Distinctive Tooth-Extraction Socket Healing: Bisphosphonate Versus Parathyroid Hormone Therapy

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Background: Patients with osteoporosis who receive tooth extractions are typically on either oral bisphosphonate or parathyroid hormone (PTH) therapy. Currently, the consequence of these therapies on hard- and soft-tissue healing in the oral cavity is not clearly defined. The aim of this study is to determine the differences in the therapeutic effect on tooth-extraction wound healing between bisphosphonate and PTH therapies.

Methods: Maxillary second molars were extracted in Sprague Dawley rats (n = 30), and either bisphosphonate (zoledronate [Zol]), PTH, or saline (vehicle control [VC]) was administered for 10 days (n = 10 per group). Hard-tissue healing was evaluated by microcomputed tomography and histomorphometric analyses. Collagen, blood vessels, inflammatory cell infiltration, and cathepsin K expression were assessed in soft tissue using immunohistochemistry, quantitative polymerase chain reaction, and immunoblotting.

Results: Both therapies significantly increased bone fill and suppressed vertical bone loss. However, considerably more devital bone was observed in the sockets of rats on Zol versus VC. Although Zol increased the numbers of blood vessels, the total blood vessel area in soft tissue was significantly smaller than in VC. PTH therapy increased osteoblastic bone formation and suppressed osteoclasts. PTH therapy promoted soft-tissue maturation by suppressing inflammation and stimulating collagen deposition.

Conclusion: Zoledronate therapy deters whereas PTH therapy promotes hard- and soft-tissue healing in the oral cavity, and both therapies prevent vertical bone loss. *J Periodontol 2014;85:24-33.*

KEY WORDS

Parathyroid hormone; tooth extraction; wound healing; zoledronic acid.

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Nearly one of four females and one of eight males aged >50 years are diagnosed with osteoporosis in the United States.[1,2] Patients with osteoporosis are typically treated with either bisphosphonate or teriparatide (recombinant human parathyroid hormone [1-34]) therapy. Bisphosphonates are antiresorptives widely used for the management of osteoporosis. More than 150 million prescriptions were written for oral bisphosphonates from 2005 to 2009.[3] Conversely, teriparatide, a recombinant human parathyroid hormone (PTH), is a bone anabolic agent used for the treatment of osteoporosis. More than 1 million patients around the world have received teriparatide therapy since 2002.[4] Hence, a large elderly population is currently on either bisphosphonate or teriparatide therapy for osteoporosis. Because tooth extractions are common dental procedures in the elderly,[5,6] more patients on bisphosphonate or teriparatide therapy are likely to receive tooth extractions and subsequent implant therapy. Therefore, it is crucial for dentists to understand the effect of these therapies on tooth-extraction wound healing to establish better informed consent and postextraction care. Currently, how these drugs influence tooth-extraction wound healing is not clearly defined.

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Tooth extractions cause tissue injuries, such as mechanical damage in bony socket walls and soft-tissue lacerations.[7] Extraction wounds are typically left exposed to the oral cavity in which many microorganisms reside. Accordingly, intense inflammatory responses occur after tooth extractions.[8] Mechanical damage-induced osteocyte death in bony socket walls manifests as necrotic bone that prompts osteoclastic bone resorption for repair. If this process does not occur, necrotic bone is retained and the healing is incomplete. In rats, osteoclasts emerge in the crestal portion of socket walls to resorb damaged bone at 2 days after extractions, resulting in a reduction of inflammatory responses.[9] The resolution of inflammation is indeed necessary to advance wound healing and subsequent bone formation. In a previous study in which osteopetrotic rats received tooth extractions, more than one third of the rats developed infections in tooth-extraction wounds and, even in non-infected extraction wounds, bone fill was considerably compromised compared with control rats.[10] Although mutant rats were used in the study, the findings suggest that osteoclastic bone resorption plays a pivotal role during healing of tooth-extraction sockets.

Bisphosphonates suppress bone remodeling and resorption by targeting osteoclasts.[11] Because osteoclastic resorption of damaged bone is an essential step in tooth-extraction socket healing, it is theorized that bisphosphonate therapy would alter the normal osseous healing process in tooth-extraction sockets. However, the incidence of healing complications, such as infection and osteonecrosis of the jaw (ONJ) after tooth extractions among patients on oral bisphosphonates is low.[12] In the case of ONJ, the incidence is estimated at ≈0.02%.[13] Therefore, the majority of tooth-extraction wounds heal successfully and uneventfully in patients on oral bisphosphonates, although osteoclasts are suppressed in these patients. Teriparatide (PTH) therapy increases bone mass by promoting bone remodeling in favor of bone formation versus resorption.[14] The bone anabolic effect of PTH therapy appears to be enhanced when applied to osseous tissues in which bone metabolism is active, such as fracture sites[15] and rapidly growing bones.[16] In the craniofacial region, PTH therapy improves the outcome of periodontal surgeries[17] and grafting procedures in rat tooth-extraction sockets.[18] Because bisphosphonate therapy suppresses and PTH therapy enhances bone remodeling, the healing pattern of tooth-extraction sockets would be distinct between hosts on bisphosphonate or PTH therapy.

