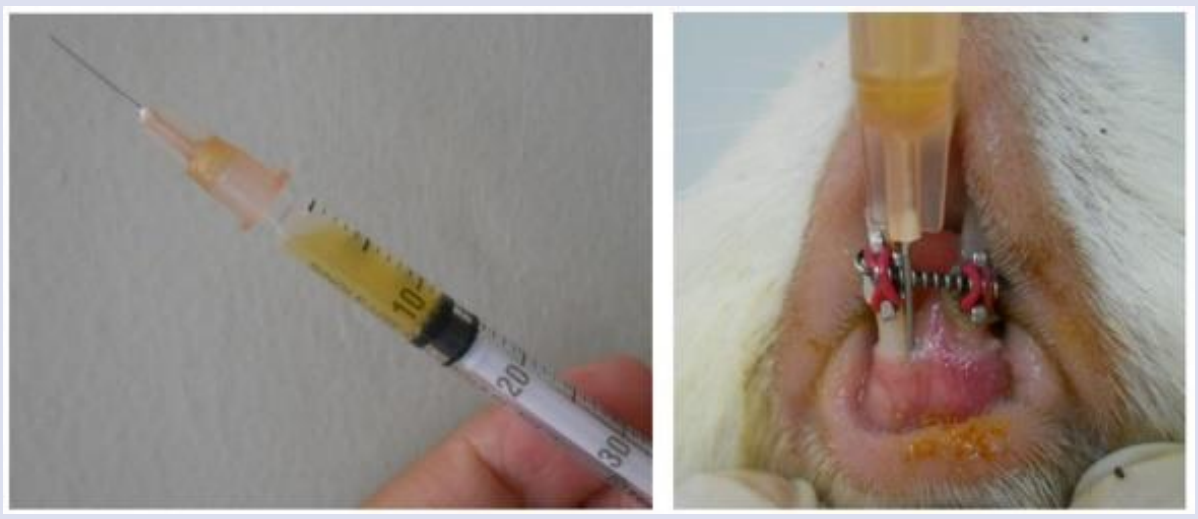

Figure 1. Bisphosphonate risedronate hydrogel is injected intraligamentary

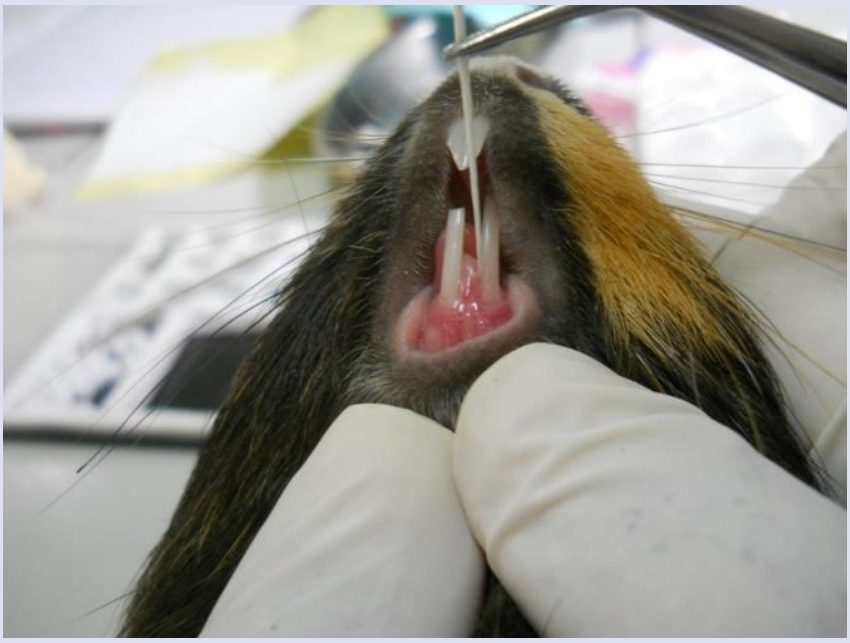

Figure 2. Gingival crevicular fluid withdrawal

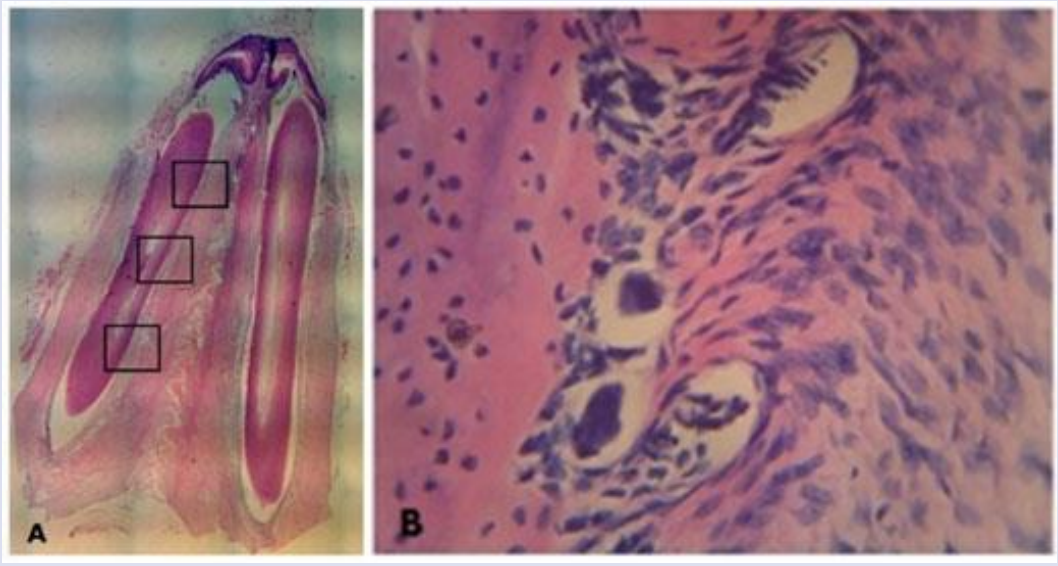

Figure 3. H&E: A. Staining three fields of view in one slide of the mesial side. B. Image of osteoclasts and osteoblasts in one field of view.

Measurement of the osteoclast/osteoblast ratio

The guinea pig was decapitated, and the mandibular alveolar bone was dissected until all the mesial and distal sides of the right and left lower incisors and the root ends of the teeth were removed. Preparations and staining were carried out at the Histology Laboratory of the Faculty of Medicine using hematoxylin and eosin (H&E) staining. The number of osteoclasts and osteoblasts was measured at the Pathology Laboratory of the Faculty of Veterinary Medicine. The data were obtained by calculating the mean number of osteoclasts and osteoblasts from three fields of view that were randomly taken on the slices of the preparation. Observations were carried out by two observers, namely, researchers (TR) and veterinarians at the anatomical pathology from Faculty of Veterinary Medicine (YN). The results of the first observer's observations were clarified to the second observer. Osteoclasts are multinuclear cells containing 4–20 nuclei and are found in contact with bone surfaces and within the lacunae. Osteoblasts were found in cuboidal cell clusters along the cell margins of the new bone (Figure 3). Osteoblasts are flat and round, essentially one or mononuclear cell usually lined up on the bone surface. Osteoblasts are usually found on the surface of solid bones, while osteoclasts are usually present in basins because the bone area has been resorbed and the cells are large and multinuclear/multinucleated.

Statistics

The data obtained in this study were statistically analyzed by SPSS. The homogeneity and normality test was carried out followed by one-way ANOVA test to determine differences in ALP levels and osteoclast/osteoblast ratios on days 0, 3, 7, 14, and 21 in groups A (without risedronate), B (250 µmol/L), and C (500 µmol/L). To find out which group of days had the most influence, the least significant difference (LSD) test was used. There is a significant difference if $p<0.05$.

Results

Effect of bisphosphonate risedronate hydrogel on alkaline phosphatase levels

The results of measuring the levels of ALP on days 0, 3, 7, 14, and 21 in the group without risedronate bisphosphonate (A), group that was given bisphosphonate risedronate at a dose of 250 µmol/L (B), and group that was given bisphosphonate risedronate at a dose of 500 µmol/L (C). The results showed that there was a significant difference in the increase in ALP levels on days 14 and 21 (Figure 4).

