Is Tissue Oxygenation Causes Jaw Osteonecrosis in Osteoporotic Bone?

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Abstract

The pathogenesis of the occurrence of BRONJ until now cannot be determined with certainty. This paper investigates the effects of zoledronic acid (Z) on tissue oxygenation concerning the healing process of post-extraction tooth sockets. 24 female Sprague Dawley mice that had an ovariectomy were divided into three groups: a dose of 1x Z (n=8), a group of 6x Z (n=8), and a control group (n=8) who got 0.1mg/kg of Z through the intravena pathway. After six weeks, the extraction of the first molar teeth of the mandible. IHK and Elisa examinations were carried out on H+0 and H+14 post-extraction to observe HIF1-alpha, BMP2, and VWF expression concentrations.

Statistical analysis results using the Kruskal-Wallis nonparametric test, the concentration of HIF1-alpha did not get a significant difference ($p = 0.073$). Likewise for the concentration of BMP2 ($p = 0.123$). As for the expression of also found no significant results ($p = 0.076$).

The concentrations of HIF1-alpha and BMP2 tended to be higher in the Z group, although the results were not statistically significant. For VWF expression, the higher trend in the Z group was statistically insignificant. Therefore, caution is needed when interpreting the results of these three types of analysis with follow-up research dan clinical studies.

Experimental article (J Int Dent Med Res 2022; 15(3): 1001-1005)

Keyword: Hypoxia-Inducible Factor 1, Bone Morphogenetic Proteins, Osteonecrosis, Zoledronic Acid.

Received date: 12 April 2022 **Accept date:** 15 July 2022

Introduction

Osteoporosis is a systemic skeletal disease that has detrimental effects on bone mass and bone microarchitecture, leading to increased fracture risk. [1] The prevalence of osteoporosis in East Asian countries is growing due to a rapid rise in the percentage of elders in the population. The amino-bisphosphonates are first-line therapy for the treatment of most patients with osteoporosis, with proven efficacy to reduce fracture risk at the spine, hip, and other nonvertebral skeletal sites, that significantly decrease morbidity and increase survival.[2]

In terms of the stability of the jawbone structure, osteoporosis and loss of bone density due to estrogen deficiency can hurt tooth stability.[3] Tooth extraction is thought to be one of the etiological factors underlying osteonecrosis of the jaw (ONJ). This invasive procedure causes alveolar bone trauma and long-term use of BPs, slowing the extraction site's healing (tooth socket).

In 2003, Marx et al. were the first to report osteonecrosis of the jaw (ONJ) linked to antiresorptive drugs, such as bisphosphonates (BPs). Sugiyama reported that ZOL administered at an optimal dose was the most effective therapy for osteoporosis patients requiring dental extractions, regardless of ZOL-related side effects.[4] In 2016, a study reported that zoledronate in topical use could penetrate into alveolar bone rats and increase apoptosis osteoclast which is useful for orthodontic tooth movement.[5]

The International Task Force for ONJ recently reported that the worldwide prevalence of ONJ in osteoporosis patients treated with oral BPs ranged from 0 to 0.04%, with intravenous

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administration of BPs associated with a significantly higher prevalence (0−0.348%) of the disease.[6] Administration by oral route will only be stored in the bones < 1 % while intravenously, 70% will be held in the bones.[7]

Hypoxia-inducible factor – 1 alpha (HIF1-alpha) is a major transcriptional regulator of the cellular response and the development of hypoxia.[8] Overexpression of HIF1-alpha in tissues causes angiogenesis. It increases oxygenation as a physiologic mechanism,[9] so it can be investigated as the basis of molecular mechanisms to obtain new therapies related to oxygen homeostasis associated with ONJ.[8]

Bone morphogenic protein - 2 (BMP-2), plays a role in angiogenesis during bone formation and tooth socket healing postextraction.[10] Under physiological conditions, the vascular endothelium produces substances involved in hemostasis, fibrinolysis, growth factor synthesis, and vascular tone and permeability.[11] These substances include the von Willebrand factor (VWf), synthesized and stored in endothelial cells.[11] The levels of VWf increase in response to endothelial cell damage as an indicator of endothelial dysfunction. They may have potential value as a biomarker for developing pathological conditions.[11]