In the present study, the effect of bisphosphonate and PTH therapies on tooth-extraction wound healing is investigated. The objectives are as follows: 1) to characterize, contrast, and compare how bisphosphonate and PTH therapies affect the soft-tissue healing process; and 2) to determine the advantages and disadvantages of each therapy on tooth-extraction wound healing.

MATERIALS AND METHODS

Animals, Tooth Extractions, and Antiresorptives

Thirty Sprague Dawley rats† (40 days old) were obtained and randomly divided into three groups (n = 10 per group). Sample size was determined by power calculations to obtain 85% statistical power by referring to a published study in which similar experiments were performed.[19] Rats were maintained at 22°C and in 12-hour light/dark cycles. Rats were allowed access to water and standard rodent diet *ad libitum*. The maxillary left and right second molars were extracted under general anesthesia (ketamine and xylazine mixture) using dental instruments. PTH‡ was subcutaneously administered daily at 80 μg/kg for 10 days, and zoledronate (Zol)§ was subcutaneously administered every 2 days at 0.1 mg/kg/week for 10 days (total cumulative dose of 0.15 mg/kg) after tooth extraction. These doses have been used previously and reported.[20,21] The vehicle control (VC) group was administered an equivalent volume of saline. The experimental design and protocol were reviewed and approved by the University of Michigan Committee on Use and Care of Animals.

Microcomputed Tomography (micro-CT)

The maxillae (right half) were dissected, fixed in 10% formalin, and scanned using micro-CT‖ at 10-μm voxel resolution with an energy level of 70 kV. The trabecular compartment in the extraction sockets and interradicular bone between the mesial and distal roots of the maxillary first molars were segmented by the semi-manual contouring method and assessed using the built-in software program.¶ The vertical alveolar bone loss (ABL) was also assessed by measuring the distance between the cemento-enamel junction (CEJ) level of the neighboring tooth and the crest of the bone in the extraction wounds.

Immunohistochemistry

To understand the systemic effect of treatment, proximal tibiae were histomorphometrically assessed

† Charles River Laboratories, Wilmington, MA.
‡ H4835, Bachem, Torrance, CA.
§ Zometa, Novartis, Basel, Switzerland.
‖ μCT 100, Scanco Medical, Bruttisellen, Switzerland.
¶ Scanco software, Scanco Medical.

in addition to the maxillae. Tibiae were isolated, fixed in 10% formalin, and then, along with the maxillae, demineralized in 10% EDTA, paraffin embedded, and sectioned at 5 μm. For tibial sections, hematoxylin and eosin (H&E) staining was performed using a standard staining protocol. Tartrate-resistant acid phosphatase (TRAP) staining was also performed following the instructions of the manufacturer.# For the maxillae, 15 serial sections were prepared from each extraction wound, and three serial sections were used for each staining. Masson's trichrome staining was conducted to visualize collagen fibers following the instructions of the manufacturer** in addition to H&E and TRAP staining. Blood vessels and macrophages were immunohistochemically visualized in the wounds using von Willebrand factor (vWF) and CD68 antibodies, respectively. Briefly, sections were deparaffinized, and enzymatic epitope retrieval was performed in 0.05% trypsin. Non-specific protein was blocked with 10% goat serum. Sections were incubated at 4°C overnight with a rabbit polyclonal vWF antibody†† at 1:800 and a mouse monoclonal CD68 antibody‡‡ at 1:200. Endogenous peroxidase activity was then blocked with 0.3% hydrogen peroxide. Goat antirabbit immunoglobulin G (IgG)§§ and goat antimouse IgG‖‖ conjugated to horseradish peroxidase (HRP) were used for secondary antibody. Proteins were developed with 3,3-diaminobenzidine,¶¶ and sections were counterstained with hematoxylin and mounted.

Hard- and soft-tissue healing in the extraction wounds were histomorphometrically assessed in three serial-stained sections.## Bone area fraction (BAF) and osteoclast numbers per bone perimeter (OC.N/BS) were analyzed in the proximal tibiae. OC.N/BS, osteoblast surface per bone perimeter, and empty osteocyte lacunae were analyzed in the extraction socket walls as described previously.[22] Empty osteocyte lacunae were quantified within 100-μm depth of bone surface in the sockets. Soft-tissue healing was assessed by quantifying collagen fibers, inflammatory cell (polymorphonuclear leukocyte [PMN]) infiltration, blood vessels, and $CD68^+$ mononuclear cells in the soft tissue overlying the tooth-extraction socket (0.4 mm height × 2.5 mm width). The results of the histomorphometric measurement were averaged for the three serial sections for each staining and used for analyses.

Quantitative Real-Time Polymerase Chain Reaction (qPCR)

Approximately 8 mm^3 (2 × 2 × 2 mm) of fresh soft tissue overlying the tooth-extraction socket was isolated and immediately frozen in liquid nitrogen. Fresh bone marrow was isolated from femurs using centrifugation[23] and immediately frozen in liquid nitrogen. A subset of soft-tissue specimens and bone marrow were processed,*** and total RNA was extracted by the phenol/chloroform method. First-strand cDNA was synthesized.††† qPCR was performed.‡‡‡§§§ Samples were run in triplicate, and relative quantification of data generated using this system was performed using the standard curve method. The sets of primers used for qPCR were as follows: 1) *Cathepsin K* (*CTSK*), 5′-TGTCT GAGAACTATGGCTGTGG-3′ and 5′-ATACGGG-TAAGCGTCTTCAGAG-3′; 2) *Vitronectin*, 5′-TTA-GAACACGGCGAGTGGAC-3′ and 5′-CTGTGTG GGTGCTGATTCCT-3′; and 3) *GAPDH*, 5′-ACAA-GATGGTGAAGGTCGGTGTGA-3′ and 5′-AGCTT CCCATTCTCAGCCTTGACT-3′.

