Role of parathyroid hormone in regeneration of irradiated bone in a murine model of mandibular distraction osteogenesis

Stephen Y. Kang, MD,[1,3] Sagar S. Deshpande, BS,[1] Alexander R. Zheutlin, BS,[1] Alexis Donneys, MD,[1] Jose J. Rodriguez, MD,[1] Noah S. Nelson, BS,[1] Peter A. Felice, MD,[1] Douglas B. Chepeha, MD, MSPH,[4] Steven R. Buchman, MD[1,2]*

[1]Craniofacial Research Laboratory, University of Michigan Medical School, Ann Arbor, Michigan, [2]Section of Plastic Surgery, University of Michigan Health System, Ann Arbor, Michigan, [3]Department of Otolaryngology–Head and Neck Surgery, The Ohio State University Wexner Medical Center, Columbus, Ohio, [4]Department of Otolaryngology–Head and Neck Surgery, University of Toronto, Toronto, Ontario, Canada.

Accepted 5 September 2016
Published online 25 October 2016 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/hed.24612

ABSTRACT: *Background.* The purpose of this study was to measure the histologic and histomorphometric effects of parathyroid hormone (PTH) treatment on irradiated bone undergoing distraction osteogenesis (DO).

Methods. Thirty-four rats were divided into 3 groups. The control group underwent DO and the radiation control group underwent radiotherapy (RT) before DO. The PTH group underwent RT and received PTH during DO. Quantitative histology and histomorphometry were performed.

Results. RT resulted in a depletion of osteocytes and increase in empty lacunae. Treatment with PTH resulted in an increase in osteocyte counts and decrease in empty lacunae ($p < .05$), restoring osteocytes to levels seen in nonradiated bone ($p = .121$). RT decreased bone volume to tissue volume (BV-TV) ratio and increased osteoid volume to tissue volume (OV-TV) ratio, signifying increased immature bone formation. PTH treatment restored OV-TV ratio to that observed in nonradiated bone.

Conclusion. PTH treatment of irradiated bone enhanced bone regeneration and restored osteocyte counts and OV-TV ratio to levels comparable to nonradiated bone. © 2016 Wiley Periodicals, Inc. *Head Neck* **39:** 464–470, 2017

KEY WORDS: parathyroid hormone, distraction osteogenesis, radiation, mandible, union

INTRODUCTION

Radiotherapy (RT) is a critical component of the multidisciplinary management of head and neck cancer. However, RT also severely damages surrounding tissue, impairs wound healing, and degrades bone within the treatment field.[1–3] RT delivered to bone has been shown to cause immediate and delayed osteocyte death,[1,2,4] decrease bone mineral density,[5] decrease biomechanical strength,[6] and impair osseous vascularity on a macroscopic and microscopic level.[6–9] The most severe form of osseous damage from RT occurs in the form of osteoradionecrosis,[2,10] which remains a challenging clinical entity.

Adjunctive agents that enhance osseous healing in the irradiated field could be quite beneficial. Parathyroid hormone (PTH) has emerged as a potential therapeutic agent as it has been shown to promote bone anabolism and is Food and Drug Administration approved in the treatment of osteoporosis.[11] However, although PTH has shown tremendous anabolic potential in patients with osteoporosis, more data are needed to determine if PTH can enhance osseous regeneration within irradiated bone. If PTH were to enhance bone regeneration within an irradiated field, it could have tremendous therapeutic potential for clinical translation to patients with advanced oral cavity cancers requiring osseous free tissue transfer and adjuvant RT, as well as patients with osteoradionecrosis.

In this study, we use a murine model of distraction osteogenesis in order to study the histologic and histomorphometric effects of RT and intermittent PTH on the regenerate bone. We hypothesize that RT will result in decreased osteocyte levels, increased empty lacunae, and replacement of mature mineralized bone with immature osteoid formation. We further hypothesize that treating irradiated bone with intermittent PTH will reverse these radiation-induced effects, restoring histological and histomorphometric architecture similar to that of nonradiated bone.

***Corresponding author:** S. R. Buchman, University of Michigan Health System, Pediatric Plastic Surgery Section, 4-730 C.S. Mott Children's Hospital, 1540 E. Hospital Drive, Ann Arbor, MI 48109-4215. E-mail: sbuchman@med.umich.edu

This work was presented as an oral presentation at the 2014 American Society of Plastic Surgeons meeting, Chicago, IL, October 10–14, 2014.

Contract grant sponsor: This work was supported both by a grant from the National Institute of Health (NIH-R01 CA 125187-01) to Steven R. Buchman and by a research grant "Effect of Anabolic PTH on Distracted Mandibular Bone" from the AAO-HNS to Douglas B. Chepeha. The authors have no commercial associations or financial disclosures to declare.

