

The Effect of Systemically Administered Bisphosphonates on Bony Healing After Tooth Extraction and Osseointegration of Dental Implants in the Rabbit Maxilla

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Purpose: To evaluate the effects of bisphosphonates on bone healing after tooth extraction and osseointegration of dental implants in a rabbit model. **Materials and Methods:** Twenty-four rabbits were divided into four groups; one control and three experimental. The experimental were treated with intravenous zoledronic acid (ZA, 0.1 mg/kg) twice per week starting 4 (Z4 group) and 8 (Z8 group) weeks before surgery until the end of the experiments. The experimental ZD4 group was treated with intravenous ZA (0.01 mg/kg) and intramuscular dexamethasone (1 mg/kg) twice per week starting 4 weeks before surgery until the end of the experiments. The maxillary first premolar was extracted, and an implant with a diameter of 1.5 mm was placed between the incisor and the premolar of each maxilla. Healing of the extraction socket was evaluated and histomorphometric analysis around the implant was performed, using the bone-to-implant contact ratio (BIC) and bone area ratio (BA) 4 and 8 weeks after the surgery. **Results:** The control group underwent a normal healing process, but all experimental groups showed necrotic bone with hollow lacunae. BIC and BA in the control group increased from the 4- to 8-week evaluations, but decreased in the experimental groups from 4 to 8 weeks. BIC and BA of the Z8 and ZD4 groups were higher than those of the control group at the 4-week evaluation, but were lower than the control at the the 8-week evaluation. **Conclusions:** This study showed that administration of bisphosphonates interferes with normal bone remodeling after tooth extraction. The experimental groups showed good initial stability, but long-term healing around the implants was impaired. Within the limits of this study, it may be suggested that patients taking bisphosphonates should be treated with caution when performing tooth extraction or placing dental implants. INT J ORAL MAXILLOFAC IMPLANTS 2013; 28:1194–1200. doi: 10.11607/jomi.2685

Key words: bisphosphonates, bisphosphonate-related osteonecrosis of the jaws (BRONJ), dental implant, initial stability, tooth extraction, wound healing

Bisphosphonate-related osteonecrosis of the jaws (BRONJ) has generated great interest in the study of both dental and bone biology in recent years.¹ Bisphosphonate (BP) is a strong suppressor of osteoclasts, and it is commonly used to treat diseases that affect bone metabolism including multiple myeloma, secondary

hypercalcemia caused by malignant tumors, bone metastasis in metastatic prostate cancer or metastatic breast cancer, and Paget's disease.² Highly efficient intravenous BPs have extended their use from treatment of malignant bone diseases to prevention of osteoporosis.^{3–5} The Food and Drug Administration (FDA)

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recently approved the annual injection of zoledronic acid (Reclast, Novartis) for the prevention of osteoporosis in menopausal populations.⁶ Earlier reports in the dental field suggested that the use of BPs may be beneficial in dental implantation and bone transplantation due to their strong inhibitory effects on bone resorption.^{7,8} However, later reports showed that BPs caused side effects such as acute reactions, gastrointestinal disturbances, and renal disorders.^{9–11} Moreover, osteonecrosis of the jaws is one of the most severe adverse effects,¹² and BRONJ was first reported in 2003.¹¹ Since then, a number of case reports and studies on BRONJ have been published,^{13–15} and the American Association of Oral and Maxillofacial Surgeons defined BRONJ as exposed, necrotic bone in the maxillofacial region that has persisted for more than 8 weeks with no history of radiation therapy to the jaws and with current or previous BP treatment.¹⁶ The number of dental patients undergoing long-term administration of BPs is increasing, and the incidence of osteonecrosis of the jaws is increasing accordingly.^{17–19} However, the pathogenesis of BRONJ has not been clearly established, although various hypotheses have been suggested.^{1,20,21} The most supported is that BPs suppress the remodeling of jaw bones, which may lead to necrosis.¹ A direct cytotoxic effect of BPs on osteocytes, anti-angiogenic effects, and infection have also been proposed as causes of BRONJ. Diabetes, smoking, dental extraction, and concurrent medications have been thought to be indirectly associated with BRONJ by affecting remodeling, angiogenesis and infection.^{1,22,23}