The potential role of ZOL use in the pathogenesis of ONJ is unclear. Biochemical analyses of HIF1-alpha, BMP-2, and VWF levels following ZOL treatment are needed to shed light on their potential roles in the pathogenesis of ONJ and to detect the risk of post-tooth extraction osteonecrosis. In case of osteonecrosis, piezosurgery allows an effective osteotomy under local anesthesia with minimized surgical trauma and initiated a good healing response.[12]

There have been many studies on experimental osteoporosis animal models treated with BPs.[13] However, these studies did not investigate the potential role of BPs in the pathophysiology of BRONJ. Thus, we used an experimental animal osteoporosis model treated with ZOL before tooth extraction. This study aimed to investigate the effect of ZOL administration on osteoporotic bone in the early stages of socket healing after tooth extraction.

Materials and methods

Nine-week-old female Sprague Dawley (SD) rats with a mean weight of 200 g purchased from the Indonesian Center for Veterinary Research (Bogor, Indonesia) were used in this study. The research sample consisted of 24 randomly selected animals. The study proceeded in two stages. All the animals underwent a bilateral ovariectomy to create an osteoporosis model in the first stage. In the second stage, the animals were divided into three groups, as follows: a single-dose injection group (ZAS, n=8), a repeated-dose injection group (ZAR, n=8), and a control group (VEH, n=8). The ZAS and ZAR groups were treated with ZOL. The animals in the placebo group were treated with sodium chloride solution. Following an ovariectomy, the first molar was extracted in all three groups. Based on the time of euthanasia, the groups of rats were divided into two days. Namely, D-0 is done shortly after tooth extraction, and D-4 is done 14 days after tooth extraction to observe the healing period.

The research was conducted in the laboratory of the Veterinary Hospital, Faculty of Veterinary Medicine, Bogor Agricultural University (Bogor, Indonesia) and the animal ethics committee approved the study of the Faculty of Veterinary Medicine of Bogor Agricultural University (ethical approval No: 163/KEH/SKE/XII/2019).

Zoledronic acid (Zometa®, Novartis Pharma AG, Basel, Swiss) was administered intravenously, with a dose (100 µg/kg BW) used by Kim et al.[14] The treatment commenced eight weeks after the ovariectomy. The ZAS group received a one-time injection, the ZAR group received six injections, and the VEH group received a single sterile 0.9% sodium chloride solution.

The mandibular right first molar was extracted with consideration of access and visibility on day 42 after treatment, using a dental explorer to loosen the tooth from the alveolar bone.

Alveolar mucosal tissue samples in the tooth socket are removed for analysis because of the difficulty of taking the bone sample from the socket tooth extraction. HIF1-alpha, and BMP-2 antibodies were analyzed according to standard protocols using a standard protocol rat HIF1-alpha , ELISA kit, catalog no: E-EL-R0513 and

rat BMP-2 ELISA kit, catalog no: E-EL-R0002, Elabscience®, Texas, United States. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Paraffin pieces were processed before the IHC. The antibody to be detected is off, performed according to the standard protocol kit (catalog no: ab6994, Abcam, United Kingdom) to see the expression of biological markers of the angiogenesis.

For statistical analysis, SPSS, version 11 was used. Nonparametric tests were applied. Between-group data were analyzed using the Kruskal-Wallis test, and the Mann-Whitney test was used for intergroup comparisons. Differences were taken as being statistically significant for a p-value < 0.05.

Results

The results of the Kruskal-Wallis revealed no significant between-group difference in HIF1-alpha concentration (p = 0.07). The results of the Mann-Whitney showed no significant intergroup differences (p = 0.07) (Figure 1).