MATERIALS AND METHODS

Experimental groups

Thirty-four 12-week-old isogenic male Lewis rats were randomly assigned to 1 of 3 experimental groups: distraction osteogenesis (DO), xDO, and xDO-PTH, as defined herein (see Figure 1). All groups underwent surgical placement of

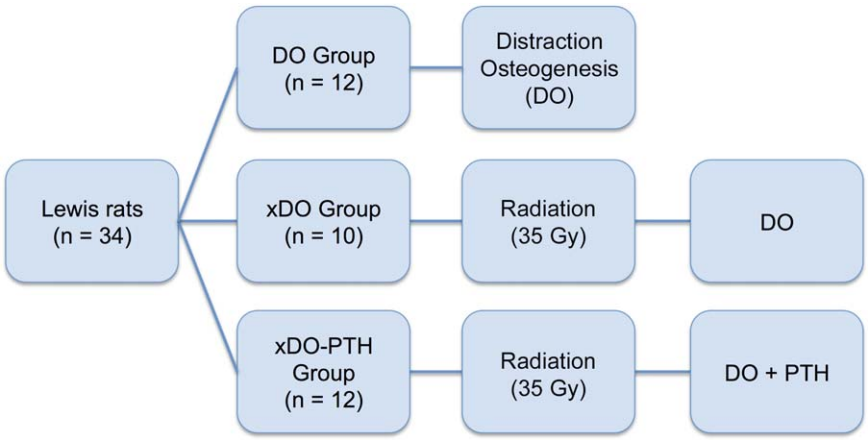

FIGURE 1. Experimental groups. The distraction osteogenesis (DO) group underwent mandibular distraction alone, without radiotherapy (RT). The xDO group underwent RT followed by mandibular distraction. The xDO-parathyroid hormone (PTH) group underwent RT, mandibular distraction, and intermittent PTH treatment for 21 days. [Color figure can be viewed at wileyonlinelibrary.com]

mandibular distractors and mandibular DO. The DO group (n = 12) was the normal control group and underwent mandibular DO. The DO group did not receive RT. The xDO group (n = 10) was the radiation control group and received preoperative RT followed by mandibular DO. The xDO-PTH group (n = 12) received preoperative RT followed by mandibular DO and intermittent subcutaneous PTH (60 μg/kg, once daily) beginning on the first day of distraction for a total duration of 21 days. Our animal protocol has been reviewed and approved by the University Committee on the Use and Care of Animals. The experimental timeline is shown in Figure 2.

Radiotherapy

Rats were acclimated for 7 days in light- and temperature-controlled facilities and given hard chow and water without restriction. Radiation was delivered to the xDO and xDO-PTH groups; the DO group did not receive radiation. Before radiation, rats were anesthetized with inhaled isoflurane. Induction was begun at 4%, after which anesthesia was maintained at 1.5%.

All RTs were performed in the Irradiation Core at the University of Michigan Cancer Center using a Philips R250 orthovoltage unit (250kV, 15 mA; Kimtron Medical, Woodbury, CT). Radiation was delivered to the left hemimandible, 2-mm posterior to the third molar. Lead shielding was used to ensure localized delivery and to protect surrounding tissue. Seven Gray was delivered daily for 5 days for a total fractionated treatment dose of 35 Gy, which is the bioequivalent dose of 70 Gy in humans. Dosimetry was carried out using an ionization chamber connected to an electrometer system, which was directly traceable to a National Institute of Standards and Technology calibration.

After completion of radiation, the xDO-PTH and xDO groups were allowed to recover for 14 days. During this period, all 3 groups were acclimated to a soft chow high-calorie diet (Hills-Columbus Serum; Columbus, OH). The percent of ration of calcium and phosphorus were 0.95% and 1.05%, respectively, and the content of vitamin D was 4.5 IU per gram.

Surgery and postoperative care

All 3 groups underwent surgical placement of a mandibular distraction device with unilateral left mandibular osteotomy. The details of the surgical procedure have been previously described in detail.[12] The surgery was performed 14 days after completion of radiation in the xDO-PTH and xDO groups. Anesthesia was achieved with inhalational isoflurane (4% induction, 1.5% maintenance) and subcutaneous buprenorphine (0.3 mg/kg). A single dose of subcutaneous gentamicin (5 mg/kg) was given preoperatively.

Postoperatively, rats were given 2 doses of subcutaneous gentamicin (5 mg/kg) every 12 hours to prevent infection. Subcutaneous buprenorphine (0.03 mg/kg) was given every 12 hours through postoperative day 5. Staples were removed at postoperative day 10. Weights were measured daily and subcutaneous lactated ringer solution was delivered as needed. Pin care was provided with Silvadene

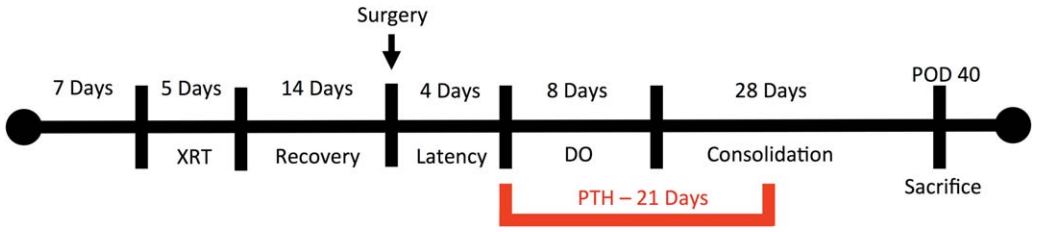

FIGURE 2. Experimental timeline. In the distraction osteogenesis-parathyroid hormone (xDO-PTH) group, intermittent subcutaneous PTH treatment was given daily for 21 days beginning on the first day of mandibular distraction. XRT, radiotherapy treatment. [Color figure can be viewed at wileyonlinelibrary.com]

(Monarch Pharmaceuticals, Bristol, TN). Maxillary incisors were clipped weekly because of overgrowth from crossbite.

