Effects of alendronate in relation to the administration period on bone metabolism around implants in rats

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(Directed by Professor Hong Seok Moon, D.D.S., M.S.D., Ph.D.)

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감사의 글

본 논문을 완성하는 데 있어 아낌없는 격려와 세심한 지도로 저를 이끌어 주신 문홍석 지도교수님께 마음 깊이 감사 드립니다. 바쁘신 와중에도 논문 심사를 맡아 주시고 바른 방향으로 논문이 작성될 수 있도록 귀중한 조언을 해 주신 박영범 교수님, 이재훈 교수님, 정한성 교수님, 그리고 조성원 교수님께 깊은 감사를 드립니다. 또한, 치과의사로서 올곧게 성장할 수 있도록 늘 관심으로 지켜봐 주시고 가르침을 주시는 정문규 교수님, 한동후 교수님, 이근우 교수님, 심준성 교수님, 김선재 교수님, 그리고 김지환 교수님께 감사드립니다. 윤기준 선배님을 비롯하여 함께 의국 생활을 했던 보철과의 의국원들과, 시편의 제작 등에 많은 도움을 주신 박상현 기사 및 이채은 연구원에게도 감사의 마음을 전합니다. 앞으로도 낮은 자세에서 끊임없이 정진하는 치과의사가 되도록 노력하겠습니다.

마지막으로, 언제나 곁에서 제 편이 되어주시고 헌신과 사랑으로 키워주신 아버지, 어머니, 그리고 사랑하는 동생 이철이에게 감사의 마음을 전하며 이 기쁨을 함께 나누고 싶습니다.

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Alendronate belongs to the bisphosphonate class of drugs that has been widely prescribed to prevent or treat various bone disorders. Its increased clinical application has raised concerns about complications such as failure of implant osseointegration and medication-related osteonecrosis of the jaw (MRONJ) in dentistry. A few previous animal studies that have attempted to determine the effects of bisphosphonates on the bone around

the implant area were performed on sites other than jaw bones or used bisphosphonates other than alendronate. The present study aimed to determine the effects of alendronate in relation to the administration period on bone metabolism around implants placed in the rat maxilla from a multidisciplinary point of view, by employing microcomputed tomographic, histologic, and biochemical analyses.

Thirty-six male Sprague-Dawley rats received periodic subcutaneous injections of either alendronate (alendronate group, n=18) or normal saline (control group, n=18) 4 weeks after the extraction of the maxillary first molars. The custom-made titanium implants were placed bilaterally into the extraction sites 4 weeks after the commencement of injection protocol. The rats were sacrificed at either 4, 8, or 12 weeks after implantation (4-, 8-, and 12-week groups, respectively; n=6 rats per group). Microcomputed tomographic and histologic analyses were conducted for all rats. Biochemical analyses were additionally carried out at four time points for the 12-week groups.

There were no statistically significant differences between the groups on microcomputed tomographic and histologic analyses. The measured biochemical parameters showed a tendency of decrease over time except for the serum osteocalcin (s-OC) level of the control group, with significant differences among some time points within each group. The serum osteocalcin (s-OC) level was significantly lower in the 12-week alendronate group than in the control group ($p < 0.05$).

Within the limitations of the present study, alendronate administration did not cause significant differences at the bone–implant interface during the early phase after

implantation. However, alendronate might have affected bone metabolism around the implants during the late phase of the experimental period. Further researches that address the long-term validation and clinical availability of the results are required.

Key words: alendronate, bisphosphonate, bone metabolism, implant failure, medication-related osteonecrosis of the jaw, serum osteocalcin

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I. Introduction

Bisphosphonates have become widely prescribed medications for not only inhibiting bone resorption but also treating various bone disorders, including Paget's disease of bone, metastatic bone diseases, multiple myeloma, osteopenia, and osteoporosis.[1-3]

The chemical structure of bisphosphonates shares similarities with those of naturally occurring pyrophosphate compounds, and serves as the basis for understanding their clinical effects (Figure 1).[4,5] A bisphosphonate is composed of a carbon atom in the core linked to four side chains: two of these side chains are phosphonate groups that allow the chelation of calcium ions, and the other two (designated as the R1 and R2 side chains) result in structural variations and thus in different pharmacologic activities.[6-8] When the R1 side chain has a hydroxyl group, it will enhance the binding to bone mineral surfaces together with the two phosphonate groups by acting as a "bone hook."[3,9] The R2 side chain determines the antiresorptive potency of bisphosphonates depending on the presence of nitrogen or amino groups, since these groups dramatically promote the inhibition potency of bone resorption.[7,10,11] Due to this property, alendronate is now one of the most-widely prescribed nitrogen-containing bisphosphonates for treating osteoporosis in the United States.[12]

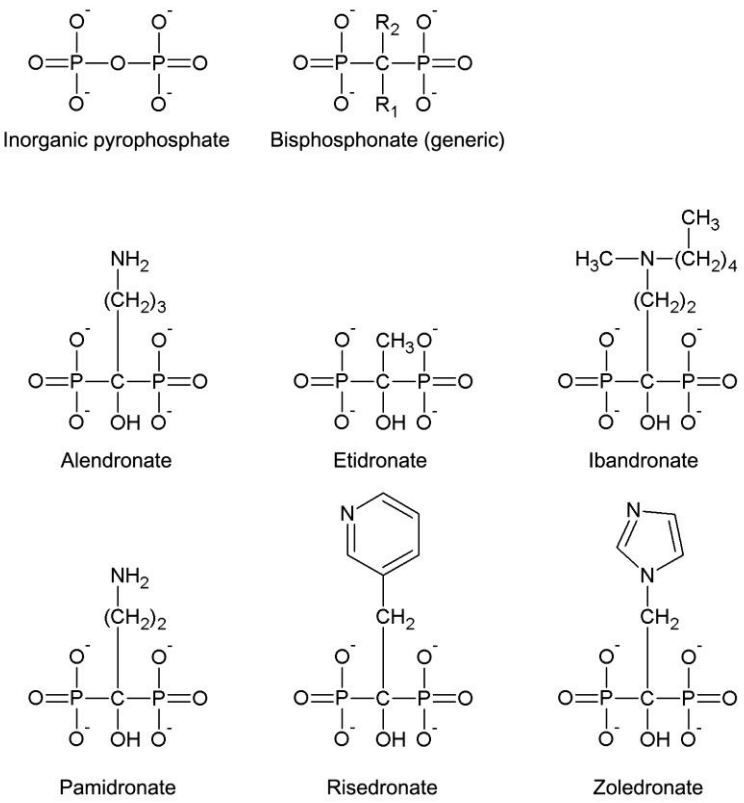

Figure 1. Chemical structure of inorganic pyrophosphate and bisphosphonates.