A highly efficient BP was recently approved, and the use of this drug is gradually increasing as society is aging.^{24,25} Therefore, it is necessary to completely understand and identify the pathogenesis of osteonecrosis of the jaws with long-term use of BPs after tooth extraction and dental implant installation. This study was performed to evaluate the impact of BPs on bone healing after tooth extraction and osseointegration of dental implants by administration of zoledronic acid (ZA) along with dexamethasone (DX). DX was used simultaneously in some of the experimental groups to induce the best resemblance of BRONJ.^{26,27}

MATERIALS AND METHODS

Twenty-four female New Zealand white rabbits weighing 2.5 to 3.0 kg were used for the experiments. They were bred under the same conditions in accordance with the regulations of the Clinical Research Institute of Uijeongbu St Mary's Hospital, Catholic University of Korea (Gyeonggi-do, Korea). All animal experiments were performed in strict accordance with a protocol approved by the Ethics Committee for Animal

Fig 1 The implant used in this study (C-implant). It comprised two parts: a 6.5-mm SLA surface screw part and a 2-mm smooth-surfaced transgingival part. The diameter of the implant was 1.8 mm.



Experiments of Uijeongbu St Mary's Hospital Catholic University of Korea (no. UJA2010-04A).

The BP used in this study was ZA (Zometa, Novartis) and DX was purchased from Sigma-Aldrich. The implants used in this study had a diameter of 1.8 mm and length of 8.5 mm (C-Implant, Cimplant) (Fig 1). The implant had a sand-blasted, acid-etched (SLA) surface, and 2 mm of the transgingival part had a smooth surface.

Surgical Procedures

Twenty-four rabbits were divided into four groups: one control and three experimental groups. The experimental Z4 group was treated with intravenous administration of ZA (0.1 mg/kg) using the rostral auricular vein twice a week starting 4 weeks before surgery until the end of the experiments. The experimental Z8 group was treated with intravenous administration of ZA (0.1 mg/kg) from 8 weeks before surgery to the end of the experiments. The ZD4 group was treated with intravenous administration of ZA (0.1 mg/kg) and intramuscular administration of DX (1 mg/kg) from 4 weeks before surgery to the end of the study.

General anesthesia was induced by administering a mixture of tiletamine/zolazepam (15 mg/kg) (Zoletil 50, Virbac) and Xylazine (5 mg/kg) (Xylazine 20 Inj, Kepro) into the femoral muscle. Lidocaine (2%) with 1:100,000 epinephrine (Yuhan) was used for local anesthesia. A crestal incision was made between the incisor and the premolar of the rabbit maxilla. A full-thickness flap was elevated to expose the alveolar bone. A hole was made in the implantation site using a guide drill with a diameter of 1.5 mm. The implant was inserted until the SLA surface was completely submerged in the alveolar bone. The flap was repositioned and sutured with 4-0 nylon around the smooth surface of

the implant. Extraction of the maxillary first premolar was performed immediately after implant placement (Fig 2). Gentamycin (4 mg/kg) (Kukje Pharm) and ketoprofen (1 mg/kg) (Bukwang Pharm) were administered intramuscularly twice per day for 2 days postoperatively. The animals were sacrificed at 4 or 8 weeks following surgery by intravenous administration of potassium chloride (Dai Han Pharm).

Preparation of Tissue Samples and Histologic Evaluation

The specimens retrieved from the extraction sockets were fixed in 10% neutral formalin solution for 7 days, decalcified with ethylenediaminetetraacetic acid for more than 3 months, and embedded in paraffin using a universal method. Hematoxylin-eosin staining was performed and examination was conducted using a microscope (IX71 Inverted Microscope, Olympus).

Histomorphometric Evaluation of Tissue Samples

Specimens retrieved from the implantation sites were fixed in 10% neutral buffered formalin fixative, and the non-decalcified samples were prepared according to a method reported previously.²⁸ The dehydration process was carried out using an ethanol series, and the tissues were placed in embedding media (Technovit 7200 VLC; Kulzer). The embedded tissue block was dissected with a band saw (EXAKT) and polished by applying #800, #1200, #2500, and #4000 sandpaper. The thickness of the final samples was 40 to 50 μm . Evaluations were performed using a light microscope (BX51, Olympus) after hematoxylin-eosin staining. Briefly, the samples were washed with deionized water for 10 minutes. The specimen was stained with hematoxylin for 15 to 20 minutes and then washed with deionized water for 10 minutes. The samples were then stained with eosin for 3 minutes. The dehydration process was carried out using an ethanol series (three times in 95% ethanol and an additional three times in 100% ethanol). Samples were dried and mounted.