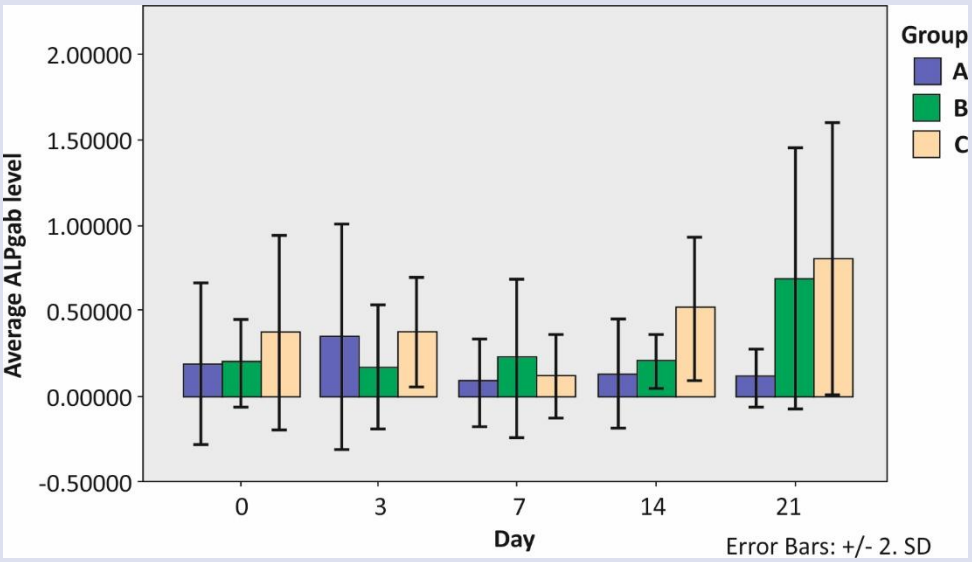

Figure 4. Effect of bisphosphonate risedronate hydrogel on ALP levels. There is a significant difference in the increase in ALP levels on days 14 and 21.

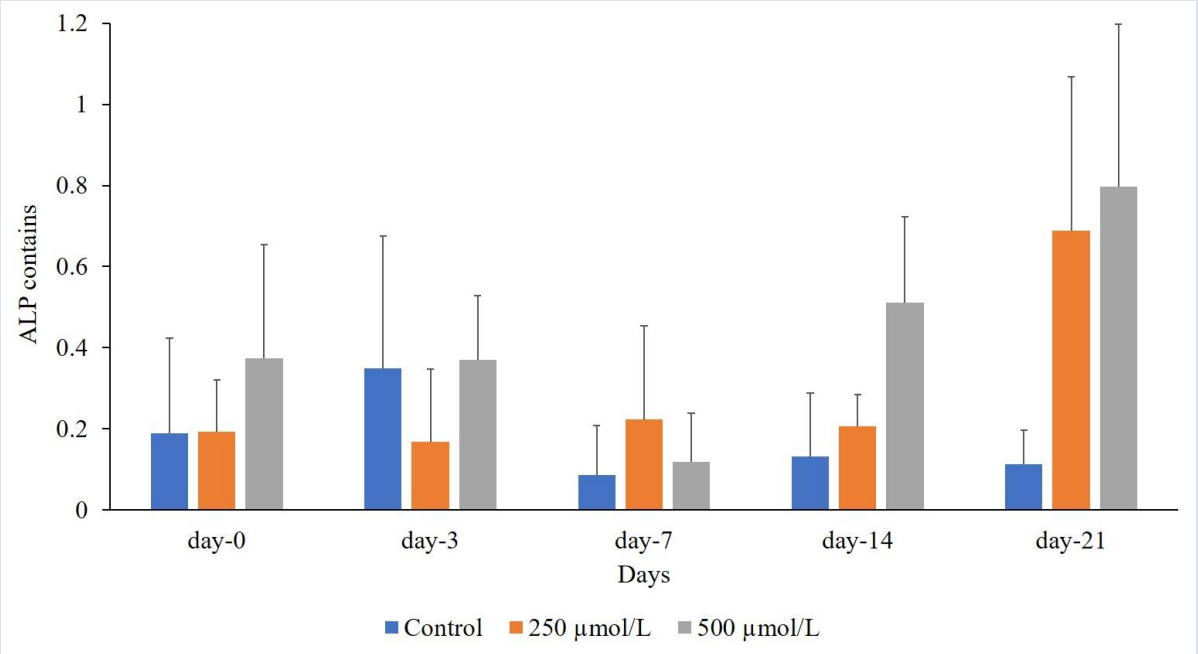

Figure 5. The effect of bisphosphonate risedronate hydrogel treatment to ALP level. There is a significant increase of ALP in 14th and 21st day.