Both non-nitrogen-containing and nitrogen-containing bisphosphonates are thought to suppress bone resorption by inducing the apoptosis of osteoclasts via an intracellular process. However, the contemporary point of view is that these two classes of bisphosphonates exert different effects on osteoclasts at the molecular level.[13,14] Non-nitrogen-containing bisphosphonates induce osteoclast death by inhibiting the adenosine triphosphate (ATP)-dependent intracellular pathways inside the osteoclasts.[15] In contrast,

nitrogen-containing bisphosphonates cause the death of osteoclasts by interfering with several key enzymes involved in the mevalonate pathway that contributes to the production of cholesterol, other sterols, and isoprenoid lipids (Figure 2).[16-18] Farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) are isoprenoid lipids synthesized via the mevalonate pathway, and are required for the posttranslational modification (prenylation) of small guanosine triphosphatase (GTPase) signaling proteins such as Ras, Rho, and Rab.[19,20] These small GTPases play important roles in regulating the cellular activities of osteoclasts, such as membrane ruffling, trafficking of intracellular vesicles, and cytoskeletal arrangement.[21-23]

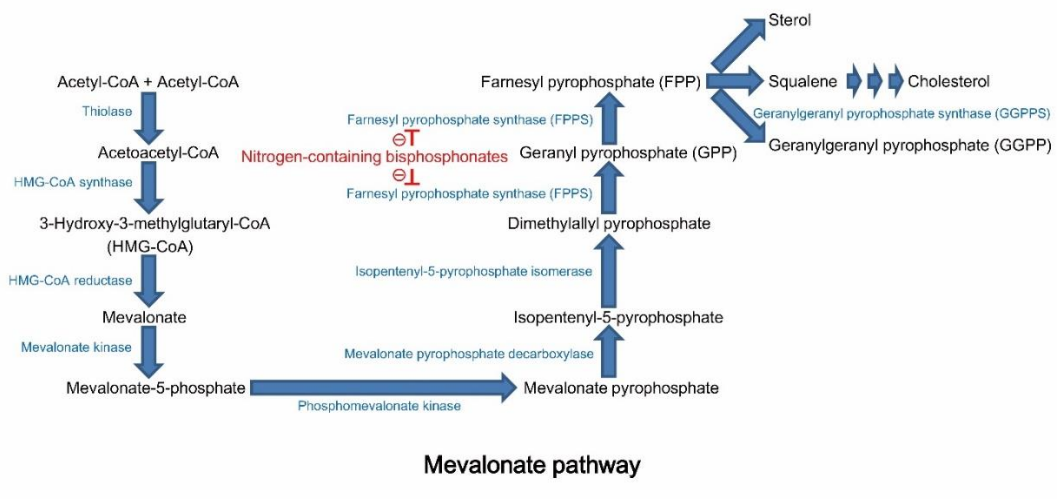

Figure 2. Mevalonate pathway. Nitrogen-containing bisphosphonates primarily inhibit farnesyl pyrophosphate synthase (FPPS).

Nitrogen-containing bisphosphonates mainly target, bind, and inhibit the enzyme farnesyl pyrophosphate synthase (FPPS), among many key enzymes, thereby directly inhibiting the synthesis of FPP and indirectly inhibiting the synthesis of GGPP.[20] This blocks the prenylation of small GTPases and induces the apoptosis of osteoclasts. Meanwhile, bone resorption and formation are known to be coupled since osteoclasts and osteoblasts communicate with each other via an array of crosstalk networks.[24] In addition, several osteoblast-stimulating cytokines are released from osteoclasts.[25] Hence, nitrogen-containing bisphosphonates may also suppress osteoblastic bone formation.[26]

The increased clinical application of bisphosphonate has raised concerns about complications such as esophageal irritation, atrial fibrillation, frozen bone, severe musculoskeletal pain, hypocalcemia, and flu-like symptoms.[5] Failure of implant osseointegration and medication-related osteonecrosis of the jaw (MRONJ) have been common clinical concerns in dentistry.[27,28]

The first report of medication-related implant failure addressed the failure of five osseointegrated endosseous implants in the anterior mandibular area after bisphosphonate therapy for osteoporosis.[29] Although the associations between specific medications and implant survival or success rates were not well understood at that time, it was postulated that bisphosphonate could affect implant osseointegration to some extent. Since then there have been many controversial findings regarding the effects of bisphosphonates on implant maintenance: some clinical studies found that the use of bisphosphonates did not affect implant osseointegration,[30-33] while others found that patients who received bisphosphonate

therapy were significantly more vulnerable to implant failure.[34-36] Furthermore, some researchers found that patients undergoing bisphosphonate therapy developed MRONJ.[37,38]

The main features of MRONJ involve exposed bone or bone that can be probed through an intraoral or extraoral fistula in the maxillofacial region that is present for longer than 8 weeks in patients undergoing or who have previously received treatments with antiresorptive or antiangiogenic agents and who do not have a history of radiation therapy or obvious metastatic disease to the jaws.[2] Although the pathophysiology of MRONJ remains unclear, it has been suggested to be caused by the suppression of bone metabolism related to traumatic events such as dental extraction, implant placement, and denture irritation.[39-42]

A few animal studies have attempted to determine the effects of bisphosphonates on the bone around the implant area.[43-46] However, most of these studies were performed on sites other than jaw bones or used bisphosphonates other than alendronate over relatively short experimental periods. The present study therefore aimed to determine the effects of alendronate in relation to the administration period on bone metabolism around implants placed in the rat maxilla from a multidisciplinary point of view, by employing microcomputed tomographic, histologic, and biochemical analyses.

II. Materials and methods

1. Experimental design and procedures

Thirty-six Sprague-Dawley rats (4-week-old males, typical body weight of 130–140 g) were used in the study.