Western Blotting

The remaining soft-tissue specimens and bone marrow were used for protein analysis. The specimens were homogenized‖‖‖ to extract protein lysate. Proteins (50 μg) were separated by a 4% to 10% gradient polyacrylamide gel¶¶¶ and transferred to nitrocellulose membranes.### The blots were blocked with 5% non-fat milk for 1 hour at room temperature and incubated with 1:750 rabbit anti-CTSK antibody**** and 1:10,000 anti-tubulin antibody†††† overnight at 4°C in blocking buffer, washed three times, followed by 1:10,000 HRP-conjugated donkey antimouse‡‡‡‡ or antirabbit secondary antibody.§§§§ The protein bands were visualized on x-ray film‖‖‖‖ after using an enhanced chemiluminescence reagent.¶¶¶¶ Bands were then densitometrically assessed using a software program.####

Serum Calcium

Blood was collected from the tail vein before tooth extractions and at euthanasia in anesthetized rats. Serum samples were prepared and kept at –80°C

\# 386A, Sigma-Aldrich, St. Louis, MO.
** HT15, Sigma-Aldrich.
†† ab6999, Abcam, Cambridge, MA.
‡‡ MAB1435, Millipore, Billerica, MA.
§§ AP307, Millipore.
‖‖ AP340P, Millipore.
¶¶ Vector Laboratories, Burlingame, CA.
Image-Pro Plus, Media Cybernetics, Bethesda, MD.
*** TRIzol, Invitrogen, Grand Island, NY.
††† SuperScript First-Strand System, Invitrogen.
‡‡‡ iCycler iQ, Bio-Rad, Hercules, CA.
§§§ SYBR Green mix, Invitrogen.
‖‖‖ C3228CelLytic, Sigma-Aldrich.
¶¶¶ Bio-Rad.
Bio-Rad.
**** 30056, Santa Cruz Biotechnology, Santa Cruz, CA.
†††† T5168, Sigma-Aldrich.
‡‡‡‡ 2314, Santa Cruz Biotechnology.
§§§§ 2313, Santa Cruz Biotechnology.
‖‖‖‖ CL-Xposure Film, Pierce, Rockford, IL.
¶¶¶¶ Pierce.
NIH Image J Version 1.45, Bethesda, MD.

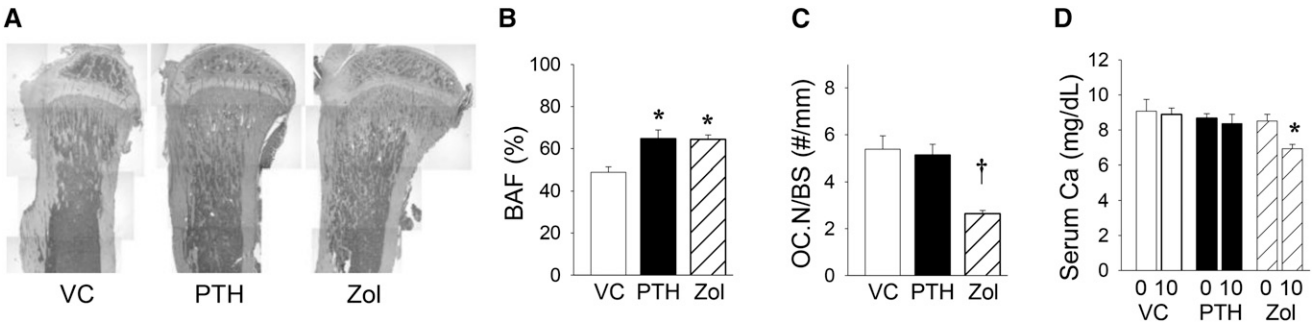

Figure 1.
Histomorphometric analysis of the proximal tibiae and serum calcium. ***A)*** *Representative images of H&E-stained sections of the proximal tibiae.* ***B)*** *Histomorphometric analysis showed significantly higher BAF in the proximal tibiae of the PTH and Zol groups compared with VC.* ***C)*** *TRAP staining was performed, and TRAP-expressing multinucleated cells (nucleus more than two) on the bone surfaces were counted. Zol therapy significantly suppressed OC.N/BS versus VC. PTH did not alter OC.N/BS versus VC.* ***D)*** *Serum calcium levels were measured before and after the 10-day treatment regimen, and results were compared using paired t test. Zol therapy significantly lowered serum calcium level after 10 days, but no change was noted in the VC and PTH groups. n ≥8 per group.* *P <0.01, †P <0.001.

until use. Serum calcium levels were measured***** and compared before and after therapy.