Mandibular distraction osteogenesis and region of interest

All rats began left mandibular DO on postoperative day 4. Distraction was performed at a rate of 0.3 mm every 12 hours to a total distraction gap of 5.1 mm. The 5.1-mm distraction gap was chosen because it is a critical size defect in the murine mandible and was previously shown to be reliably distracted with complete bony union.[12] The final distraction was completed on postoperative day 12. Subjects then underwent a 28-day consolidation period before euthanization and histological analysis.

Parathyroid hormone

Intermittent recombinant human PTH (1–34) (Bachem, CA) was administered to the xDO-PTH group in the postoperative period, beginning on the first day of distraction (postoperative day 4). PTH (60 μg/kg) was delivered in intermittent fashion, once daily, and was injected subcutaneously over the right hindlimb. Treatment duration was 3 weeks; a total of 21 doses were delivered and treatment was completed on postoperative day 25. The PTH treatment dose and duration used in this study has previously been shown to improve vascularity, union quality, bone mineral density, and bone volume fraction in a similar murine model of DO.[5,9]

Tissue harvest

Specimens were processed and embedded following a protocol previously described.[13] Bone formed within the 5.1-mm distraction gap was isolated and selected as our region of interest (ROI) for histologic analysis. Coronal sections of the ROI were performed at a thickness of 7 μm, using a Leica Reichert–Jung 2030 Biocut Microtome (Leica, Wetzlar, Germany). After the sections were mounted on glass slides, every seventh slide was stained with Gomoro 1-step trichome. Two slides representative of the mid gap of the ROI were selected per specimen for histologic analysis.

Quantitative histomorphometry

Digital color analysis. Using the imaging analysis software program Bioquant NOVA Osteo version 7 (R&M Biometrics, Nashville, TN), digital images of each slide were obtained for the placement of a standard template over the ROI and color thresholding. The digital color analysis thresholds a blue-green color to the mature, mineralized bone color, and a red-pink color to the osteoid, or immature bone. Two images for each slide are then created, one image in which mature mineralized bone is replaced by red overlay, and another image in which osteoid is replaced by red overlay. Three independent reviewers, who were blinded with respect to the treatment groups, reviewed these images and obtained the following: (1) tissue volume, representing the total volume of the ROI template; (2) bone volume, representing the volume of mineralized bone within the ROI; and (3) osteoid volume, representing the volume of nonmineralized, immature bone, and vacuoles within the ROI.

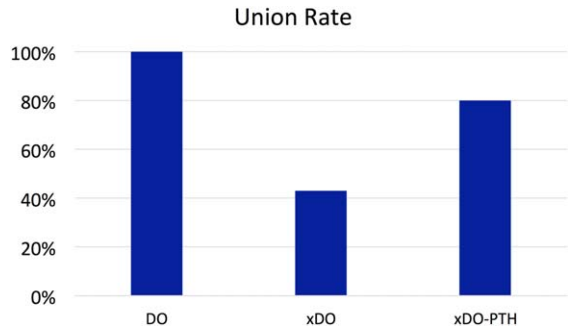

FIGURE 3. Union rate in the distraction osteogenesis (DO), xDO, and distraction osteogenesis-parathyroid hormone (xDO-PTH) groups. Union rate was ascertained based on clinical assessment of the regenerate for bony bridging without motion in the distraction osteogenesis gap. The DO (no radiotherapy [RT]) group had a 100% bony union rate, whereas the xDO (RT) group only formed a bony union in 43% of specimens. The xDO-PTH group (RT + PTH) formed complete unions in 80% of specimens. [Color figure can be viewed at wileyonlinelibrary.com]

Bone volume to tissue volume and osteoid volume to tissue volume ratios were calculated using the Bioquant software.

Union after distraction osteogenesis

Union of the regenerate region was determined by 3 blinded and independent reviewers. The presence of a union was defined as a solid bony bridging, in addition to an absence of motion across the DO gap.

Point counting of osteocytes and empty lacunae

Using the Bioquant software, the ROI was superimposed onto the digital image. Nine high-power-field images at 16× magnification were randomly selected and stored as TIFF files. Point counting of osteocyte and empty lacunae was performed by 3 independent reviewers who were blinded with respect to the treatment groups. Osteocytes were single cells that resided within lacunae.