All of the surgical interventions were basically performed under general anesthesia induced by intramuscular injection of an anesthetic mixture composed of Rompun® (xylazine, 20 mg/ml, 0.5 ml/kg body weight; Bayer, Leverkusen, Germany) and Zoletil® (tiletamine and zolazepam, 100 mg/ml, 0.5 ml/kg body weight; Virbac Lab., Carros, France).[47] All rats were given free access to food pellets and tap water during the experimental period.

The maxillary first molars of 4-week-old rats were extracted by using dental explorers and forceps under further local infiltrative anesthesia (2% lidocaine with 1:100,000 epinephrine). After having a postextraction healing period of 4 weeks, the rats were randomly divided into two groups and received subcutaneous injections of either alendronate sodium salt (1.0 mg/kg, alendronate group, n=18; Merial Inc., Parramatta, New South Wales, Australia) or normal saline (control group, n=18) twice per week until sacrifice.

Implants were placed bilaterally into the extraction sockets at 4 weeks after the start of the injection protocol. A 1.5-mm-deep osteotomy was performed with several sets

of round and fissure burs under additional local infiltrative anesthesia (2% lidocaine with 1:100,000 epinephrine) at the extraction sites after flap elevation. Profound saline irrigation was maintained throughout this procedure. The custom-made sterile, machined-surface implants (1.5 mm in diameter and 1.5 mm in length), fabricated from grade IV titanium, were embedded into the drilled cavities by tapping them with a mallet until the top surfaces of the implants became parallel with the level of the cortical surface of the adjacent bone (Figure 3).

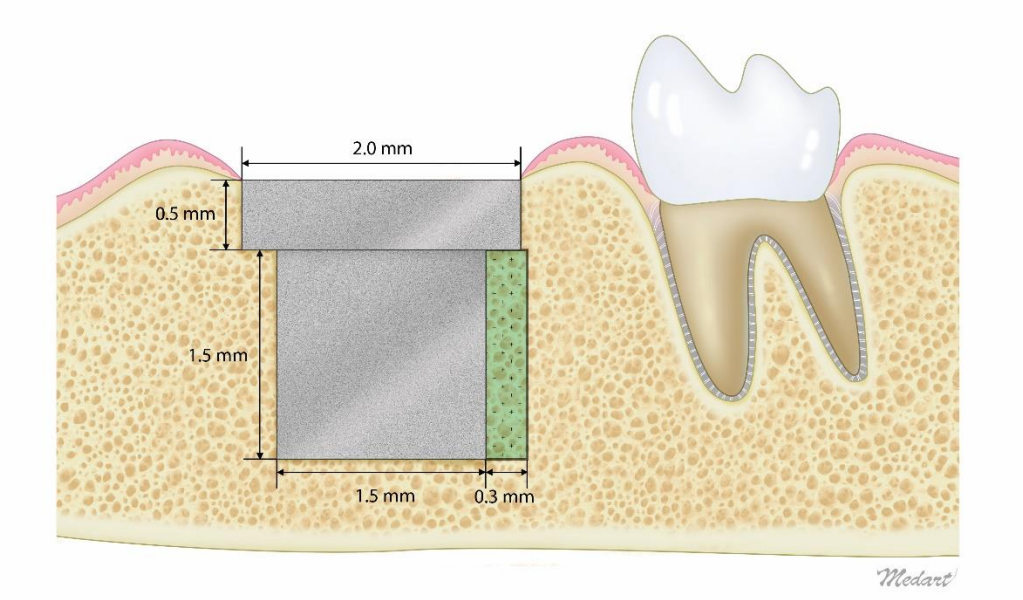

Figure 3. Custom-made implants placed into the extraction sockets. Green color indicates the region of interest (ROI).

The rats were sacrificed at either 4, 8, or 12 weeks after implantation (4-, 8-, and 12-week groups, respectively; *n*=6 rats per group) by transcardial perfusion of the fixative containing 4% paraformaldehyde.[48] The maxillae and implants were removed *en bloc* and immersed in the 4% paraformaldehyde solution for 24 hours. Additional procedures were carried out for the rats in the 12-week alendronate and control groups. Blood samples were taken from these groups until sacrifice since the commencement of the subcutaneous injection protocol.

The overall experimental design is briefly summarized in Figure 4. Microcomputed tomographic, histologic, and biochemical analyses were performed as described below.

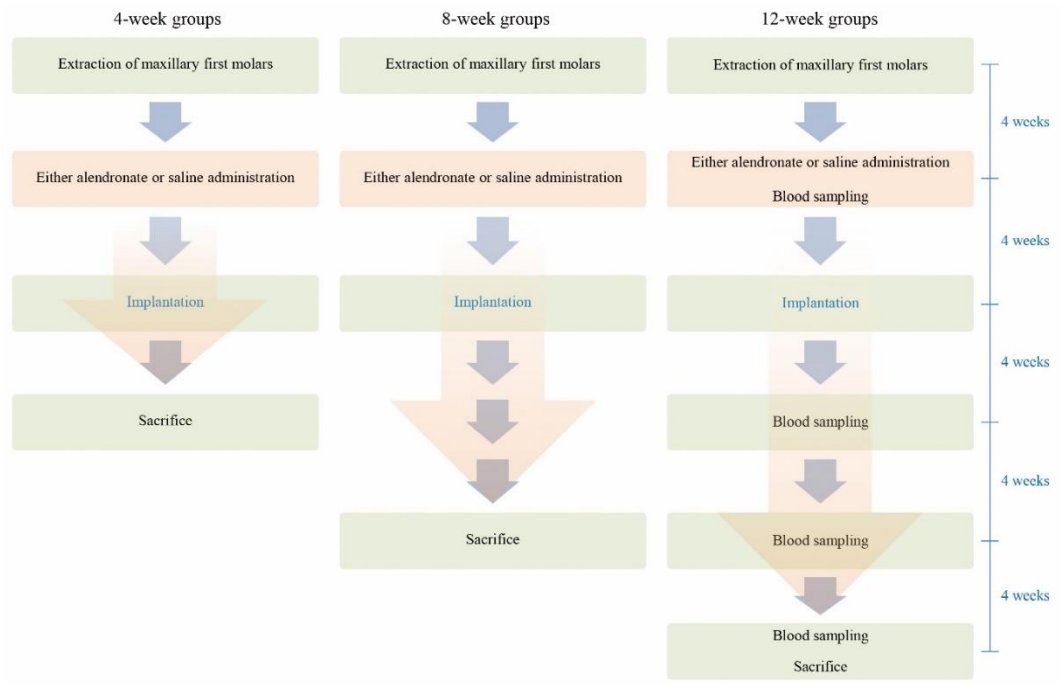

Figure 4. Schematic diagram of the overall experimental design.