Statistics

Data were analyzed with the Shapiro-Wilk test for normality. Analysis of variance was performed for multiple groups. Tukey's test was used as a post hoc test. For comparisons within the group, a paired *t* test was conducted. No non-parametric tests were used because the Shapiro-Wilk test revealed that all datasets were parametric. Statistical analysis was conducted with a statistics software program.††††† An α level of 0.05 was used for statistical significance, and results are presented as mean ± SEM unless otherwise specified.

RESULTS

Zoledronate Suppressed Osteoclasts and Increased Bone Mass

PTH and Zol were administered to rats for 10 days, and the proximal tibiae were assessed to determine their systemic effects in bone. Significantly higher bone mass was noted in the PTH and Zol groups versus VC (Figs. 1A and 1B). No differences were noted in bone mass (BAF) between the PTH and Zol groups. OC.N/BS was no different with PTH administration but was significantly lower in the Zol group (Fig. 1C), suggesting that the high bone mass in the Zol group is, at least in part, at the expense of osteoclastic bone resorption. In accordance with the suppressed osteoclast surface, serum calcium levels were significantly decreased by Zol (Fig. 1D). Thus, Zol treatment for 10 days exerted significant anti-resorptive effects in bone.

Zoledronate Increased Osteoclasts in Tooth-Extraction Sockets

There were no differences noted in clinical gross healing among groups (data not shown). Representative images of the trichrome-stained sections of the healing wounds are shown (Fig. 2A). PTH significantly increased osteoblast number and markedly decreased OC.N/BS. Zoledronate had no effect on osteoblasts (Figs. 2B and 2C) but substantially increased OC.N/BS in the healing wounds compared with VC. The numbers of empty osteocyte lacunae were significantly higher in the Zol group compared with other groups (Fig. 2D).

PTH and Zoledronate Enhanced Bone Fill in Tooth-Extraction Sockets

To elucidate the effects of PTH and Zol on hard tissue during tooth-extraction socket healing, wound areas were assessed using micro-CT. Both PTH and Zol significantly enhanced bone fill in the sockets compared with VC (Fig. 2E) and also increased trabecular bone thickness (Tb.Th). In addition, the vertical ABL was significantly suppressed by both PTH and Zol (Fig. 2F). Although both therapies affected hard-tissue healing at the wound site, no effects were noted in the interradicular intact bone (Fig. 3). Hence, both therapies exerted their effects in osseous wounds but not in intact bone of the maxillae.

PTH Promoted Soft-Tissue Healing

In the soft tissue overlying the tooth-extraction socket, newly formed blood vessels were visualized by vWF staining and quantified (Figs. 4A through 4C). Although Zol significantly increased the numbers of blood vessels, the total area of blood vessels

***** Calcium Reagent Set (C7503), Pointe Scientific, Canton, MI.
††††† SYSTAT v.12, Systat Software, Chicago, IL.

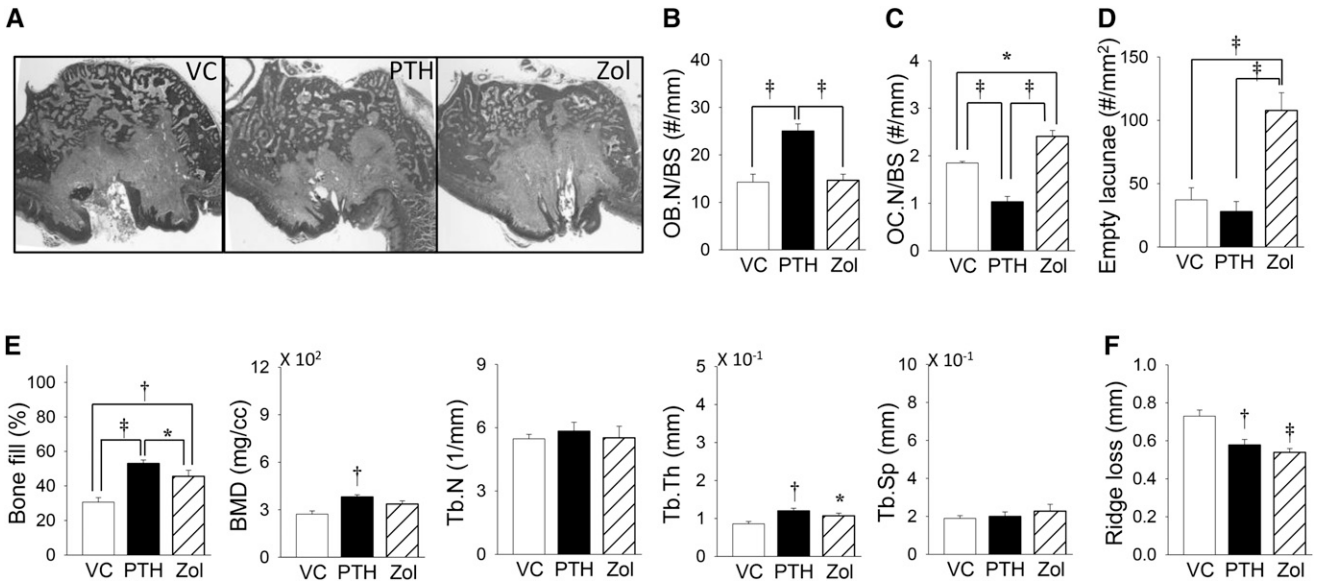

Figure 2.

