Intermittent Parathyroid Hormone Administration Improves Periodontal Healing in Rats

Daniel Fernando Pereira Vasconcelos,* Marcelo Rocha Marques,† Bruno Braga Benatti,‡ Silvana Pereira Barros,§ Francisco Humberto Nociti Júnior,‖ and Pedro Duarte Novaes†

Background: Intermittent administration of parathyroid hormone (PTH) promotes new bone formation in patients with osteoporosis and bone fractures. It was shown previously that PTH also reduces periodontitis-related bone loss. The aim of this study is to evaluate the effect of treatment with PTH on periodontal healing in rats.

Methods: Fenestration defects were created at the buccal surface of the distal root of the mandibular first molars, and both periodontal ligament (PDL) and cementum were removed. Animals were then assigned to two groups (eight animals per group): group 1: control, placebo administration; and group 2: test, human PTH (hPTH) 1-34 administration at a concentration of 40 μg/kg. For both groups, the animals were injected every 2 days, and the animals were sacrificed at 14 and 21 days after surgery. Specimens were harvested and processed for routine decalcified histologic sections. The following parameters were assessed: 1) remaining bone defect extension (RBDE); 2) newly formed bone density (NFBD); 3) total callus area (TCA); 4) osteoclast number (ON) in the callus region; and 5) newly formed dental cementum-like tissue (NFC). Birefringence of root PDL reattachment was also evaluated.

Results: Birefringence analysis showed root PDL reattachment for both groups 21 days after treatment. Intermittent hPTH 1-34 administration decreased RBDE ($P < 0.01$) and increased NFBD ($P < 0.01$), TCA ($P < 0.01$), area of NFC ($P < 0.01$), and ON in the callus region ($P < 0.01$).

Conclusion: Within the limits of the present study, intermittent administration of hPTH 1-34 led to an enhanced periodontal healing process compared with non-treated animals. *J Periodontol 2014;85:721-728.*

KEY WORDS

Dental cementum; microscopy, polarization; parathyroid hormone; periodontium; rats; wound healing.

* Division of Histology and Embryology, Federal University of Piauí, Parnaíba, Piauí, Brazil.
† Department of Morphology, Division of Histology, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil.
‡ Federal University of Maranhão, São Luís, Maranhão, Brazil.
§ Center for Oral and Systemic Diseases, Department of Periodontology, University of North Carolina School of Dentistry, University of North Carolina at Chapel Hill, Chapel Hill, NC.
‖ Department of Prosthodontics and Periodontics, Division of Periodontics, Piracicaba Dental School, State University of Campinas.

The ultimate goal of periodontal therapy is to achieve the regeneration of periodontal tissue destroyed by the progression of periodontal diseases. Some success has been achieved in suppressing the progression of periodontitis by mechanically removing the cause of the disease. However, no conventional periodontal and/or surgical treatments can regenerate whole periodontal tissue lost because of periodontal diseases.[1]

Regenerative periodontal therapy uses specific techniques designed to restore those parts of the tooth-supporting structures that have been lost because of periodontitis or gingival trauma.[2] Currently, the methods used to reconstitute the lost parts of periodontal structures (i.e., alveolar bone, periodontal ligament [PDL], and root cementum) rely on conventional mechanical anti-infective modalities, followed by a range of regenerative procedures, such as guided tissue regeneration, bone replacement grafts, and exogenous growth factors.[3]

Parathyroid hormone (PTH) is a peptide related to calcium metabolism, and although PTH is physiologically known to promote bone resorption, it has been shown that PTH may promote new bone formation when administrated intermittently. PTH is the only anabolic bone agent approved by the Food and Drug Administration for use in

doi: 10.1902/jop.2013.130155

humans.[4] Intermittent administration of the amino-terminal fragment of human PTH (hPTH) 1-34 can increase bone mass in humans and animals,[4,5] and it has also been reported to enhance fracture healing in both intact and ovariectomized rats[6,7] and postmenopausal females.[8]

The present authors have reported previously that intermittent hPTH 1-34 administration can protect against bone loss from periodontitis in rats.[9-11] In addition, hPTH 1-34 administration can also promote bone gain in alveolar bone affected by periodontitis in humans.[12] Therefore, in the present study, the effect of intermittent hPTH 1-34 administration on periodontal healing/regeneration is histologically determined using the surgically created fenestration defect in rats.

MATERIALS AND METHODS

Animals

Thirty-two 8-week-old male Wistar rats with a mean ± SD weight of 254.3 ± 16.6 g were obtained from the Animal Facility Center of the University of Campinas, Piracicaba, São Paulo, Brazil. The animals were kept in plastic cages and had access to food and water *ad libitum*. Before surgical procedures, all animals were acclimatized to the laboratory environment for 5 days. This study was approved by the University of Campinas Institutional Animal Care and Use Committee (protocol 1338-1).