2. Microcomputed tomographic analyses

Microcomputed tomographic images were made using a microcomputed tomography scanner (Skyscan 1076; Skyscan, Kontich, Belgium) at a tube voltage of 100 kV and a tube current of 100 μA with rotation steps of 0.5° over a trajectory of 360°. The images were reconstructed with the aid of the reconstruction software (NRecon software version 1.6.1.4; Skyscan).

The volume of interest (VOI) was defined as a hollow cylinder with an outer diameter of 2.1 mm and an inner diameter of 1.5 mm over the entire length of each implant (Figure 5).

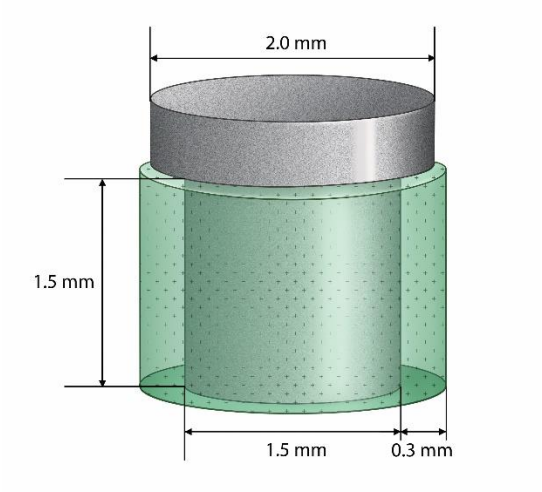

Figure 5. Volume of interest (VOI) for microcomputed tomographic analyses. Green color indicates the VOI.

Image-analysis programs (CTAn software version 1.12.0.0 and CTVol software version 2.2.1.0; Skyscan) were used for morphometric analysis and three-dimensional rendering. The ratio of bone volume to tissue volume (BV/TV, %) and the bone mineral density (BMD, mg/ml) were calculated within each VOI.

3. Histologic analyses

The specimens were decalcified with 10% ethylenediaminetetraacetic acid (EDTA) and stored at 4℃ for 2 months. A series of 3-μm-thick sections was prepared from the specimens using standard protocols. Two slides were selected for each tissue block and subjected to hematoxylin-eosin (H&E) or tartrate-resistant acid phosphatase (TRAP) stains separately. An acid phosphatase leukocyte kit (Sigma, St. Louis, MO, USA) was used for the TRAP stain. The slides were examined at two magnifications (×12.5 and ×100) with the aid of a light microscope (Leica DM LB; Leica Microsystems, Wetzlar, Germany). The images were captured and saved in tagged image file format (TIFF).

The numbers of empty lacunae and TRAP-positive cells were counted in H&E- and TRAP-stained sections, respectively, within the region of interest (ROI) by using a computer-based histologic analysis program (IMT i-Solution Lite software version 8.1; IMT i-Solution Inc., Vancouver, Canada). The ROI was defined as a rectangular area at the distal surface of the implant with a width of 0.3 mm and a height of 1.5 mm (Figure 3).

4. Biochemical analyses

Blood samples (5 ml from each 12-week rat) were obtained from the cardium of the 12-week rats using an injection syringe at four time points (Figure 4). These samples were transferred to Eppendorf tubes to centrifuge the serum at 2000 rpm for 10 minutes at 4℃.

The tubes containing the serum were stored at -200℃ in a freezer until they were used for laboratory assays. The levels of serum carboxy terminal telopeptide of type I collagen (s-CTX) and serum osteocalcin (s-OC) were measured with the SpectraMax190 (Molecular Devices, Sunnyvale, CA, USA) using a RatLaps^{TM} enzymeimmunoassay (EIA) kit (Immunodiagnostic Systems, Boldon, UK) and a Rat Osteocalcin EIA kit (Biomedical Technologies Inc., Stoughton, MA, USA), respectively.

5. Statistical analyses

A repeated-measures, single-factor analysis of variance model was applied to the data derived from the analyses. The data are presented as mean ± standard deviation values and the level of statistical significance was set at $\alpha = 0.05$. All calculations were performed with SPSS version 20.0 software (IBM SPSS Statistics, IBM Corp., Somers, NY, USA).

6. Ethics statement

All experimental procedures were performed in accordance with the guidelines for animal experiments at Yonsei University College of Dentistry, and approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Republic of Korea (approval number 09-224).

III. Results

Five rats died of unknown reasons (4 in the alendronate group and 1 in the control group). In the remaining 31 rats, a few implants were excluded for analyses because of the retained roots (n = 9), injury during the implantation procedure (n = 5), and inappropriate implant stability (n = 5). Hence, 43 well-fixed implants were available for analyses (Table 1).

Table 1. Number of implants used for analyses.

	Control	Alendronate
4-week group	10	6
8-week group	5	10
12-week group	6	6

The BV/TV tended to have higher values in the alendronate groups than in the control groups at each time point, while the values of BMD did not show such tendency. However, the values for both BV/TV and BMD at each time point did not show significant differences between the alendronate and control groups (Figure 6, Table 2). The numbers of empty lacunae and TRAP-positive cells did not differ significantly between the two groups (Figure 7, Table 3). There were no statistically significant differences over time in both groups with regard to microcomputed tomographic and histologic findings.