Digital images were obtained with a digital camera (SPOT RT3, Diagnostic Instruments). Histomorphometric analysis of the bone-to-implant contact (BIC) ratio and the bone area (BA) ratio between the first and the second threads was performed with an image analysis system (Kappa Image Base Noah 2.5; Kappa Optronics) (Figs 3 and 4). The BIC and BA were calculated as follows:

BIC (%) = length of implant surface attached to the bone / length of implant surface \times 100

BA (%) = bone area between first and second threads of the implant / total area between first and second threads of the implant \times 100

Statistical Methods

Data are presented as means \pm standard deviations. The Mann-Whitney *U* test was used to test for differences with commercially available statistical software (SPSS version 12 for Windows, IBM). Statistically significant differences were evaluated with significance set at a value of $P < .05$.

RESULTS

A total of 24 implantation sites and 24 extraction sockets from 24 female New Zealand white rabbits were used in this experiment. No exposed necrotic bone was observed in either the control group or the experimental groups.

The control group underwent a normal healing process (Figs 5 and 6). However, all experimental groups had bone with hollow lacunae (Figs 5d and 7).

BIC Results

The mean BIC value of the control group 4 weeks after implant installation was $36.75\% \pm 17.62\%$ with a median of 38.28%. The BIC values of the Z4, Z8, and ZD4 groups were $17.69\% \pm 4.34\%$ (median, 16.31%), $78.75\% \pm 10.20\%$ (median, 82.58%), and $59.315 \pm 12.41\%$ (median, 60.80%), respectively (Fig 8). The BIC values of the Z8 and ZD4 groups were higher than those of the control group, but only the Z8 group showed a significant difference ($P < .05$).

The mean BIC of the control, Z4, Z8, and ZD4 groups at the 8-week evaluation were $61.44\% \pm 12.52\%$ (median, 59.44%), $11.64\% \pm 1.10\%$ (median, 12.11%), $10.20\% \pm 12.16\%$ (median, 41.50%), and $27.33\% \pm 12.53\%$ (median, 26.97%), respectively (Fig 8). All three experimental groups showed a significantly lower BIC at 8 weeks compared with that of the control group.

The BIC of the control group increased from the 4- to 8-week evaluations ($P > .05$). However, the BIC of the Z4, Z8, and ZD4 groups decreased from 4 to 8 weeks, and significant decreases were identified in the Z8 and ZD4 groups ($P < .05$).

BA Results

The mean BA value of the control group 4 weeks after implant installation was $30\% \pm 10.58\%$ with a median of 25.54%. The BIC values of the Z4, Z8, and ZD4 groups were $50.17\% \pm 5.54\%$ (median, 48.40%), $42.895 \pm 8.44\%$ (median, 40.04%), and $48.755 \pm 8.45\%$ (median, 50.60%), respectively (Fig 9). The mean BA values of the experimental groups (Z4, Z8, and ZD4) at 4 weeks were higher than those of the control group, but statistical significance was reached in only the Z4 group ($P < .05$).

Figs 2a and 2b (a) Maxilla of a New Zealand white rabbit and (b) surgical area. The black arrow indicates the extraction socket, and the yellow arrow indicates the implant.