Statistical analysis

Statistical analysis was performed using SPSS Statistics software version 20 (IBM, Armonk, NY). The data were compared using 1-way analysis of variance. Post-hoc analysis was performed by either Tukey or Games–Howell method, depending on the homogeneity of variances. Significance was assigned as $p < .05$.

RESULTS

Union after distraction osteogenesis

Union rate in the DO, xDO, and xDO-PTH groups are shown in Figure 3. The nonradiated control group (DO group) exhibited complete union in all specimens (100%). In contrast, the union rate in the group that received RT (xDO group) was considerably reduced, as only 43% of these mandibles formed a complete union. However, the addition of PTH to irradiated bone (xDO-PTH group) improved the union rate to 80% (see Figure 3).

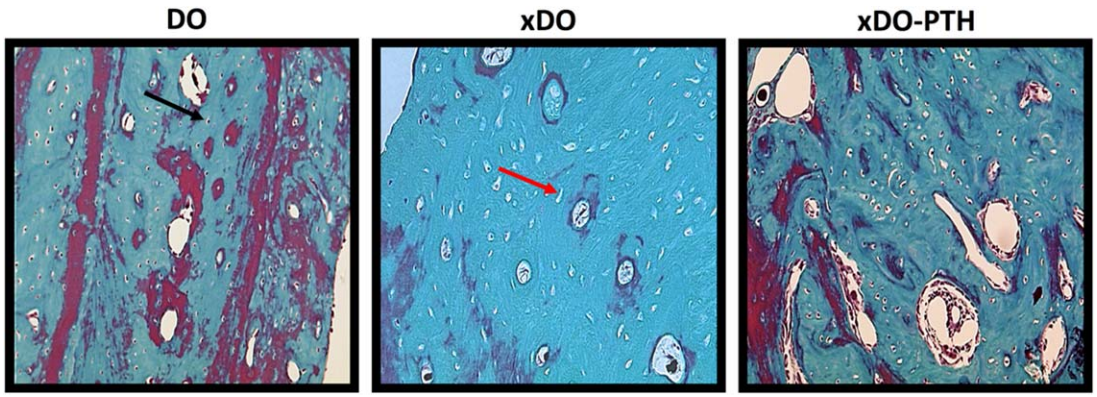

FIGURE 4. Representative sections of formation during distraction osteogenesis (DO) in non-irradiated bone, irradiated bone (xDO), and irradiated bone treated with parathyroid hormone (xDO-PTH). Specimens were stained with Gomori trichrome and are shown at 16× magnification. RT in the xDO group resulted in a significant depletion of osteocytes and increase in empty lacunae. However, treatment of irradiated bone with PTH (xDO-PTH) resulted in a significant increase in osteocyte count and decrease in empty lacunae. There was no significant difference in osteocyte count in nonradiated bone (DO) and irradiated bone treated with PTH (xDO-PTH). The black arrow shows an osteocyte residing within a lacunae and the red arrow shows an empty lacunae.

Quantitative histologic analysis

Figure 4 shows representative histologic samples from the nonirradiated group (DO group), the RT group (xDO group), and the RT group receiving intermittent PTH (xDO-PTH group). Compared with the DO group, RT resulted in a significant decrease in osteocyte count and increase in empty lacunae. The group receiving RT and PTH (xDO-PTH group) showed osteocyte counts that were restored to nonradiated levels. Empty lacunae were significantly decreased in the xDO-PTH group compared to the xDO group, although empty lacunae were not decreased to nonradiated levels (Figures 4 and 5).

In all quantitative metrics, a significant difference was identified between treatment groups as determined by 1-way analysis of variance ($p < .05$). As expected, the RT group (xDO group) resulted in a significant depletion of osteocytes and a significant increase in empty lacunae when compared to the nonradiated control group (DO group). Specifically, compared to the DO group, the xDO group showed a greater than 2-fold decrease in osteocyte count after RT (67.9 vs 139.3; $p < .001$). The xDO group showed a greater than 5-fold increase in empty lacunae after RT compared to the nonradiated DO group (12.3 vs 2.36; $p = .001$; see Figure 5).

Treatment of radiated bone with intermittent PTH resulted in significant restoration of osseous cellularity, resulting in reversal of radiation-induced hypocellularity in the xDO-PTH group (RT followed by distraction and intermittent PTH). Specifically, compared to the non-PTH treated radiation group (xDO), treatment with PTH resulted in a 1.84-fold increase in osteocyte count (125.0 vs 67.9; $p < .001$). Furthermore, compared to the xDO group, the xDO-PTH group had a 2.6-fold decrease in empty lacunae after PTH treatment (4.67 vs 12.3; $p = .005$; see Figure 5).