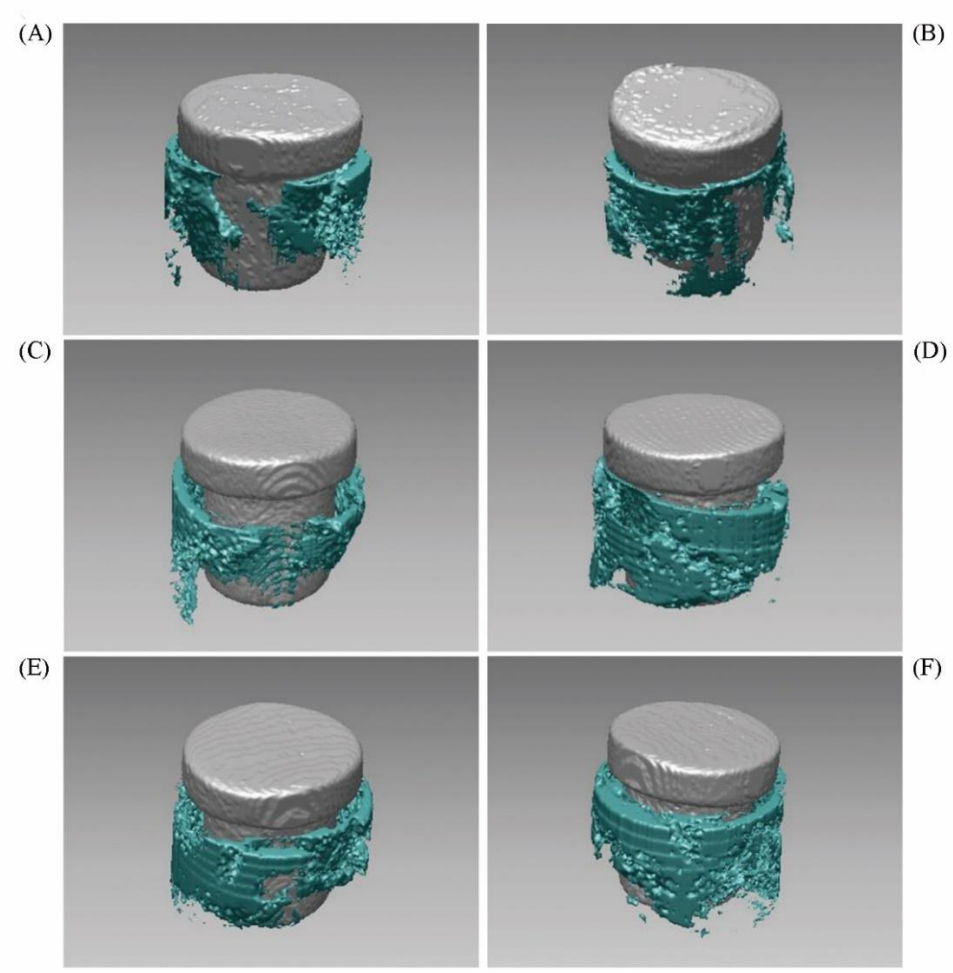

Figure 6. Three-dimensionally reconstructed images for microcomputed tomographic analyses: **(A)** 4-week control group, **(B)** 4-week alendronate group, **(C)** 8-week control group, **(D)** 8-week alendronate group, **(E)** 12-week control group, and **(F)** 12-week alendronate group.

Table 2. Microcomputed tomographic findings in the volume of interest (VOI).

	BMD (mg/ml)		BV/TV (%)	
	Control	Alendronate	Control	Alendronate
4-week group	1.53 ± 0.04	1.54 ± 0.02	13.05 ± 4.32	13.85 ± 3.23
8-week group	1.61 ± 0.06	1.57 ± 0.06	17.64 ± 3.68	18.69 ± 6.74
12-week group	1.58 ± 0.05	1.60 ± 0.08	16.04 ± 2.41	19.72 ± 4.47

BMD, bone mineral density; BV/TV, the ratio of bone volume to tissue volume.

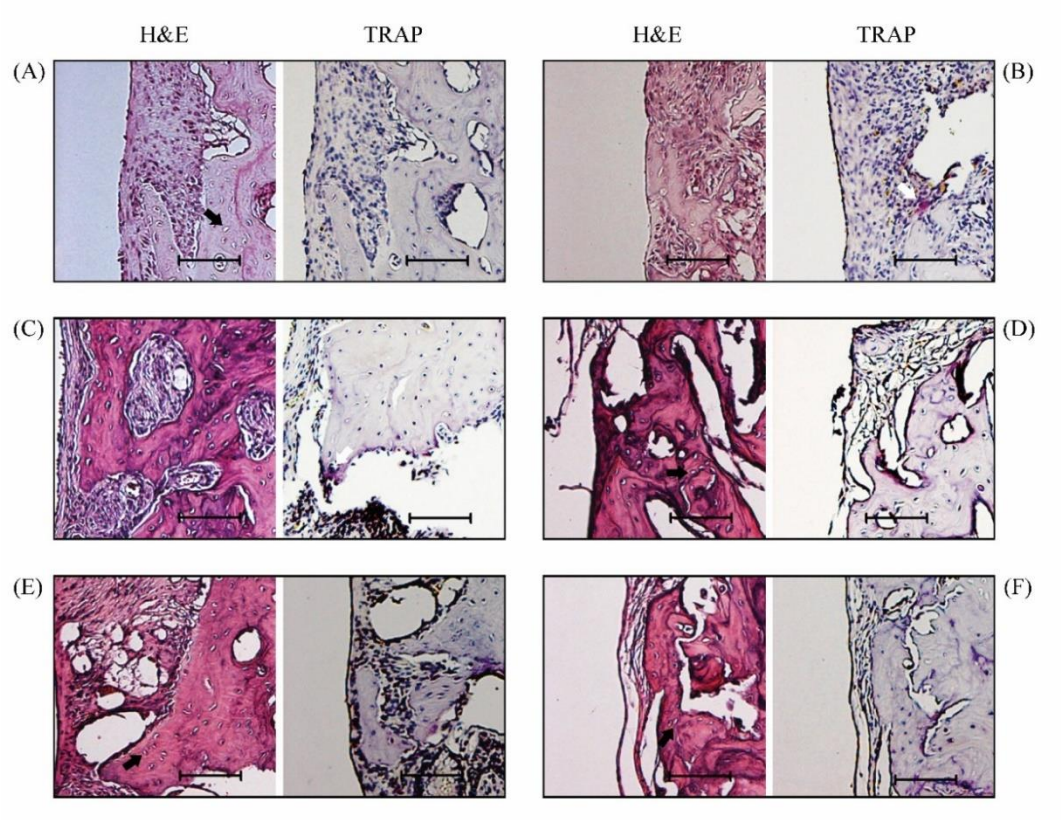

Figure 7. Histologic images in the region of interest (ROI) at higher magnification (×100): **(A)** 4-week control group, **(B)** 4-week alendronate group, **(C)** 8-week control group, **(D)** 8-week alendronate group, **(E)** 12-week control group, and **(F)** 12-week alendronate group. Scale bars = 200 μm. Black arrows indicate empty lacunae in hematoxylin-eosin (H&E)-stained sections. White arrows indicate tartrate-resistant acid phosphatase (TRAP)-positive cells in TRAP-stained sections.