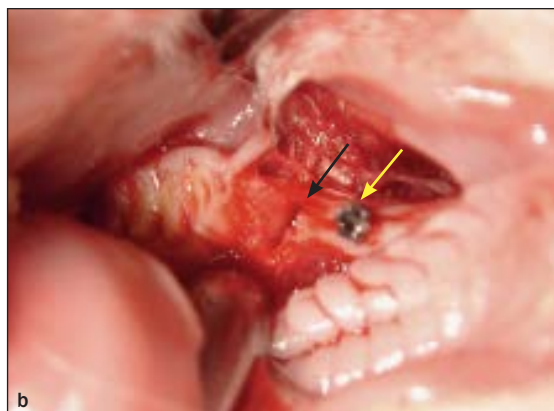
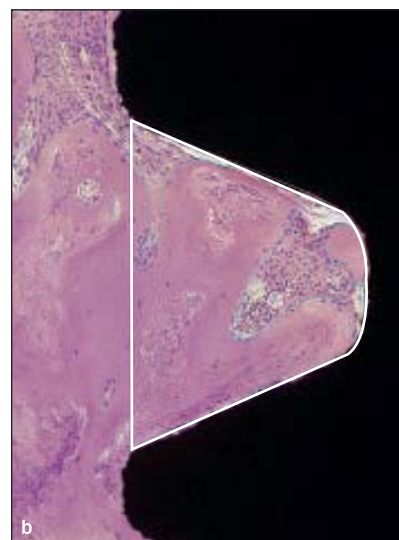
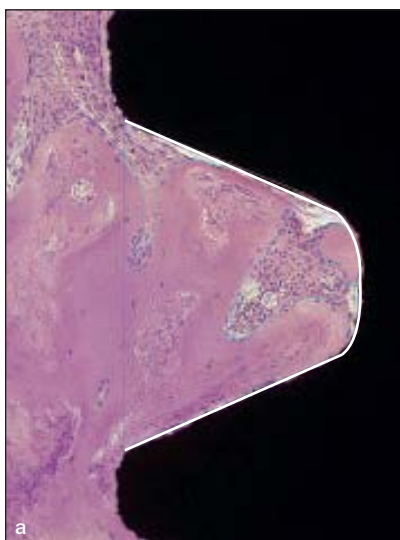
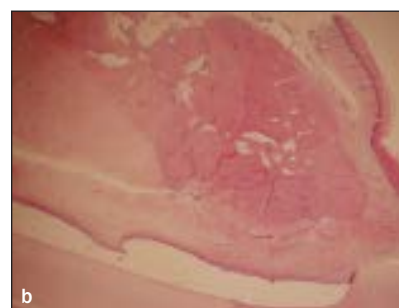
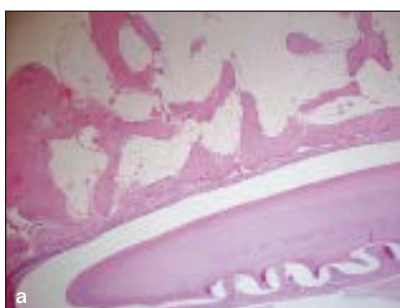


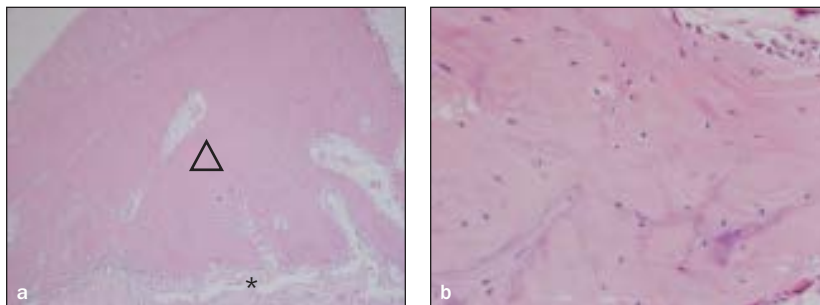
Fig 3 Histologic view of dental implant. BIC and BA were evaluated between the first and second threads of the buccal and palatal sides (hematoxylin-eosin stain, original magnification $\times 12.5$).



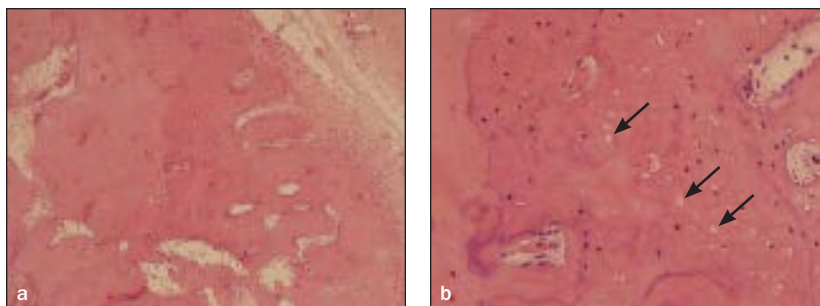
Figs 4a and 4b Histomorphometric analysis of (a) BIC ratios and (b) bone area ratios (BA) (hematoxylin-eosin stain, original magnification $\times 100$).