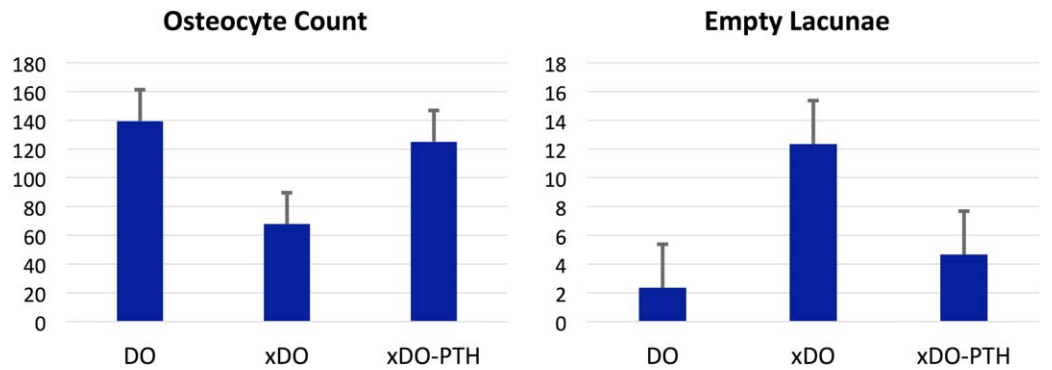

FIGURE 5. Quantitative histologic analysis. Radiotherapy (RT) treatment resulted in a significant depletion of osteocytes in the irradiated bone (xDO) group when compared to nonradiated group (DO; $p < .001$). Treatment of irradiated bone with parathyroid hormone (PTH) resulted in a significant increase osteocyte counts ($p < .001$). In fact, no significant difference in osteocyte count existed between the nonradiated group (DO) and the irradiated group treated with PTH (xDO-PTH; $p = .121$). Y axis represents osteocytes per high power field. With regard to empty lacunae, RT treatment in the xDO group resulted in a significant increase in empty lacunae compared to nonradiated bone ($p = .001$). Treatment with PTH in the xDO-PTH group resulted in a significant decrease in empty lacunae ($p = .005$), albeit not to levels seen in nonradiated bone. Y axis represents empty lacunae per high power field. [Color figure can be viewed at wileyonlinelibrary.com]