The results of the multiple comparisons (LSD) test showed that there was a significant difference on the 14th day between groups A and C and groups B and C, and on the 21st day between groups A and B and groups A and C (Figure 5). The data is significant if the p-value is <0.05.

Effect of bisphosphonate risedronate hydrogel on alkaline phosphatase levels

The results of measurements of the number of osteoclasts and osteoblasts on days 0, 3, 7, 14, and 21 in groups A, B, and C are shown in Figure 6, as well as the results of the one-way ANOVA test. There were significant differences between groups on days 3 and 14.

The significant difference in the osteoclast/osteoblast ratio only occurred on days 3 and 14; then, it was continued with the multiple comparisons (LSD) test. There was a significant difference on day 3 between groups A and B and groups A and C, and on day 14 between groups A and C and groups B and C (Figure 6).

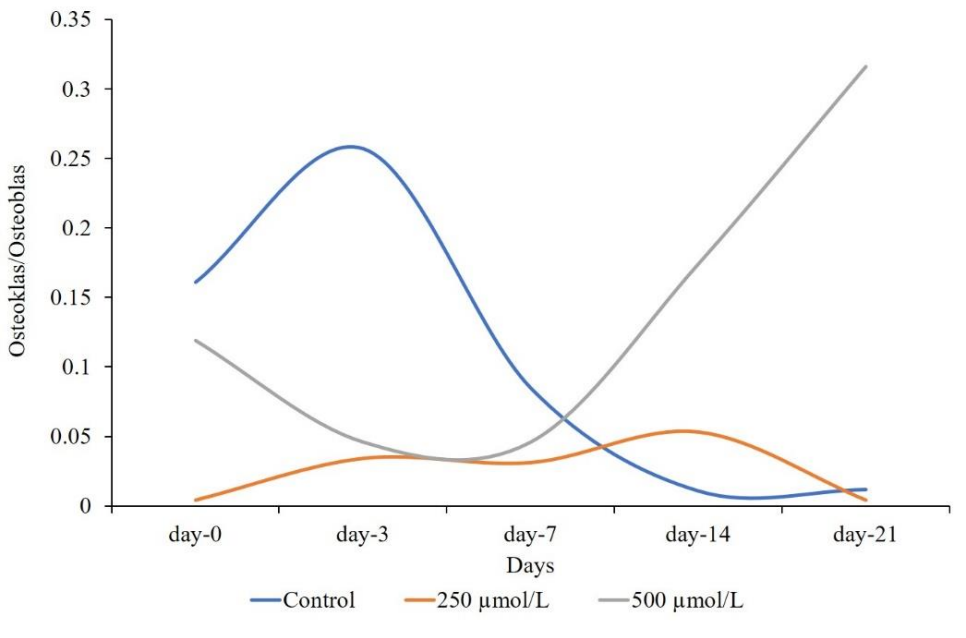

Figure 6. Osteoclast and osteoblast ration in A, B, and C groups. The significant difference was observed in 3rd and 14th day.