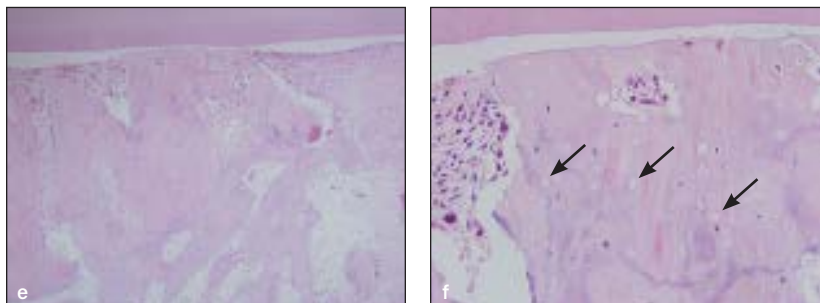
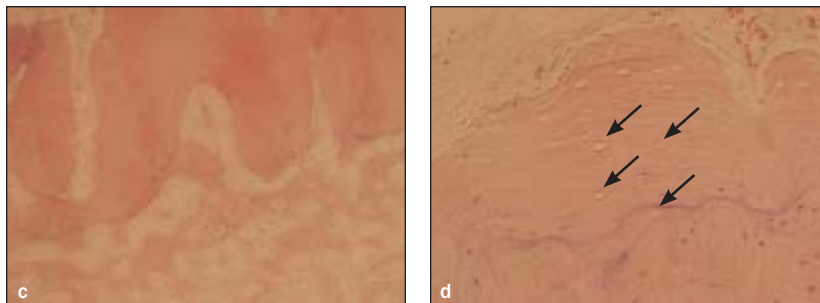
Figs 5a to 5d Histologic images of extraction socket at 4-week evaluation in (a) control group, (b) Z4 group, (c) Z8 group, and (d) ZD4 group (hematoxylin-eosin stain, original magnification $\times 40$).



Figs 6a and 6b Histologic view of the tooth extraction socket in the control group at (a) 4-week evaluation (hematoxylin-eosin stain, original magnification $\times 100$) and (b) view at higher magnification (hematoxylin-eosin stain, original magnification $\times 400$). Asterisk indicates extraction socket, and triangle indicates alveolar bone. The extraction socket showed a normal healing process.



Figs 7a to 7f Histologic images of the extraction socket in the experimental groups. (a) Z4 group at 4-week evaluation (hematoxylin-eosin stain, original magnification $\times 100$). (b) Higher magnification view of Z4 group at 4 weeks (hematoxylin-eosin stain, original magnification $\times 400$). (c) Z8 group at 4-week analysis (hematoxylin-eosin stain, original magnification $\times 100$). (d) Higher magnification view of Z8 group at 4 weeks (hematoxylin-eosin stain, original magnification $\times 400$). (e) ZD4 group at 8 weeks after extraction (hematoxylin-eosin stain, original magnification $\times 100$). (f) Higher magnification of ZD4 group at 8-week evaluation (hematoxylin-eosin stain, original magnification $\times 400$). All experimental groups had hollow lacunae in the healing site of the extraction socket. Arrows indicate hollow lacunae.



DISCUSSION

The BA increased in the control group and decreased in the experimental groups from the 4- to 8-week evaluations ($P > .05$). The mean BA of the control, Z4, Z8, and ZD4 groups at the 8-week evaluation were $49.35\% \pm 23.20\%$ (median, 38.06%), $48.80\% \pm 4.66\%$ (median, 46.45%), $32.20\% \pm 4.34\%$ (median, 32.28%), and $35.40\% \pm 23.86\%$ (median, 23.41%), respectively (Fig 9). The mean BA values of the experimental groups at 8 weeks were lower than those of the control group, but statistical significance was not achieved ($P > .05$).

This study showed the effects of BPs on bone healing after tooth extraction and osseointegration of dental implants by the administration of ZA along with DX. In addition, this study established a rabbit model for BRONJ to evaluate the bony healing and osseointegration of dental implants using the maxilla.