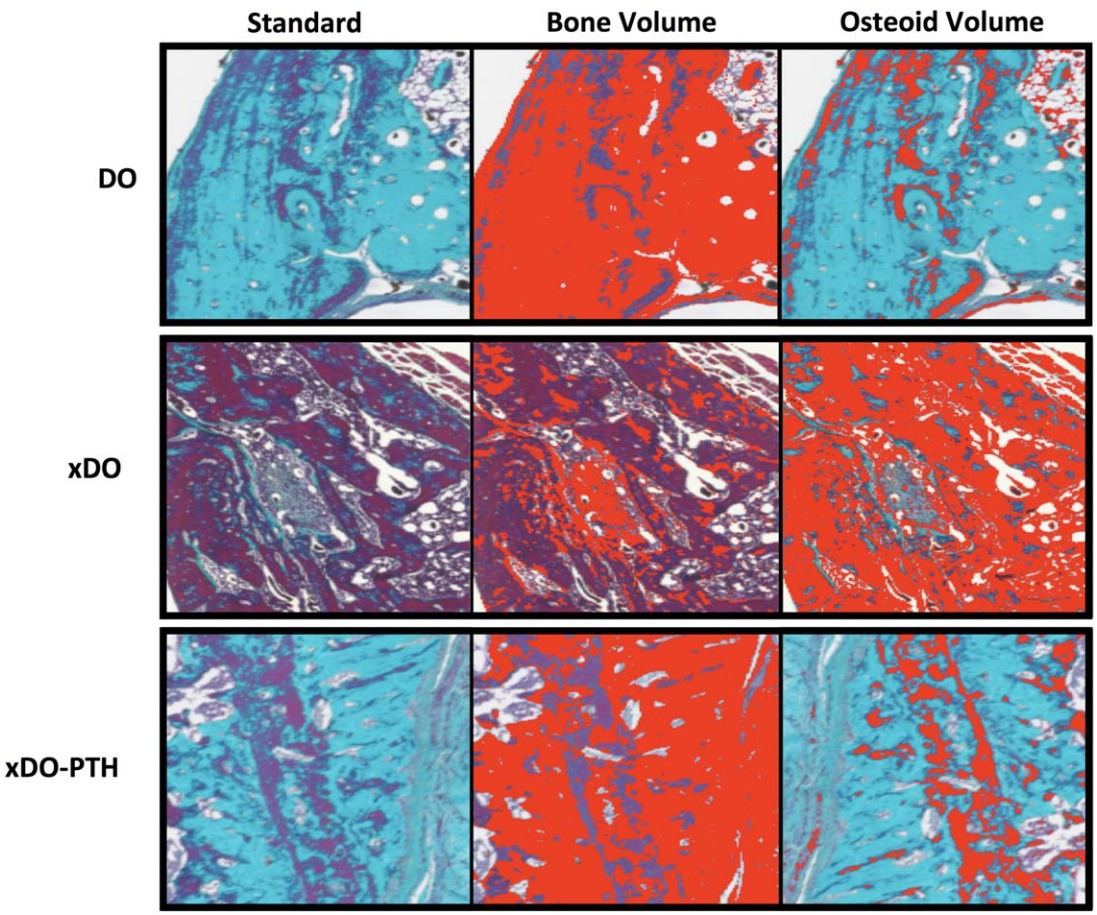

FIGURE 6. Representative digital images used for histomorphometric assessment obtained by the imaging analysis software program Bioquant NOVA Osteo version 7 (R&M Biometrics, Nashville, TN) to quantitatively assess the ratio of mature, mineralized bone formation to immature osteoid formation within the region of interest. In the Standard column, mature, mineralized bone is represented by turquoise whereas osteoid is represented in pink-purple. In the Bone Volume column, red overlay represents mature mineralized bone formation. In the Osteoid Volume column, red overlay represents immature, osteoid formation. These representative images show that compared to nonradiated bone (distraction osteogenesis [DO] group), radiation treatment (xDO group) results in decreased mature bone formation and greater osteoid formation. Treatment with parathyroid hormone (PTH; xDO-PTH group) attenuates the effect of radiotherapy (RT) treatment, resulting in greater areas of mature, mineralized bone formation, and less osteoid formation in irradiated bone undergoing DO. When treated with PTH, the pattern of bone formation in irradiated bone undergoing DO is strikingly similar to nonradiated bone.

Intermittent treatment with PTH after RT in the xDO-PTH group resulted in a significant restoration of osteocytes to levels observed in the nonradiated DO group. There were no measurable differences in osteocyte count between nonradiated and distracted bone (DO group) and bone undergoing RT and intermittent PTH treatment (xDO-PTH group). Specifically, there was no significant difference in osteocyte count between the nonradiated DO group and the group undergoing RT followed by intermittent PTH (xDO-PTH; 139.3 vs 125.0; $p = .121$; see Figure 5). Although treatment with PTH significantly decreased the number of empty lacunae compared to the RT control group, treatment with PTH did not restore empty lacunae levels to nonradiated levels (4.67 vs 2.36; $p < .05$).

Histomorphometry

Compared to the DO group, RT in the xDO group resulted in a significant decrease in mature bone formation, as determined by histomorphometry. Representative histomorphometric samples from the DO, xDO, and xDO-PTH groups are shown in Figure 6. Specifically, compared to the DO group, RT in the xDO group resulted in a 1.6-fold decrease in bone volume to tissue volume ratio (BV-TV; 0.331 vs 0.516; $p = .008$). The xDO group also showed an increase in immature bone formation as determined by osteoid volume to tissue volume ratio (OV-TV). Specifically, compared to the DO group, RT resulted in a 1.7-fold increase in immature bone formation as measured by OV-TV (0.257 vs 0.149; $p = .024$; see Figure 7).

Compared to the xDO group, treatment with PTH was able to increase the BV-TV ratio in the xDO-PTH group, but this difference was not significant (0.4171 vs 0.3483; $p = .420$). Similarly, treatment with PTH decreased immature bone formation as determined by OV-TV ratio, but this difference was also not significant (0.1837 vs 0.2572; $p = .178$; see Figure 7).

Although RT resulted in a significant increase of immature bone formation, as determined by OV-TV ratio in the

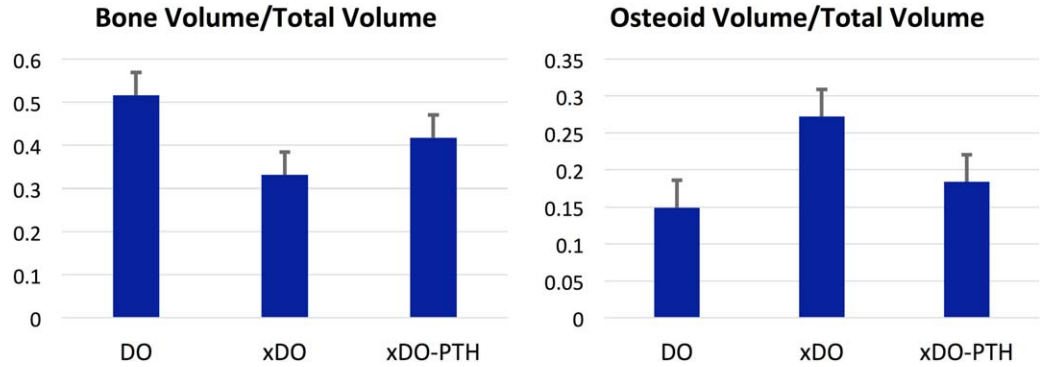

FIGURE 7. Quantitative histomorphometric analysis. Compared to the nonradiated distraction osteogenesis (DO) group, radiotherapy treatment in the xDO group resulted in a significant decrease in bone volume to total volume ratio (BV-TV; $p = .008$). Treatment of irradiated bone with parathyroid hormone (PTH) resulted in an increase in BV-TV, but this difference was not significant ($p = .420$). Radiation treatment resulted in a significantly greater osteoid volume to total volume (OV-TV) in the xDO group, compared to the nonradiated DO group ($p = .024$). Treatment of irradiated bone with PTH resulted in a decrease in OV-TV, but this difference was not significant ($p = .178$). However, there was no detectable difference in OV-TV between the nonradiated DO group and the irradiated group treated with PTH ($p = .345$). [Color figure can be viewed at wileyonlinelibrary.com]

xDO and DO groups, treatment with PTH lessened this radiation-induced effect. Specifically, in comparison to the nonradiated DO group, the xDO-PTH group showed no significant difference in OV-TV (1.837 vs 1.491; $p = .345$). However, although PTH treatment was able to reduce the effect of radiation on bone volume loss, it was unable to completely reverse the BV-TV ratio to nonradiated levels. Specifically, compared to the DO group, the xDO-PTH group showed a significant decrease in BV-TV ratio (0.4171 vs 0.516; $p = .026$; see Figure 7).