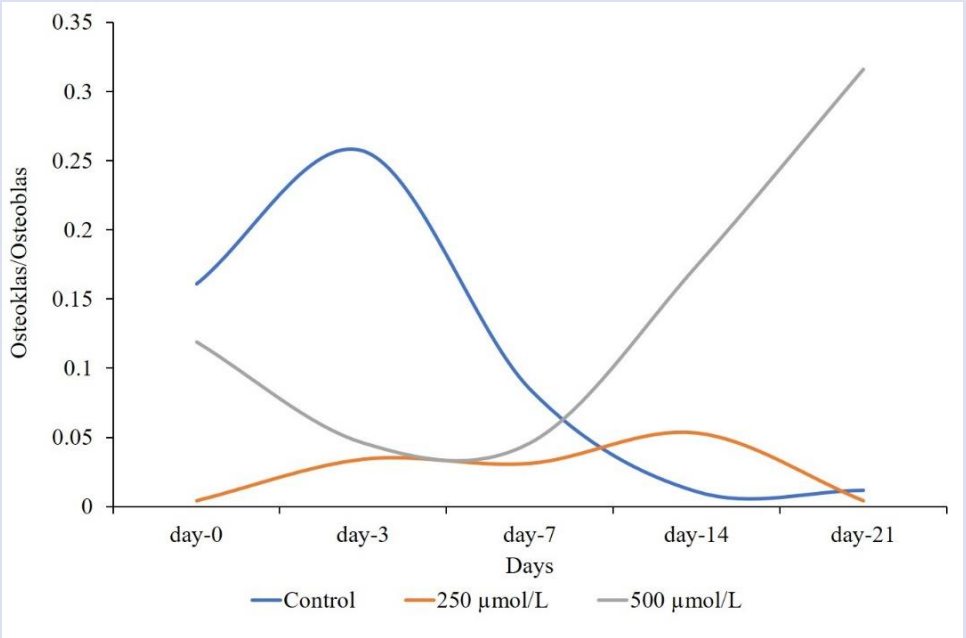

Figure 7. Histological image of osteoclasts and osteoblasts with H&E staining on days 3, 14, and 21 (x400 magnification). Black arrows indicate osteoclasts, and yellow arrows indicate osteoblasts.

The results of statistical tests showed that there was a significant difference on days 3 and 14 where the osteoclast/osteoblast ratio on day 3 in group A (without risedronate) was higher than in groups B (250 µmol/L) and C (500 µmol/L), but on day 14, the ratio was higher in group C than in groups A and B. The number of osteoblasts appeared to be more dominant in all groups (A, B, and C) on days 0, 3, 7, 14, and 21 compared to the number of osteoclasts (with a value of <1). Histological examination with H&E staining showed that the number of osteoblasts was more dominant than the number of osteoclasts (Figure 7).

Discussion

In this study, the results showed that there were no significant differences in ALP levels between groups A, B, and C on days 0, 3, and 7, but there were significant differences on days 14 and 21. The process of bone remodeling was more complex with resorptive activity at its initial phase (3–5 days) and is followed by a reversal (5–7 days). Furthermore, the final phase of bone deposition (7–14 days) occurs in both areas of stress and stress on the alveolar walls. In the early phase, bone resorption occurs more than bone deposition, but in the next phase, resorption and deposition become synchronous.[23]

In this study, ALP levels were measured with the movement of relapse when stabilization has been carried out for approximately 1 week, so there may have been an asynchronous phase between resorption and deposition; therefore, there is no significant difference on days 0, 3, and 7. On days 14 and 21, the effect of the bisphosphonate hydrogel risedronate began to show, where there was a significant difference in both ALP levels between the group without bisphosphonate (group A) and the group that received bisphosphonate injection (groups B and C). This result is not much different from the results of the study of Batra *et al.*[23], which showed that there was a significant change ($p < 0.05$) in ALP activity on days 7, 14, and 21 both on the mesial and distal sides between the experimental side and the control side. The peak of enzyme activity occurred on day 14 from the start of the retraction followed by a significant decrease in activity, especially on the mesial side.

Bisphosphonates increase the proliferation and maturation of osteoblasts.[24] Mature osteoblasts secrete osteoid, type I collagen, growth factors, and ALP. Bone formation occurs through three sequential processes: production (proliferation), maturation of the osteoid matrix, and mineralization.[25,26] During the proliferation, several extracellular matrix proteins (procollagen I, TGF-ß, and fibronectin) can be detected. The matrix maturation phase is characterized by the presence of ALP expression.[27]

The results of this study indicate that bisphosphonate risedronate with gelatin hydrogel as carrier media, caused pure sodium risedronate diffuse out when the hydrogel is degraded, so it can have a local effect. This preparation is also effective in reducing the number of osteoclasts that play a role in the resorption process and is effective in increasing the number of osteoblasts that play a role in the process of forming new bone where bisphosphonates increase the proliferation and maturation of osteoblasts, which is indicated by differences in the increase in ALP levels.[6]

The calculation of the number of osteoclasts and osteoblasts in the stress area during the movement of the relapse aims to obtain the ratio between osteoclasts and osteoblasts to determine the dominance of the activity of the two cells. The results of this study indicated that osteoblasts were more dominant than osteoclasts in the three groups both on days 0, 3, 7, 14, and 21, indicating that osteoblast activity in the bone formation process was more dominant than osteoclasts during tooth relapse movement.