Numerous animal experiments have been conducted to identify the pathogenesis of this disease, and various hypotheses have been suggested. Oral administration of alendronate (0.20 or 1.0 mg/kg per

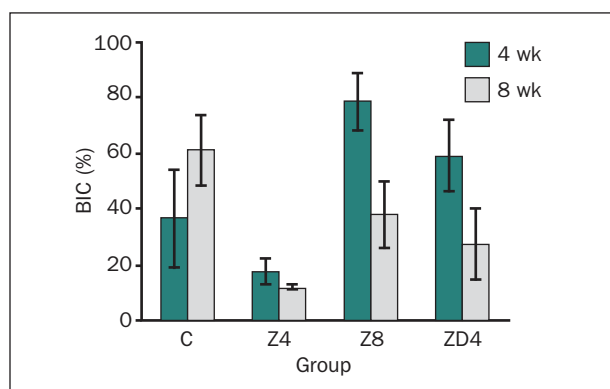


Fig 8 BIC ratios of all groups at 4 and 8 weeks after implant installation. Mean BIC of the control group increased from 4 to 8 weeks, but decreased in all experimental groups during the same time period. When the BIC values of the experimental groups were compared with those of the control group in the same periods, the Z8 group at 4 weeks and all experimental groups at 8 weeks showed significant differences ($P < .05$).

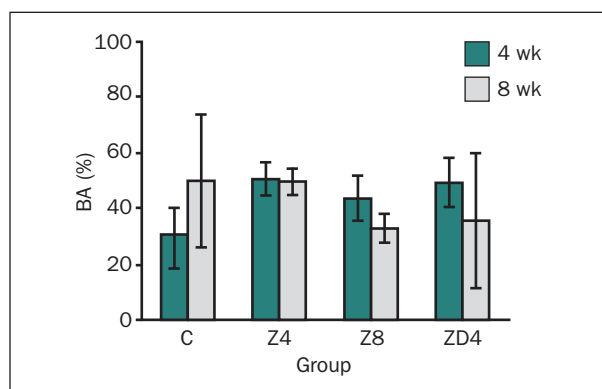


Fig 9 BA ratios of all groups at 4 and 8 weeks after implant installation. Mean BA in the control group increased from 4 to 8 weeks, but decreased in all experimental groups during the same time period.

day) resulted in matrix necrosis of the mandible in dog models.²⁹ Administration of BPs to the subperiosteum of the alveolar bone prior to tooth extraction delayed initial healing of the extraction socket in rat models.³⁰ Moreover, administration of zoledronate and pamidronate caused inflammatory and necrotic changes in both bone and soft tissue in rats.³¹ Likewise, the present study showed that administration of BPs produced hollow lacunae in necrotic bone, suggesting that BPs interfere with the normal bone healing process of the jaw bone after trauma such as tooth extraction.

Failure of osseointegration of an implant due to BRONJ was first reported in 1995.³² Implant failure due to BRONJ has been consistently reported since then. Bony defects around implants in the mandible were reported in a patient who was taking alendronate for 10 years prior to surgery.³³ Delayed implant failure was reported in a patient who was taking alendronate for 6 years prior to implant installation.³⁴ The present study showed that BIC and BA in the control group increased from the 4- to 8-week evaluations. However, BIC and BA of the Z8 and ZD4 groups decreased from 4 to 8 weeks. Interestingly, the BIC and BA of the Z8 and ZD4 groups at 4 weeks were higher than those of the control group. These results suggest that the use of BPs may have good initial stability with poor long-term results, which may be due to impaired remodeling procedures.

The frequency of BRONJ may vary depending on the administration route and the strength of drugs used.^{16,35–37} The American Association of Oral and Maxillofacial Surgeons reported that the prevalence of BRONJ was about 0.8% to 12% when BPs are administered intravenously.¹⁶ The prevalence is reportedly lower when BPs are administered orally.^{38,39} However,

it was suggested that special attention should be paid when patients undergo BPs treatment for more than 3 years because the prevalence of BRONJ increases as the period of BPs treatment increases.^{16,38}

Establishing animal models reportedly plays a key role in experiments related to this disease.⁴⁰ One researcher generated a BRONJ model in mice by injecting ZA intravenously every week for 5 weeks,⁴¹ and other investigators used ZA along with DX to establish BRONJ models in mice.²⁷ Rat models have been used to establish BRONJ models for studying changes in bone and soft tissue.³¹ In the present study, the authors used the rabbit maxilla to establish the disease model because the oral cavity of a mouse or rat may have limitations when performing tooth extraction or installation of dental implants. Although 8-week administration of ZA or 4-week treatment with ZA and DX did not produce exposed necrotic bone, the results clearly show that healing of the extraction socket and dental implant area was impaired. The results suggest that this model may be used to study BRONJ. Moreover, treatment with ZA for 8 weeks produced results similar to those obtained by administration of ZA and DX for 4 weeks, indicating that the disease model was produced within a shorter time by co-administration of DX.