Table 3. Histologic findings in the region of interest (ROI).

	Empty lacunae (*n*)		TRAP-positive cells (*n*)	
	Control	Alendronate	Control	Alendronate
4-week group	19.17 ± 8.13	21.00 ± 11.52	3.50 ± 2.17	5.90 ± 3.96
8-week group	18.70 ± 13.29	13.83 ± 8.13	3.90 ± 2.03	4.33 ± 2.50
12-week group	13.67 ± 6.71	18.50 ± 9.48	3.83 ± 2.04	3.00 ± 1.41

TRAP, tartrate-resistant acid phosphatase.

The biochemical parameters exhibited significant differences over time among some time points within each group, except for the s-OC level in the control group ($p < 0.05$). No statistically significant differences were found between the control group and alendronate group for s-CTX level (Figure 8, Table 4), while a statistically significant difference was identified at 12 weeks after implantation for s-OC level (Figure 9, Table 4): the alendronate group showed lower values than the control group ($p < 0.05$). There were no significant differences for s-OC level between the groups at the three other time points.

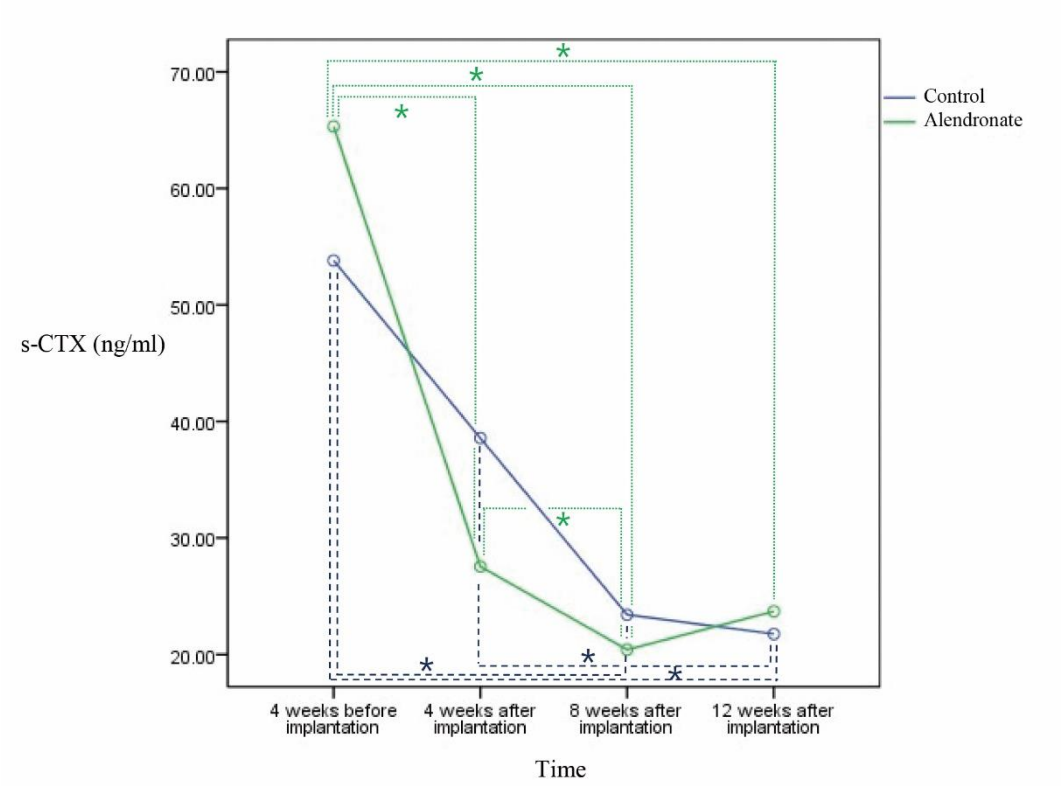

Figure 8. Serum carboxy terminal telopeptide of type I collagen (s-CTX) values according to the time points. The asterisks indicate statistically significant differences ($p < 0.05$). Statistically significant differences were not found between the alendronate and control groups.