Based on the results of their research, Franzen *et al.*[2] stated that orthodontic tooth movement and relapse would show the same process. On the side which is the tension area (tension side) during active gear movement, it will be changed to a pressure side during the relapses.[28]. After the removal of the orthodontic appliance, the tooth begins to relapse and moves to its original position. This movement is accompanied by changes in the number and distribution of osteoclasts. The number of osteoclasts significantly decreased in both mesial and distal roots of the first molars within 3 days, most likely due to apoptosis and/or decreased vascular density. The number of osteoclasts decreased further on day 14 and stabilized on days 14–21 of the relapse period.[3]

The results of this study showed a significant difference in the osteoclast/osteoblast ratio on days 3 (between groups A and B and groups A and C) and 14 (between groups A and C and groups B and C) and showed that osteoblasts were more dominant than osteoclasts in all three groups. The results of the study of Von Knoch *et al.*[29] using a clinically relevant *in vitro* model showed that bisphosphonates increase bone marrow stromal cell proliferation and initiate osteoblastic differentiation. Although the main action of bisphosphonates is inhibition of bone resorption by osteoclasts, there is increasing evidence that bisphosphonates also interact with osteoblasts.[9]

The effect of bisphosphonates inhibits not only osteoclast activity but also osteoblast formation. Bisphosphonates can stimulate osteoblast proliferation and inhibit osteocyte and osteoblast apoptosis. The results showed that bisphosphonates increased OPG expression in human osteoblastic cells, suggesting that the antiresorptive effect of bisphosphonates was mediated by the influence of osteoblasts.[9] Research by Krishnan *et al.*[6] showed that the application of nitrogenous-type bisphosphonate risedronate increased the number of osteoblasts.

It was concluded that intrasulcular application of bisphosphonate risedronate hydrogel had an effect on the osteoclast ratio of osteoblasts and increased levels of ALP on days 14 and 21. Hydrogel risedronate bisphosphonate increased the proliferation and maturation of osteoblasts, which play an important role in bone formation, thereby increasing tooth stability after orthodontic movement. These results indicate the important role of bisphosphonate risedronate hydrogel in the bone formation process; therefore, it has the potential to inhibit relapse. Although the use of risedronate hydrogel in this

study shows a good effect, it is not easy enough to apply it in the gingival sulcus, so further research is needed to make preparations that are easier to apply and have a local effect.

Conclusions

The intrasulcular application of bisphosphonate risedronate affected the osteoclast/osteoblast ratio and increased ALP levels. These results indicate the important role of bisphosphonate risedronate hydrogel in the bone formation process; therefore, it has the potential to increase tooth stability after orthodontic movement and prevent relapse.

Acknowledgements

The author would like to thank the Faculty of Dentistry, Gadjah Mada University for the 2014 Community Fund Grant.

Conflicts of Interest Statement

There is no conflict of interest with the research results and publication of this manuscript.