Hard-tissue assessment in healing wounds. ***A)*** *Representative images of trichrome-stained sections of tooth-extraction wounds.* ***B)*** *PTH therapy significantly stimulated osteoblasts compared with VC. No differences were noted in osteoblast number (OB.N/BS) between Zol and VC. n ≥ 8 per group.* ***C)*** *PTH resulted in considerably lower OC.N/BS versus VC, whereas Zol significantly increased OC.N/BS compared with VC. n ≥ 8 per group.* ***D)*** *The numbers of empty osteocyte lacunae were counted in the socket walls of tooth-extraction sockets. Significantly higher numbers of empty lacunae were noted in the Zol group compared to other groups. n ≥8 per group.* ***E)*** *Tooth-extraction sockets were semi-manually contoured and assessed. PTH and Zol therapies resulted in significantly higher bone fill in the extraction sockets compared with VC. Significantly more bone fill was observed in the PTH group versus Zol. A similar trend was noted in bone mineral density (BMD) and Tb.Th. No statistically significant differences were noted in the trabecular bone number (Tb.N) and trabecular separation (Tb.Sp) between groups. n >7 per group.* ***F)*** *An imaginary straight line between the buccal CEJs of the neighboring molars was drawn using the micro-CT software, and the distance between the line and the crestal bone in the extraction wounds was measured. Significantly smaller alveolar ridge loss was found in the both PTH and Zol groups compared with VC. n >7 per group.* *P <0.05, †P <0.01, ‡P <0.001.

was lower when compared with the other groups, suggesting that blood vessels in the Zol group were smaller. No statistically meaningful differences were noted in $CD68^+$ macrophage numbers among groups, although there was a trend noted in which $CD68^+$ macrophages were decreased in the PTH and Zol groups compared to VC (Figs. 4D and 4E). PTH significantly suppressed PMN infiltration and promoted collagen formation, indicating enhanced soft-tissue healing (Figs. 4F through 4H).

Differential CTSK Expression in Wounds Depending on Treatment

CTSK in the soft tissue of the healing wounds and the femoral bone marrow were assessed at the RNA and protein levels. In the healing wounds, PTH significantly suppressed CTSK at the RNA level, whereas Zol increased the average CTSK level, although statistical significance was not reached (Fig. 5A). Because CTSK is abundant in osteoclasts,[24] the decreased CTSK expression in the healing wounds were harmonious with decreased OC.N/BS in the PTH group (Fig. 2C). However, at the protein level, no significant differences were noted between the Zol and VC groups, although a trend was observed that Zol therapy increased CTSK (Fig. 5B). In the bone marrow, no differences were noted in the CTSK mRNA expression or protein levels among groups (Figs. 5C and 5D).

DISCUSSION

Bisphosphonates and PTH are both used to treat osteoporosis, but they are distinct in their actions in bone. Bisphosphonates suppress osteoclastic bone resorption, whereas PTH stimulates osteoblastic bone formation.[14] Because osteoclastic bone resorption is a crucial component of tooth-extraction socket healing, the fact that, clinically, tooth-extraction wounds heal uneventfully in the majority of patients on oral bisphosphonates raises the question whether healing of tooth-extraction wounds in this patient population are indeed physiologically normal. This study finds that PTH suppressed inflammation, stimulated collagen synthesis, increased osteoblastic bone formation, and suppressed osteoclasts, thereby collectively promoting soft- and hard-tissue healing in the tooth-extraction wounds. Conversely, Zol hindered osseous healing as evidenced by more empty osteocyte lacunae and had an unfavorable impact on blood vessel formation in soft tissue. Clinical significance of the increased