CONCLUSIONS

This study showed that administration of BPs interferes with normal bone remodeling after tooth extraction. The use of BPs showed good initial stability at first but long-term healing around the implants was impaired. Within the limits of this study, it may be suggested that patients taking BPs should be treated with caution when performing tooth extractions or installing dental implants.

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REFERENCES

- Allen MR, Burr DB. The pathogenesis of bisphosphonate-related osteonecrosis of the jaw: So many hypotheses, so few data. *J Oral Maxillofac Surg* 2009;67:61–70.
- Madrid C, Sanz M. What impact do systemically administered bisphosphonates have on oral implant therapy? A systematic review. *Clin Oral Implants Res* 2009;20(suppl 4):87–95.
- Russell RG. Bisphosphonates: The first 40 years. *Bone* 2011;49:2–19.
- Russell RG. Bisphosphonates: Mode of action and pharmacology. *Pediatrics* 2007;119(suppl 2):s150–s162.
- Russell RG, Xia Z, Dunford JE, et al. Bisphosphonates: An update on mechanisms of action and how these relate to clinical efficacy. *Ann N Y Acad Sci* 2007;1117:209–257.
- Khosla S. Increasing options for the treatment of osteoporosis. *N Engl J Med* 2009;361:818–820.
- Brunsvold MA, Chaves ES, Kornman KS, Aufdemorte TB, Wood R. Effects of a bisphosphonate on experimental periodontitis in monkeys. *J Periodontol* 1992;63:825–830.
- O'Uchi N, Nishikawa H, Yoshino T, et al. Inhibitory effects of YM175, a bisphosphonate, on the progression of experimental periodontitis in beagle dogs. *J Periodontol Res* 1998;33:196–204.
- Diel IJ, Bergner R, Grotz KA. Adverse effects of bisphosphonates: Current issues. *J Support Oncol* 2007;5:475–482.
- Walter C, Grotz KA, Kunkel M, Al-Nawas B. Prevalence of bisphosphonate associated osteonecrosis of the jaw within the field of osteonecrosis. *Support Care Cancer* 2007;15:197–202.
- Marx RE. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: A growing epidemic. *J Oral Maxillofac Surg* 2003;61:1115–1117.
- Agarwal P, Rao NN. Bisphosphonate-associated osteonecrosis of the jaws. *Indian J Dent Res* 2012;23:107–111.
- Hasegawa Y, Kawabe M, Kimura H, Kurita K, Fukuta J, Urade M. Influence of dentures in the initial occurrence site on the prognosis of bisphosphonate-related osteonecrosis of the jaws: A retrospective study. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2012;114:318–324.
- Petcu EB, Ivanovski S, Wright RG, Slevin M, Miroiu RI, Brinzaniuc K. Bisphosphonate-related osteonecrosis of jaw (BRONJ): An anti-angiogenic side-effect? *Diagn Pathol* 2012;7:78.
- Li YF, Hu J. Parathyroid hormone may be a promising therapy for bisphosphonate-related osteonecrosis of jaw bones. *Int J Oral Maxillofac Surg* 2013;42:149–150.
- Ruggiero SL, Dodson TB, Assael LA, Landesberg R, Marx RE, Mehrotra B. American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis of the jaws—2009 update. *J Oral Maxillofac Surg* 2009;67:2–12.
- Yamazaki T, Yamori M, Ishizaki T, et al. Increased incidence of osteonecrosis of the jaw after tooth extraction in patients treated with bisphosphonates: A cohort study. *Int J Oral Maxillofac Surg* 2012;41:1397–1403.
- Akintoye SO, Hersh EV. Risks for jaw osteonecrosis drastically increase after 2 years of bisphosphonate therapy. *J Evid Based Dent Pract* 2012;12:116–118.
- Conte-Neto N, de Souza Bastos A, Spolidorio LC, Chierici Marcantonio RA, Marcantonio E, Jr. Long-term treatment with alendronate increases the surgical difficulty during simple exodontias—An in vivo observation in Holtzman rats. *Head Face Med* 2012;8:20.
- Otto S, Hafner S, Mast G, et al. Bisphosphonate-related osteonecrosis of the jaw: Is pH the missing part in the pathogenesis puzzle? *J Oral Maxillofac Surg* 2010;68:1158–1161.