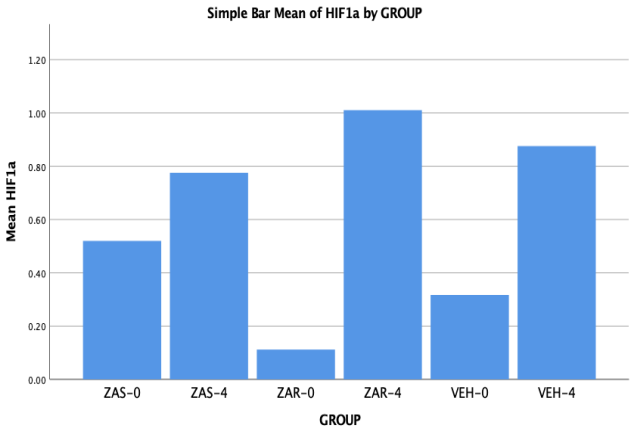

Figure 1. Concentration of HIF1-alpha. Day 0 post-extraction appeared to be the highest ZAS group compared to the ZAR and control group. Day 14 post extraction of the ZAR group was highest compared to the ZAS and control group. In all groups, there was an increase in 14 days post-extraction.

The results of the Kruskal-Wallis revealed no significant between-group difference in BMP-2 concentration (p = 0.12). The results of the Mann-Whitney showed no significant intergroup differences (p = 0.15) (Figure 2).

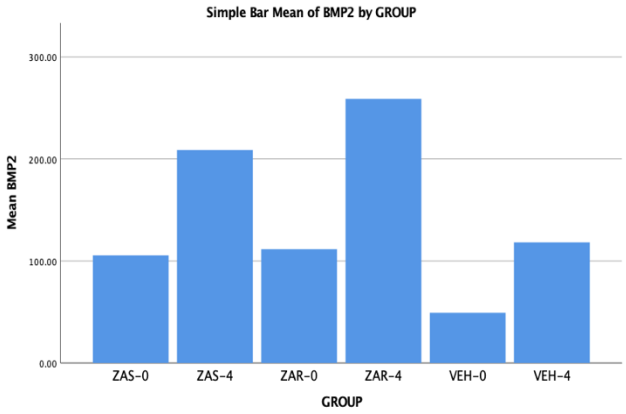

Figure 2. Concentration of BMP-2. Day 0 and Day 14 post-extraction appeared higher than the control group; there was an increase in 14 days post-extraction in all groups.

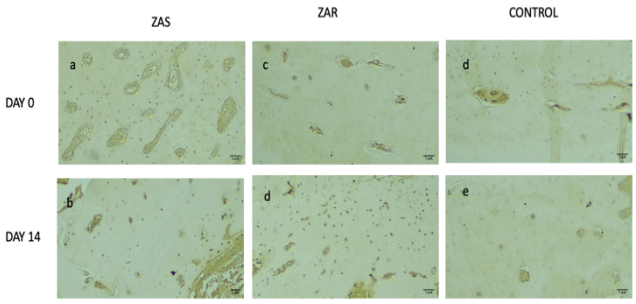

Figure 3. VWF expression. A and B groups of ZAS look quite a lot of expressions. C, D groups of ZAR are seen with fewer numbers of expressions than ZAS. The e and f control groups saw the slightest indication compared to the other groups.

The statistical analysis of the immunohistochemical examination of angiogenesis (Figure 3), as determined by endothelial cell immunoexpression and microvessel counts, revealed no significant between-group differences as the results of Kruskal-Wallis in day 0 pass extraction (p =0.17) (p > 0.05) and day 14 pass extraction (p=0.076) (p > 0.05).

Discussion

Bisphosphonates are antiresorptive drugs that bind to bone minerals, inhibit osteoclasts differentiation and function, increase osteoclastic apoptosis, and inhibit angiogenesis.[15,16] Although the activity of BPs is thought to be related to their inhibition of bone resorption, trauma, immune dysfunction, inflammation, and blood supply, the

pathophysiology underlying their activity remains unclear.[16]

This study examined HIF1-alpha, BMP-2, and VWF as markers of wound healing in osteoporotic bone after tooth extraction and different doses of ZOL. Bone formation begins with the expression of osteogenic factors, including BMP-2, BMP-4, and BMP-7, which are central players in osteoblast differentiation during osteogenesis and trigger the development of mineralized bone tissue.[17] As an alternative, for the acceleration of BMP-2 expression, one of them can apply cordifolia (Ten) so that it can induce osteoblast cells after extraction and new bone growth.[18]