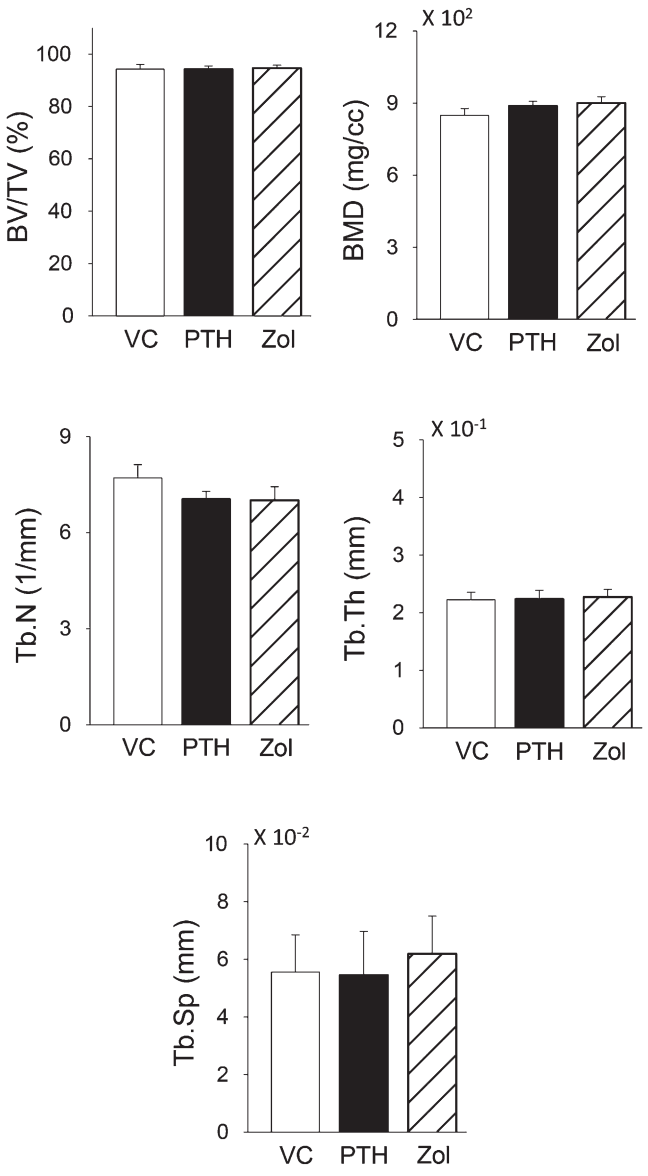

Figure 3.
Micro-CT analysis of the interradicular bone. The interradicular bone of the neighboring maxillary right first molars was assessed. No therapeutic effect was noted among groups. n >7 per group.

numbers of empty osteocyte lacunae in the sockets by Zol is unknown. However, because this is a common finding of studies about bisphosphonate effects on oral osseous wound healing,[7,25,26] bisphosphonate therapy may weaken osteocyte resistance to trauma-induced death. Under normal healthy conditions, bone with empty osteocyte lacunae is indicative of dead bone, and osteoclastic resorption initiates the repair process of such dead bone. However, it is likely that dead bone is retained because the repair process is not initiated as a result of osteoclast suppression by bisphosphonates. This would negatively affect overall tissue integrity.

The antiangiogenic property of bisphosphonates has been implicated as a causal factor of ONJ.[27] However, recent clinical trials have demonstrated no association between antiangiogenic therapy and the incidence of ONJ.[28] The current study finds that, in soft tissue overlying the tooth-extraction socket wound, Zol therapy promoted blood vessel formation yet the total area of blood vessels was significantly smaller versus VC. Therefore, short-term Zol therapy seems to have a negative effect on soft-tissue wound healing. Conversely, PTH therapy showed no effect on blood vessels in this model but did strongly suppress PMN infiltration and promoted soft-tissue repair by increasing collagen deposition. Hence, short-term PTH therapy promoted, but Zol therapy impeded, soft-tissue healing around the extraction wounds.

In rats, epithelial coverage of extraction wounds generally occurs by day 7, and the bottom half of the extraction sockets are filled with woven bone by day 10 and completely occupied by day 14.[9,29] Because the initial stability of wounds is crucial for subsequent healing events, the authors of the present study chose to assess wound healing at 10 days after extraction. It was originally thought that 10 days of treatment would be enough to alter tooth-extraction wound healing but not sufficient to induce significant systemic effects in long bones. However, both Zol and PTH resulted in significantly higher bone mass in long bones compared to VC. This is likely because young adult rats (40 days old) were used. Rats at this age are still growing skeletally, with bone formation exceeding resorption.[30] When Zol or PTH is administered in growing mice, their effects in bone becomes evident in a relatively short period of time.[16,31] This explains why the effects of Zol and PTH are so dramatic in this study. It should be mentioned that the daily PTH dose of 80 μg/kg is used in this study. The daily PTH dose for rats typically ranges from 20 to 80 μg/kg in the literature.[32-35] Therefore, the dose used in the current study is considered relatively high, and this may also help to explain the increased bone mass found in long bones. In the proximal tibiae, the numbers of osteoclasts were significantly decreased by Zol but no reduction was noted in the PTH group, suggesting that increased bone mass in the Zol group was essentially attributable to the suppression of osteoclastic bone resorption, whereas in the PTH group, the stimulation of bone formation accounted for increased bone mass. Similar effects were observed in the tooth-extraction wounds; both Zol and PTH resulted in considerably high bone fill. However, no such effects were noted in the interradicular intact bone of the maxillae. This result