- Kyrgidis A. Novel hypotheses in the etiopathogenesis of bisphosphonate-related osteonecrosis of the jaws. *J Oral Maxillofac Surg* 2009;67:2554.
- Curi MM, Cossolin GS, Koga DH, et al. Bisphosphonate-related osteonecrosis of the jaws—An initial case series report of treatment combining partial bone resection and autologous platelet-rich plasma. *J Oral Maxillofac Surg* 2011;69:2465–2472.
- Kushner GM, Alpert B. Bisphosphonate-related osteonecrosis of the jaws. *Curr Opin Otolaryngol Head Neck Surg* 2011;19:302–306.
- Roughead EE, McGeechan K, Sayer GP. Bisphosphonate use and subsequent prescription of acid suppressants. *Br J Clin Pharmacol* 2004;57:813–816.
- Salari P, Abdollahi M. Long term bisphosphonate use in osteoporotic patients; a step forward, two steps back. *J Pharm Pharm Sci* 2012;15:305–317.
- 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;45:164–172.
- Kikuri T, Kim I, Yamaza T, et al. Cell-based immunotherapy with mesenchymal stem cells cures bisphosphonate-related osteonecrosis of the jaw-like disease in mice. *J Bone Miner Res* 2010;25:1668–1679.
- Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique. *J Oral Pathol* 1982;11:318–326.
- Allen MR, Burr DB. Mandible matrix necrosis in beagle dogs after 3 years of daily oral bisphosphonate treatment. *J Oral Maxillofac Surg* 2008;66:987–994.
- Hikita H, Miyazawa K, Tabuchi M, Kimura M, Goto S. Bisphosphonate administration prior to tooth extraction delays initial healing of the extraction socket in rats. *J Bone Miner Metab* 2009;27:663–672.
- Senel FC, Kadioglu Duman M, Muci E, et al. Jaw bone changes in rats after treatment with zoledronate and pamidronate. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010;109:385–391.
- Starck WJ, Epker BN. Failure of osseointegrated dental implants after bisphosphonate therapy for osteoporosis: A case report. *Int J Oral Maxillofac Implants* 1995;10:74–78.
- Wang HL, Weber D, McCauley LK. Effect of long-term oral bisphosphonates on implant wound healing: Literature review and a case report. *J Periodontol* 2007;78:584–594.
- Bedogni A, Bettini G, Totola A, Saia G, Nocini PF. Oral bisphosphonate-associated osteonecrosis of the jaw after implant surgery: A case report and literature review. *J Oral Maxillofac Surg* 2010;68:1662–1666.
- Vescovi P, Nammour S. Bisphosphonate-Related Osteonecrosis of the Jaw (BRONJ) therapy. A critical review. *Minerva Stomatol* 2010;59:181–203, 204–113.
- Ruggiero SL, Dodson TB, Assael LA, Landesberg R, Marx RE, Mehrotra B. American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis of the jaw—2009 update. *Aust Endod J* 2009;35:119–130.
- Colella G, Campisi G, Fusco V. American Association of Oral and Maxillofacial Surgeons position paper: Bisphosphonate-Related Osteonecrosis of the Jaws—2009 update: The need to refine the BRONJ definition. *J Oral Maxillofac Surg* 2009;67:2698–2699.
- Grant BT, Amenedo C, Freeman K, Kraut RA. Outcomes of placing dental implants in patients taking oral bisphosphonates: A review of 115 cases. *J Oral Maxillofac Surg* 2008;66:223–230.
- Goss A, Bartold M, Sambrook P, Hawker P. The nature and frequency of bisphosphonate-associated osteonecrosis of the jaws in dental implant patients: A South Australian case series. *J Oral Maxillofac Surg* 2010;68:337–343.
- Yoneda T, Hagino H, Sugimoto T, et al. Bisphosphonate-related osteonecrosis of the jaw: Position paper from the Allied Task Force Committee of Japanese Society for Bone and Mineral Research, Japan Osteoporosis Society, Japanese Society of Periodontology, Japanese Society for Oral and Maxillofacial Radiology, and Japanese Society of Oral and Maxillofacial Surgeons. *J Bone Miner Metab* 2010;28:365–383.
- Biasotto M, Chiandussi S, Zacchigna S, et al. A novel animal model to study non-spontaneous bisphosphonates osteonecrosis of jaw. *J Oral Pathol Med* 2010;39:390–396.