Osteoclasts are the main targets of ZOL therapy. ZOL stimulates the synthesis of osteoclast resorption inhibitor, which inhibits the recruitment and activation of osteoclasts, thereby aiding the process of new bone formation.[7] The different effects on the osteoclast, ZOL therapy in osteoblasts, and osteosit exerts antiapoptosis effects, preventing an increase in apoptosis involving rapid activation of kinases in extracellular signal-regulated kinases.[19]

This study reported that the expression of BMP-2 in the ZOL treatment group appeared to be higher than that in the control group. The authors concluded that ZOL therapy in osteoporotic bones could maintain osteoblast viability.

In addition to osteoblasts and osteoclasts, vascularization plays a role in bone healing and mineralized tissue formation. When this balance is disturbed, the bone healing process is hampered. The involvement of the vascular formation processes in mineralized tissue formation cannot be ignored.

Inadequate vascularization interferes with metabolism and the supply of nutrients to tissue, resulting in impaired healing field.[20] In tissue injury, there will be an increase in HIF1-alpha to maintain cellular life until the healing process stops,[21] then HIF1-alpha will be strongly expressed to stimulate angiogenesis, leading to an increase in new bone formation and bone mineral density.[22]

In the present study, HIF1-alpha expression increased in the ZAS group compared to that in the control group compared to that in the ZAR and control group on the day of the extraction. Two weeks post-extraction, high HIF1-alpha expression was detected in the ZAR group versus the control and ZAS groups. This reflects that the administration of zoledronate causes the atmosphere of a hypoxic environment to occur up-regulated expression of HIF1-alpha. HIF 1 maintains cellular life with angiogenesis needed for the healing process can be adequate, which continues with the mineralization of bone so that bone volume can be achieved.

This may indicate that the bone receiving zoledronic acid therapy is hypoxic. Still, the up-regulation of HIF1-alpha to not affect osteoblast activity characterized by BMP-2 expression, which is also high in the ZAS and ZAR groups.

In the process of new bone formation, angiogenesis and mineralization occur. Research conducted by Allen et al. stated that bones that get bisphosphonate therapy are not supported by an adequate blood supply, which causes necrosis and superinfection.[23] The present study results revealed higher expression of VWF in the ZOL treatment groups than in the control group on days 0 and 14. However, there was no statistically significant between-group difference. Thus, VWF expression in the group that received ZOL appeared lower on day 14 post-extraction than in the control group, although statistically, no significant differences were found. This can indicate no new blood vessels (angiogenesis) formation, so further research is needed. Ziebart et al. also found impaired migration and proliferation of endothelial cells during angiogenesis after administration of zoledronic acid.[24]

Although ZOL has antiangiogenic properties and its effect on endothelial cells regarding vascularization and the angiogenesis processes, further research needs to be done.

Limitations in this study in the form of a relatively small number of rats, where the investigation began by making osteoporotic bone models until osteonecrosis occurred in the jaw due to tooth extraction took a long time, 112 days (16 weeks), so the maintenance of mice for this study requires a high level of attention because it takes a long time to avoid the occurrence of rat deaths before the study period is completed.

Conclusions

This study used the ELISA examination to determine the concentration of HIF1-alpha and BMP-2 and the IHC examination to determine the expression of VWf. Concentrations of HIF1-alpha

and BMP-2 tended to be higher in the ZOL group, while VWF expression, the higher trend in the ZOL group also. Therefore, caution is needed when interpreting the results of these three types of analysis with follow-up research dan clinical studies.

Acknowledgements

We would like to thank all Teaching Veterinary Hospital and Pathology Laboratory of Veterinary Medicine IPB University staff for their support during the experiment.

Declaration of Interest

All authors have made a substantive contribution to this study and manuscript, and all have reviewed the final paper prior to its submission. No conflict of interest in this study.

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