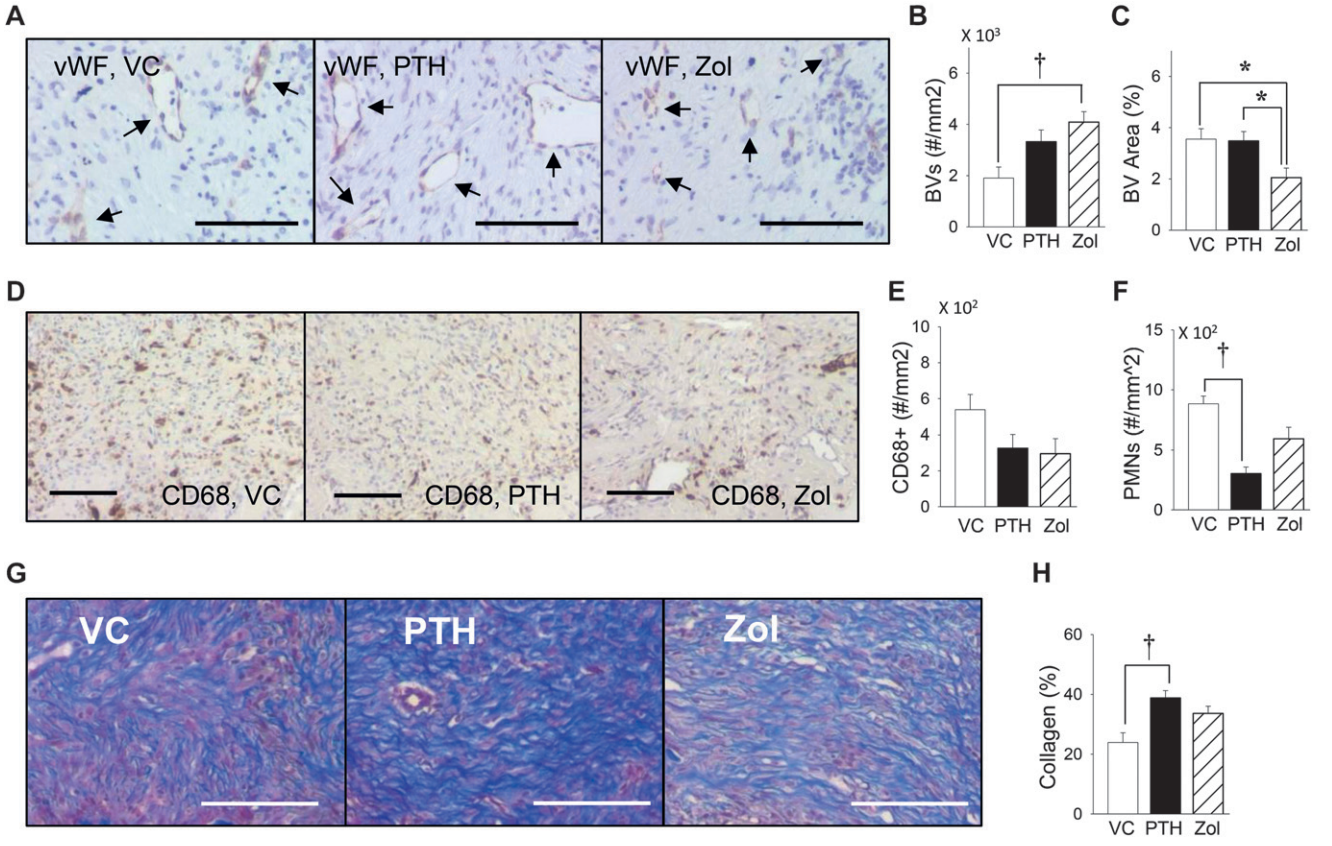

Figure 4.

Soft-tissue assessment in healing wounds. ***A)*** *Representative images of the vWF-stained sections of soft tissue in the healing wounds. Arrows indicate vWF-expressing blood vessels. Scale bar = 0.1 mm. Sections were counterstained with hematoxylin.* ***B*** *and* ***C)*** *Zol therapy induced significantly more blood vessels compared with VC. However, total blood vessel area was significantly smaller than the PTH and VC groups.* ***D)*** *Representative images of the CD68-stained sections of the healing wounds are shown. Scale bar = 0.1 mm.* ***E)*** *Histomorphometric analysis revealed a non-significant decrease in CD68^{+} macrophages in the PTH and Zol group compared to VC.* ***F)*** *PMN infiltration in the healing wounds was assessed in H&E-stained sections. Both PTH and Zol therapies significantly reduced PMN infiltration with a substantial reduction by PTH therapy.* ***G)*** *Representative images of the trichrome-stained sections of soft tissue in the healing wounds. Scale bar = 0.1 mm. Collagen fibers were stained with blue color.* ***H)*** *Collagen fibers were histomorphometrically assessed. PTH considerably increased total collagen amount in the overlying soft tissue. n >7 per group.* *****P <0.05, †P <0.01.

was anticipated because the sensitivity of the craniofacial bone to PTH is lower than that of long bones.[36] Contrary to the tibiae, PTH and Zol administration for 10 days was not long enough to alter the microarchitecture of the intact maxillary bone; in tooth-extraction wounds, bone metabolism was so vigorous that clear therapeutic effects were detected even in the maxillae.

It was interesting to note that Zol increased OC.N/BS in tooth-extraction wounds despite the fact that they were strongly suppressed in the tibiae. Although OC.N/BS were elevated, Zol significantly promoted bone fill in the extraction sockets, with no impact on osteoblast numbers. This suggests impaired osteoclastic resorption of bone. In fact, the vertical loss of the alveolar ridge was significantly less in the Zol group versus VC. Because osteoclasts are responsible for the vertical bone loss of the alveolar crest,[9,29,37] osteoclastic bone resorption is suppressed at this site in the present study. Therefore, Zol administration for 10 days did suppress osteoclastic bone resorption in tooth-extraction sockets. The discrepancy between the increased OC.N/BS and the promoted bone fill in tooth-extraction sockets in the Zol group can be explained by increased numbers of non-functioning osteoclasts. Weinstein et al.[38] reported that OC.N/BS increased despite significantly suppressed bone resorption in the biopsies of patients on bisphosphonate therapy. When osteoclasts internalize bisphosphonates, GTPase signaling is disrupted and osteoclasts detach from bone surfaces because of loss of the sealing zone.[39-41] Therefore, in this study, it is speculated that osteoclasts were generated but detached from the bone surfaces attributable to the internalization of Zol, resulting in