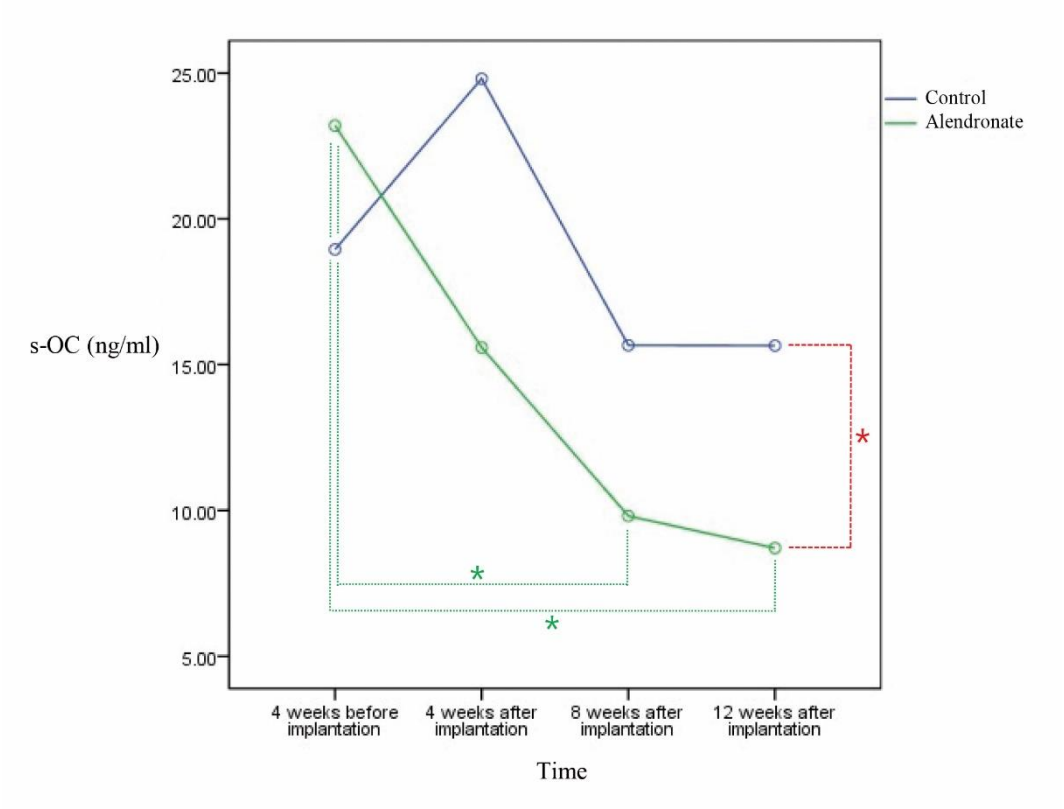

Figure 9. Serum osteocalcin (s-OC) values according to the time points. The asterisks indicate statistically significant differences ($p < 0.05$). The level of s-OC was significantly lower in the alendronate group than in the control group at 12 weeks after implantation.

Table 4. Biochemical findings. The level of s-OC was significantly lower in the alendronate group than in the control group at 12 weeks after implantation.

	s-CTX (ng/ml)		s-OC (ng/ml)	
	Control	Alendronate	Control	Alendronate
4 weeks before implantation	53.81 ± 15.42	65.32 ± 25.43	18.95 ± 4.50	23.20 ± 6.58
4 weeks after implantation	38.57 ± 8.04	27.55 ± 12.86	24.81 ± 10.32	15.59 ± 7.47
8 weeks after implantation	23.40 ± 11.31	20.41 ± 7.60	15.66 ± 8.29	9.81 ± 0.60
12 weeks after implantation	21.76 ± 5.28	23.70 ± 9.00	15.65 ± 2.32	8.71 ± 1.95

s-CTX, serum carboxy terminal telopeptide of type I collagen; s-OC, serum osteocalcin.

IV. Discussion

Animal models have played an important role in revealing the effects of drugs. Rats have historically been one of the preferred experimental animals in many biomedical fields. Rats are easy to manipulate and have a high genetic similarity with humans. Compared with mice, they are more appropriate for dental research since their jaws are larger, which facilitates access for surgical procedures.[49] The rat maxilla was chosen as the site for implant placement in this study since bone remodeling in the rat alveolar bone follows a similar sequence to that in human jaw bone.[50] Moreover, the higher remodeling activity of the alveolar bone compared with the femur is beneficial for studying the effects of drugs.[51,52]

The dose of alendronate was determined based on previous studies using weekly doses ranging from 30 μg/kg to 15 mg/kg.[53-56] The healing period for the extraction sites was set as 4 weeks based on a histologic study finding that newly formed bone in rats completely filled the extraction sockets at approximately 1 month after extraction.[57] Implant osseointegration was also achieved at 1 month postimplantation in rats.[57,58] Therefore, the shortest survival time after implantation was set as 4 weeks. The duration of alendronate or saline administration before implant placement was also set as 4 weeks based on another previous study.[46]

Following its introduction in the late 1980s, microcomputed tomographic analysis has been utilized for three-dimensional evaluations of the microarchitecture of trabecular

bone.[59-61] Although this analysis method requires relatively complex image-processing procedures, it is rapid, nondestructive, and unbiased, allowing the calculation of morphometric parameters such as volumes and surface areas in a large number of sections. On the other hand, histologic slides provide clear-cut information on cellularity with high resolution and image contrast.[62] However, histologic analysis is a destructive method, and the use of discrete slides has the limitation that the observed area may differ depending on the angle and location of the cutting plane, which could cause results to be misinterpreted.[63] The present study applied both histologic and microcomputed tomographic analyses in an effort to produce more reliable results.

While BV/TV appeared to be higher in the alendronate groups than the control groups at each time point, there were no statistically significant differences. BMD was another microcomputed tomographic analytic parameter applied in this study that did not show significant intergroup differences. These findings suggest that alendronate administration did not induce marked changes in bone quality or quantity around the implants. However, these data need to be interpreted with caution since microcomputed tomography may only actually measure mineralized tissues that exceed a certain mineralization threshold.[64]

Empty osteocytic lacunae may appear as a result of traumatic procedures, including dental extraction or implantation.[65] The number of empty lacunae reduces as the healing process progresses under normal physiologic circumstances, since dead osteocytes are replaced by new ones via bone remodeling.[48,66] Previous studies found that this

replacement process appeared to be inhibited when bisphosphonates were administered, since the numbers of empty lacunae did not decrease.[67,68] Furthermore, based on the osteocytes forming a dense network through canaliculi and playing a crucial role in supplying nutrients to bone tissues, it was proposed that the presence of these nonviable empty lacunae can be utilized when evaluating bone metabolism, and may represent evidence for MRONJ.[39,69,70]

TRAP is reportedly an osteoclastic marker, since it is expressed by osteoclasts, but there have been diverse findings regarding the association between the use of bisphosphonate and TRAP-positive cell counts.[53,56,67] The present study found that the numbers of empty lacunae and TRAP-positive cells did not differ significantly between the alendronate and control groups. This suggests that alendronate did not directly disturb osteoclast function. However, it should be noted that apoptotic structures were not found inside osteoclasts in the present study.

A previous study found that the injured bone occurred usually within 100 μm of where an implant was placed, while another found that dead bone can exist 500 μm beyond the margin of the bone cavity.[48,71] The horizontal width of the measurement area or volume was set at 300 μm for both the microcomputed tomographic and histologic analyses in the present study.