References

1. Littlewood SJ, Kandasamy S, Huang G. Retention and relapse in clinical practice. Aust Dent J 2017; 62:51–57.
2. Vaida L, Todor BI, Lile IE, Mut A-M, Mihaiu A, Todor L. Contention following the orthodontic treatment and prevalence of relapse. HMV Bioflux 2019; 11:37-42.
3. Franzen TJ, Brudvik P, Vandevska-Radunoviv V. Periodontal tissue reaction during orthodontic relapse in rat molars. Eur J Orthod 2013; 35:152-159.
4. Schneider DA, Smith SM, Campbell C, Hayami T, Kapila S, Hatch NE. Locally limited inhibition of bone resorption and orthodontic relapse by recombinant osteoprotegerin protein. Orthod Craniofac Res 2015; 18:187-195.
5. Zhao N, Lin J, Kanzaki H, Ni J, Chen Z, Liang W, et al. Local osteoprotegerin gene transfer inhibits relapse of orthodontic tooth movement. Am J Orthod Dentofacial Orthop 2012;141:30-40.
6. Krishnan S, Pandian S, Kumar SA. Effect of bisphosphonates on orthodontic tooth movement—an update. J Clin Diagn Res 2015; 9: ZE01-ZE05.
7. George EL, Lin Y-L, Saunders MM. Bisphosphonate-related osteonecrosis of the jaw: a mechanobiology perspective. Bone Reports 2018; 8:104-109.
8. Otto S. Medication-related osteonecrosis of the jaws: bisphosphonate, denosumab, and new agents. Germany: Springer-Verlag Berlin Heidelberg 2015: 27-42.
9. Maruotti N, Corrado A, Neve A, Cantatore FP. Bisphosphonates: effects on osteoblast. Eur J Clin Pharmacol 2012;68:1013-1018.
10. Branco Santos, J.C., de Melo, J.A., Maheshwari, S., de Medeiros, W.M.T.Q., de Freitas Oliveira, J.W., Moreno, C.J., Mario Amzel, L., Gabelli, S.B., Sousa Silva, M. Bisphosphonate-Based Molecules as Potential New Antiparasitic Drugs. Molecules 2020; 25: 2602.
11. Russell, R. Graham G. Bisphosphonates: the first 40 years. Bone 2011; 49(1): 2-19.
12. Abtahi J. Bisphosphonates and implants in the jaw bone. Dissertations. Linköping University; 2011)
13. Florencio-Silva R, Silva Sasso GR, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of bone tissue: structure, function, and factors that influence bone cells. Bio Med research international 2015;2015.
14. Kini U, Nandeesh BN. Physiology of bone formation, remodeling, and metabolism. Radionuclide and hybrid bone imaging 2012: 29-57.
15. Sanikop S, Patil S, Agrawal P. Gingival crevicular fluid alkaline phosphatase as a potential diagnostic marker of periodontal disease. J Indian Soc Periodontol 2012;16.(4): 513
16. Ameer SAA, Alhuwaizi AF. The effect of orthodontic force on salivary levels of alkaline phosphatase enzyme. Journal of Baghdad College of Dentistry 2015; 27(4): 175-179.
17. Flórez-Moreno GA, Marín-Restrepo LM, Isaza-Guzmán DM, Tobón-Arroyave SI. Screening for salivary levels of deoxypyridinoline and bone-specific alkaline phosphatase during orthodontic tooth movement: A pilot study. Eur J Orthodon 2013; 35(3):361-368.
18. Saito T, Tabata Y. Preparation of gelatin hydrogels incorporating low-molecular-weight heparin for anti-fibrotic therapy. Acta Biomater 2012; 8:646-652.
19. Li J, Mooney, DJ. Designing hydrogels for controlled drug delivery, Nat Rev Mater. 2016;1(12):16071.
20. Santoso MIE. Buku ajar etik penelitian kesehatan [Health research ethics textbook]. Malang: Universitas Brawijaya Press 2011. [in Indonesian]
21. Ikada Y, Tabata Y. Protein release from gelatin matrices. Adv Drug Deliv Rev 1998; 31:288-301.
22. Asma AAA, Rohaya MAW, Hisham ZAS. Crevicular alkaline phosphatase activity during orthodontic tooth movement: canine retraction stage. J Med Sci 2008;8:228–233.
23. Batra P, Kharbanda Op, Duggal R, Singh N, Parkash H. Alkaline phosphatase activity in gingival crevicular fluid during canine retraction. Orthod Craniofac Res 2006;9:44-51.
24. Manzano-Moreno FJ, Ramos-Torrecillas J, Melguizo-Rodríguez L, Illescas-Montes R, Ruiz C, García-Martínez O. Bisphosphonate modulation of the gene expression of different markers involved in osteoblast physiology: Possible implications in bisphosphonate-related osteonecrosis of the jaw. Int J Med Sci 2018; 15(4):359-367.
25. Baron R, Neff L, Tran-Va P, Nefussi JR, Vignery A. Kinetic and cytochemical identification of osteoclast precursors and their differentiation into multinucleated osteoclast. Am J Pathol 1986; 122:363-378.
26. Lerner UH. New molecules in the tumor necrosis factor ligand and receptor superfamilies with importance for physiological and pathological bone resorption. Crit Rev Oral Biol Med 2004;15:64-81.
27. Hanna H, Mir LM, Andre FM. In vitro osteoblastic differentiation of mesenchymal stem cells generates cell layers with distinct properties, Stem Cell Res Ther 2018;9:203.
28. Maltha JC, Vandevska-Radunovic V, Kuijpers-Jagtman AM. The biological background of relapse of orthodontic tooth movement. In: Krishnan V, Davidovitch Z. Biological mechanisms of tooth movement. NJ: John Wiley & Sons Ltd 2015.
29. Von Knoch F, Jaquiery C, Kowalsky C, Schaeren S, Alabre C, Martin I, et al. Effects of bisphosphonates on proliferation and osteoblast differentiation of human bone marrow stromal cells. Biomaterials 2005; 26: 6941–6949.