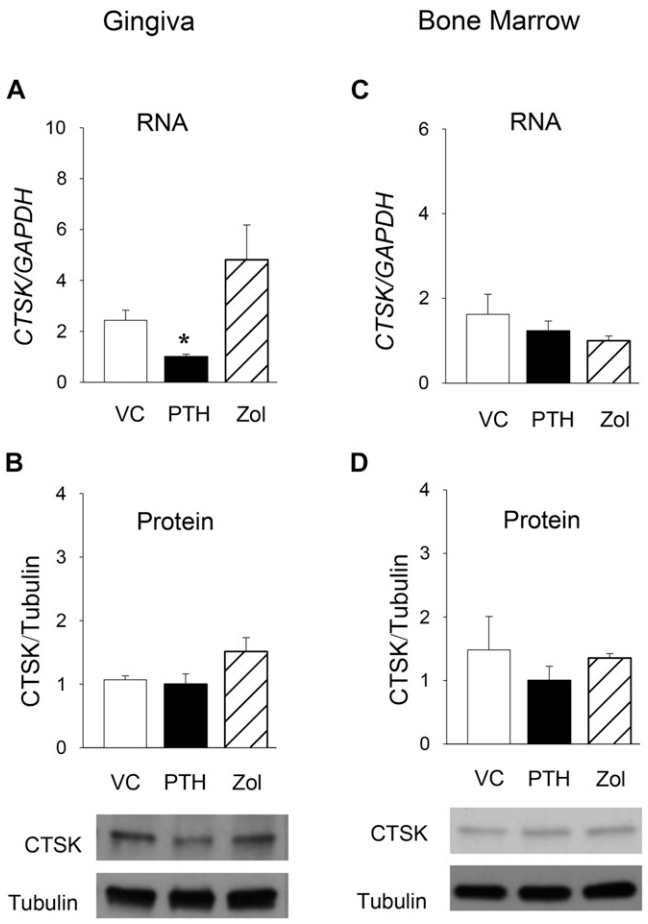

Figure 5.
CTSK expression in healing wounds. Soft tissue was resected from the healing wounds, and the expression of CTSK was analyzed at the RNA level using qPCR and at the protein level using densitometric analysis of immunoblots. CTSK levels were also assessed in the bone marrow of the femurs for comparing local versus systemic effects. PTH therapy significantly suppressed CTSK in the healing wounds at the RNA level ***(A)*** *but not at the protein level* ***(B)****, whereas in the bone marrow, PTH therapy did not alter the expression of CTSK at the RNA and protein levels* ***(C*** *and* ***D)****. There was a trend in which Zol therapy increased the expression of CTSK in the healing wounds at the RNA (A) and protein (B) levels, but statistically meaningful differences were not achieved. In the bone marrow, no differences were noted in the expression of CTSK at the RNA and protein levels comparing Zol versus VC (C and D). n = 4 per group.* ******** *P <0.05.*

increased non-resorbing osteoclasts in the wounds. Hence, osteoclastic bone resorption was inefficient, although many osteoclasts were noted in the extraction sockets. In this study, the impacted soft tissue overlying the tooth-extraction socket is assessed for CTSK at the RNA and protein levels to get an insight into osteoclasts in the extraction wounds. PTH suppressed and bisphosphonate increased CTSK expression at the RNA level, but no significant differences were noted at the protein level. Histomorphometric assessment revealed that OC.N/BS were suppressed by PTH and increased by Zol treatment. The discrepancy in the result between protein and histomorphometric assessments is likely attributed to difficulties in the tissue collection. It was a great challenge to locate and isolate the impacted soft tissue without also including intact soft tissue in the small tooth-extraction wounds. Therefore, protein lysates could be contaminated from the intact tissue, which may affect the expression of CTSK in the results. Also, the connective tissue deep in the extraction wounds near the bone could be missed during tissue collection, and this too could cause a decrease in differences among the groups because osteoclasts exist on and near the bone surfaces.

Osteoporosis patients are generally being treated with either bisphosphonate or teriparatide before tooth extractions. In this study, Zol and PTH administration are initiated when tooth extractions were performed. To make the study more clinically relevant, Zol and PTH could be given before the tooth extractions. Nonetheless, with the present approach, the effects of bisphosphonate and PTH on tooth-extraction wound healing are explained in detail. Moreover, this study discovered that PTH administration starting immediately after tooth extractions not only promoted osseous healing but also enhanced soft-tissue integrity in the extraction wounds. Hence, although the present approach could be improved, it was sufficient to achieve the study goals.

CONCLUSIONS

This study affirms that the healing pattern of tooth-extraction sockets is distinct between Zol and PTH therapies. Zoledronate therapy for 10 days suppressed bone resorption with retention of dead bone and appeared to have a negative effect on soft-tissue healing. PTH therapy for 10 days promoted soft-tissue repair and integrity and hard-tissue healing. These findings emphasize the importance of minimizing trauma and, hence, dead bone accumulation when invasive periodontal procedures, such as implant placement, osseous surgeries, and tooth extractions, are performed in osteoporosis patients taking bisphosphonates.

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