The s-CTX and s-OC, among several biochemical indicators that are correlated with bone metabolism, were used in the present study.[72] Since the levels of both s-CTX and

s-OC show large diurnal variations, blood samples for the tests were collected at the same time of day in an effort to reduce these variations.[73,74]

s-CTX specifically measures a particular crosslink peptide of type I collagen in bone, and is known to be a useful surrogate bone resorption marker. Some authors have correlated the level of s-CTX with osteoclastic activity and the future risk of MRONJ development, since the telopeptide fragment of type I collagen is cleaved from its main chain by the action of osteoclasts.[42,75] In contrast, other authors reported that they were unable to estimate predictive values of s-CTX for managing the cares of patients undergoing bisphosphonate therapy.[76,77] The values did not differ at each time point in the present study, which is consistent with the findings of recent systematic reviews.[78,79]

While the precise function of s-OC is yet not known, it is the most abundant noncollagenous protein in bone.[80] It has been postulated that s-OC is released into the circulation after being secreted from osteoblasts during osteoid mineralization.[81] Studies of calcium kinetics and bone histomorphometry indicate that the level of s-OC can serve as a marker of bone formation.[82,83]

In contrast with the levels of s-CTX, which did not differ significantly between the alendronate and control groups, the level of s-OC was significantly lower in the alendronate group than in the control group at 12 weeks after implantation. This suggests that the bone metabolism was disturbed at the end point of the experimental period. These results are also partially consistent with a previous clinical report of s-OC values being significantly lower in an MRONJ-diagnosed group.[84]

The present animal study combined multiple analysis methods (1) due to the difficulty of establishing a MRONJ model, (2) due to the low incidence of MRONJ, especially when using lower bisphosphonate doses, and (3) in order to evaluate the effects of alendronate from a multidisciplinary point of view. In addition, this study simulated a situation where discontinuation of alendronate administration was not possible before implant placement. Within the limitations of this study, alendronate might have affected bone metabolism around implants in the late phase of the experimental period if it was administered periodically not only before but also after implant placement.

This study was subject to several limitations: (1) the sample was small and the analyzed samples were not uniformly distributed, (2) the implants were not involved in masticatory function in the oral cavity, and (3) rats in this stage of development may exhibit quite variable biochemical bone markers.[85] Further researches to overcome these limitations are warranted. Placing implants designed to occlude with the opposing teeth in adult rats for longer follow-up periods may supplement the present results. Comparing the effects of alendronate on bone metabolism by altering the alendronate administration period before implantation may also yield useful information. Combining the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) method with TRAP staining could aid in identifying the apoptotic structures inside osteoclasts.[86] Researching differences in reversal lines in histologic slides could facilitate the understanding of the activity of bone remodeling. Investigating genetic biomarkers related to alendronate administration could also be valuable for elucidating its effects. With regard

to practical applications, the most appropriate clinical s-OC level also needs to be determined.

V. Conclusion

This experimental study administered alendronate to rats followed by implant placement to determine its effects on bone metabolism around implants. Within the limitations of the present study, alendronate administration did not result in significant differences at the bone–implant interface during the early phase after implantation. However, alendronate might have affected bone metabolism around the implants during the late phase of the experimental period, and so long-term validation of the results is still required.

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Abstract (in Korean)

알렌드로네이트의 투여 기간이 실험용 쥐의 임플란트 주위 골 대사에 미치는 영향

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알렌드로네이트는 비스포스포네이트 계열에 속하는 약제로서 골다공증 등의 골질환에 대한 대표적인 치료제로 널리 사용되고 있다. 하지만, 근래 들어 그에 따른 부작용으로 치의학 영역에서 임플란트 골유착의 실패와 약물 연관 악골괴사(medication-related osteonecrosis of the jaw, MRONJ) 등이 보고되고 있다.

비스포스포네이트가 임플란트 주위의 골조직에 미치는 영향에 대해 분석한 과거의 여러 동물 실험들에서는 알렌드로네이트 이외의 약제를 대부분

이용하였으며 실험용 쥐의 악골에서 수행된 동물 실험은 적었다. 이에 본 연구에서는 실험용 쥐에서 알렌드로네이트의 투여 기간을 다양하게 설정하고 상악에 임플란트를 식립함으로써 알렌드로네이트가 임플란트 주위 골 대사에 미치는 영향에 대하여 다각적으로 살펴보고자 하였다.

36 마리의 실험용 쥐의 상악 양측 제 1 대구치를 발치하였다. 약 4 주 간의 치유기간을 가진 후 이들을 임의로 두 군으로 나누어 일정한 주기로 알렌드로네이트(알렌드로네이트군, n=18) 또는 식염수(대조군, n=18)를 피하주사하였다. 약제 주입 후 4 주가 경과한 다음, 맞춤 제작된 임플란트를 양측의 발치 부위에 식립하였다. 임플란트 식립 후 4주, 8주, 또는 12주 뒤에 쥐를 희생시켰으며, 희생시킨 시점에 따라 각각 4주 군, 8주 군, 그리고 12주 군으로 분류(각 군에 대해 n=6)하였다. 조직학적 분석과 미세 단층촬영을 통한 방사선학적 분석을 실시하였으며, 12 주 군의 쥐에 대해서는 추가적으로 생화학적 분석을 시행하였다.

미세 단층촬영을 통한 방사선학적 분석과 조직학적 분석을 시행한 결과 두 군 간에는 통계학적인 유의차가 없었다. 대조군의 serum osteocalcin 값을 제외하면 생화학적 지표들은 각 군 내에서 몇몇 시점 간에 통계학적 유의차를 보였으며, 시간이 경과함에 따라 감소하는 경향성을 나타냈다. 12 주 알렌드로네이트 군의 serum osteocalcin 값은 대조군의 serum osteocalcin 값보다 통계학적으로 유의미하게 낮았다($p < 0.05$).

이러한 결과들을 종합해 볼 때 알렌드로네이트는 임플란트 식립 이후 초기 단계에서는 골-임플란트 계면에서 영향을 미치지 않았지만, 후기 단계에서는 임플란트 주위에서의 골 대사에 유의미한 영향을 미쳤을 가능성이 있을 것으로 생각된다. 향후 보다 장기간에 걸친 부가적인 연구가 필요하고 결과에 대한 임상적 유용성이 검증되어야 할 것이다.

핵심되는 말: 알렌드로네이트, 비스포스포네이트, 골 대사, 임플란트 실패, 약물 연관 악골괴사, serum osteocalcin