DISCUSSION

RT has been shown to result in poorer regenerate outcomes in mandibular distraction osteogenesis,[5,9,14–16] and this study confirmed that RT results in a significant depletion of osseous cellularity in a murine model of DO. However, treatment of radiated bone with intermittent PTH reversed radiation-induced hypocellularity. In fact, PTH restored osteocyte counts in irradiated bone to baseline levels, as there was no significant difference in osteocyte count between the irradiated group that received PTH (xDO-PTH group) and the group that underwent distraction alone, without RT (DO group). Treatment with PTH also increased the degree of mature bone formation within the ROI, although this was not restored to normal control levels. Previous work has shown that PTH improves biomechanical strength,[6] union quality, bone mineral density,[5] and osseous vascularity[9] in irradiated bone. This is the first report to demonstrate the ability of PTH to increase mature bone formation and restore osteocyte count to nonradiated levels in bone.

Although the complete cellular mechanism by which PTH promotes bone anabolism remains to be elucidated, PTH has been shown to have a pleiotropic anabolic effect on bone formation,[17] increasing osteoblast number by promoting osteoblastogenesis, inhibiting osteoblast apoptosis, and reactivating lining cells to resume matrix synthesizing function.[17,18] RT specifically reduces proliferation of vasculature and osteoblasts.[19] Although more studies are needed to determine the mechanism by which PTH ameliorates the effects of RT, the findings of this study clearly demonstrate the ability of PTH to preserve and restore osteocyte counts in irradiated bone to levels that are comparable to nonradiated bone.

Teriparatide, also known as recombinant human PTH (1–34), is an attractive adjunctive agent to study because it is already in clinical use and an Food and Drug Administration approved therapy for osteoporosis.[11,20,21] With regard to potential therapeutic use for osseous wounds in the head and neck, Bashutski et al[22] used intermittent PTH to accelerate alveolar bone regeneration in the oral cavity after periodontal surgery, evidenced by a significant radiographic improvement in linear resolution of osseous defects as well as a reduction in probing depth. Case reports suggest that PTH may have a role in treating bisphosphonate-related osteonecrosis of the jaw.[14,23,24] These findings suggest tremendous therapeutic potential as an adjunctive treatment to complex wounds in the head and neck, where osseous wound healing is of paramount importance to functional outcome.

However, one large clinical hurdle exists, as a history of RT delivered to bone is currently a contraindication to PTH treatment.[25,26] This contraindication exists because of the observation of osteosarcoma in rats chronically treated with PTH.[27] However, the animals in that particular study were treated at higher doses than those used in human studies, and for time spans up to 2 years, representing 80% to 90% of their normal time span, complicating comparisons to clinical trials that use short-term use of PTH. The same authors studied PTH-related osteosarcoma in monkeys treated with daily PTH, and reported zero cases of osteosarcoma[28] suggesting that this effect may be species-dependent and not applicable to human subjects.[26] Harper et al[24] reported 1 case of osteosarcoma among >300,000 patients worldwide that have been treated with teriparatide (PTH). As a result, causality between teriparatide and osteosarcoma in humans has yet to be established. However, the safety of teriparatide use in patients with a history of head and neck cancer and/or RT delivered to the mandible remains to be determined.

This study adds to a growing literature demonstrating that PTH has tremendous potential to enhance osseous regeneration in irradiated bone. PTH has previously been shown to enhance biomechanical strength, bone mineral density, and union formation in irradiated bone.[5,6] This histologic and histomorphometric study adds new data, demonstrating the ability of PTH to reverse radiation-induced hypocellularity and enhance mature bone formation in a murine model of DO, and further supports the potential use of PTH to enhance osseous regeneration in the irradiated mandible.

REFERENCES

1. Costantino PD, Friedman CD, Steinberg MJ. Irradiated bone and its management. *Otolaryngol Clin North Am* 1995;28:1021–1038.
2. Marx RE. Osteoradionecrosis: a new concept of its pathophysiology. *J Oral Maxillofac Surg* 1983;41:283–288.
3. Teknos TN, Myers LL. Surgical reconstruction after chemotherapy or radiation. Problems and solutions. *Hematol Oncol Clin North Am* 1999;13: 679–687.
4. Sanger JR, Matloub HS, Yousif NJ, Larson DL. Management of osteoradionecrosis of the mandible. *Clin Plast Surg* 1993;20:517–530.
5. Gallagher KK, Deshpande S, Tchanque–Fossuo CN, et al. Role of parathyroid hormone therapy in reversing radiation-induced nonunion and normalization of radiomorphometrics in a murine mandibular model of distraction osteogenesis. *Head Neck* 2013;35:1732–1737.
6. Deshpande SS, Gallagher KK, Donneys A, et al. Parathyroid hormone therapy mollifies radiation-induced biomechanical degradation in murine distraction osteogenesis. *Plast Reconstr Surg* 2013;132:91e–100e.
7. Cutright DE, Brady JM. Long-term effects of radiation on the vascularity of rat bone–quantitative measurements with a new technique. *Radiat Res* 1971;48:402–408.
8. Deshpande SS, Gallagher KK, Donneys A, et al. Stem cells rejuvenate radiation-impaired vasculogenesis in murine distraction osteogenesis. *Plast Reconstr Surg* 2015;135:799–806.
9. Kang SY, Deshpande SS, Donneys A, et al. Parathyroid hormone reverses radiation induced hypovascularity in a murine model of distraction osteogenesis. *Bone* 2013;56:9–15.
10. Teng MS, Futran ND. Osteoradionecrosis of the mandible. *Curr Opin Otolaryngol Head Neck Surg* 2005;13:217–221.
11. Neer RM, Arnaud CD, Zanchetta JR, et al. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N Engl J Med* 2001;344:1434–1441.
12. Buchman SR, Ignelzi MA Jr, Radu C, et al. Unique rodent model of distraction osteogenesis of the mandible. *Ann Plast Surg* 2002;49:511–519.
13. Tchanque–Fossuo CN, Monson LA, Farberg AS, et al. Dose-response effect of human equivalent radiation in the murine mandible: part I. A histomorphometric assessment. *Plast Reconstr Surg* 2011;128:114–121.
14. Fregene A, Jing XL, Monson LA, Buchman SR. Alteration in volumetric bone mineralization density gradation patterns in mandibular distraction osteogenesis following radiation therapy. *Plast Reconstr Surg* 2009;124: 1237–1244.
15. Inyang AF, Schwarz DA, Jamali AM, Buchman SR. Quantitative histomorphometric assessment of regenerate cellularity and bone quality in mandibular distraction osteogenesis after radiation therapy. *J Craniofac Surg* 2010;21:1438–1442.
16. Schwarz DA, Jamali AM, Kakwan MS, Fregene A, Arman KG, Buchman SR. Biomechanical assessment of regenerate integrity in irradiated mandibular distraction osteogenesis. *Plast Reconstr Surg* 2009;123(2 Suppl):114S–122S.
17. Jilka RL. Molecular and cellular mechanisms of the anabolic effect of intermittent PTH. *Bone* 2007;40:1434–1446.
18. Kim SW, Pajevic PD, Selig M, et al. Intermittent parathyroid hormone administration converts quiescent lining cells to active osteoblasts. *J Bone Miner Res* 2012;27:2075–2084.
19. Willey JS, Lloyd SA, Robbins ME, et al. Early increase in osteoclast number in mice after whole-body irradiation with 2 Gy X rays. *Radiat Res* 2008;170:388–392.
20. Black DM, Greenspan SL, Ensrud KE, et al. The effects of parathyroid hormone and alendronate alone or in combination in postmenopausal osteoporosis. *N Engl J Med* 2003;349:1207–1215.
21. Finkelstein JS, Hayes A, Hunzelman JL, Wyland JJ, Lee H, Neer RM. The effects of parathyroid hormone, alendronate, or both in men with osteoporosis. *N Engl J Med* 2003;349:1216–1226.
22. Bashutski JD, Eber RM, Kinney JS, et al. Teriparatide and osseous regeneration in the oral cavity. *N Engl J Med* 2010;363:2396–2405.
23. Cheung A, Seeman E. Teriparatide therapy for alendronate-associated osteonecrosis of the jaw. *N Engl J Med* 2010;363:2473–2474.
24. Harper KD, Krege JH, Marcus R, Mitlak BH. Osteosarcoma and teriparatide? *J Bone Miner Res* 2007;22:334.
25. Girotra M, Rubin MR, Bilezikian JP. The use of parathyroid hormone in the treatment of osteoporosis. *Rev Endocr Metab Disord* 2006;7:113–121.
26. Pleiner–Duxneuner J, Zwettler E, Paschalis E, Roschger P, Nell–Duxneuner V, Klaushofer K. Treatment of osteoporosis with parathyroid hormone and teriparatide. *Calcif Tissue Int* 2009;84:159–170.
27. Vahle JL, Sato M, Long GG, et al. Skeletal changes in rats given daily subcutaneous injections of recombinant human parathyroid hormone (1-34) for 2 years and relevance to human safety. *Toxicol Pathol* 2002;30:312–321.
28. Vahle JL, Zuehlke U, Schmidt A, Westmore M, Chen P, Sato M. Lack of bone neoplasms and persistence of bone efficacy in cynomolgus macaques after long-term treatment with teriparatide [rhPTH(1-34)]. *J Bone Miner Res* 2008;23:2033–2039.