Surgery and Treatment

Ketamine[¶] (10 mg/kg) and xylazine[#] (5 mg/mL) were used for general anesthesia. Periodontal fenestration defects (4 mm in width, 3 mm in length, and ≈1 mm deep) were created at the buccal aspect of the distal root of the mandibular right first molar of each animal (Fig. 1A), as described previously.[13,14] Briefly, the buccal surface of the mandibular bone, in the first molar region, was removed using a round dental bur,[**] 2 mm in diameter, at a slow speed under saline solution irrigation. The distal root of the mandibular first molar was denuded of its PDL, cementum, and superficial dentin using a chisel, avoiding excessive damage to the root that could compromise the endodontic tissues. Neither antibiotics nor anti-inflammatory drugs were administered after the surgery. Acetaminophen[††] was used for pain control. After the surgery, every 2 days, 40 μg/kg hPTH (1-34)[‡‡][15] was subcutaneously administered in 16 rats (group 2: treated group) for 14 days (T14; n = 8) and 21 days (T21; n = 8), whereas another 16 (group 1: control group) rats received a PTH vehicle (0.01% acetic acid[§§]) for 14 days (C14, control; n = 8) and 21 days (C21; n = 8). The animals were sacrificed at days 14 or 21, and their right hemimandibles were retrieved for histologic evaluation.

Histology Preparation and Histometric Analyses

For tissue fixation, the right hemimandibles of all animals were kept in 2.5% cacodylate-buffered glutaraldehyde[‖‖] (pH 7.4) containing 7% sucrose[¶¶] for 24 hours. The samples were decalcified in 10% EDTA,[##] dehydrated in ethanol,[***] and embedded in paraffin.[†††] Serial transversal tissue sections (4 μm) were obtained from the region in which the periodontal defects were created. The apical and coronal margins of the defect were determined as the portion of the first molar distal root planed by chisel was observed. Sections concerning the middle portion of each defect were submitted to histologic and histometric analyses. Five sections per animal were stained with hematoxylin and eosin[‡‡‡] (H&E), and parameters, such as extension of the initial defect (Fig. 1B), newly formed bone density (NFBD) (Fig. 1B), remaining bone defect extension (RBDE) (Fig. 1B), and total callus area (TCA) (Fig. 1C), were measured. Another three sections per animal were stained with Mallory trichrome,[§§§] and the area of the newly formed cementum-like tissue (NFC) (Fig. 1D) was measured in a 620-μm (mesio-distal direction) extension of instrumented root. For this, the NFC was measured in two different regions in each root, 310 μm from the mesial margin and 310 μm from the distal margin, of which margins were defined by the remaining cementum that was not surgically instrumented.[16] The mean values obtained were expressed as square micrometers of the NFC/620 μm of the root extension. The histomorphometric parameters were measured by one masked, previously calibrated examiner (DFPV) using image analysis software.[‖‖‖¶¶¶]

Polarizing Light Microscopy of the Newly Formed PDL

Polarizing microscopy was used to evaluate PDL fiber reattachment in the NFC. Two unstained sections per animal, obtained from the middle portion of the defect, were kept in 80% glycerin[###] for 30 minutes. The PDL birefringence was then evaluated using a polarizing light microscope[****] equipped with polarizing filters, a compensator,[††††] and polychromatic light (Fig. 1E).

¶ Vetbrands, Jacareí, São Paulo, Brazil.
\# Virbac, Rio de Janeiro, Rio de Janeiro, Brazil.
** SS White, Lakewood, NJ.
†† Paracetamol, Abbott Laboratories, São Paulo, São Paulo, Brazil.
‡‡ Sigma-Aldrich, St. Louis, MO.
§§ Merck, Darmstadt, Germany.
‖‖ Electron Microscopy Sciences, Fort Washington, PA.
¶¶ Merck.
Merck.
*** Merck.
††† McCormick Scientific, St. Louis, MO.
‡‡‡ MPL, Piracicaba, São Paulo, Brazil.
§§§ MPL.
‖‖‖ Image-Pro Plus, Media Cybernetics, Silver Spring, MD.
¶¶¶ KS400 v.2.0, Kontron Electronics, Eching, Germany.
Synth, São Paulo, São Paulo, Brazil.
**** Leica Microsystems, Bannockburn, IL.
†††† Brace-Köhler compensator, Wild Leitz, Wetzlar, Germany.

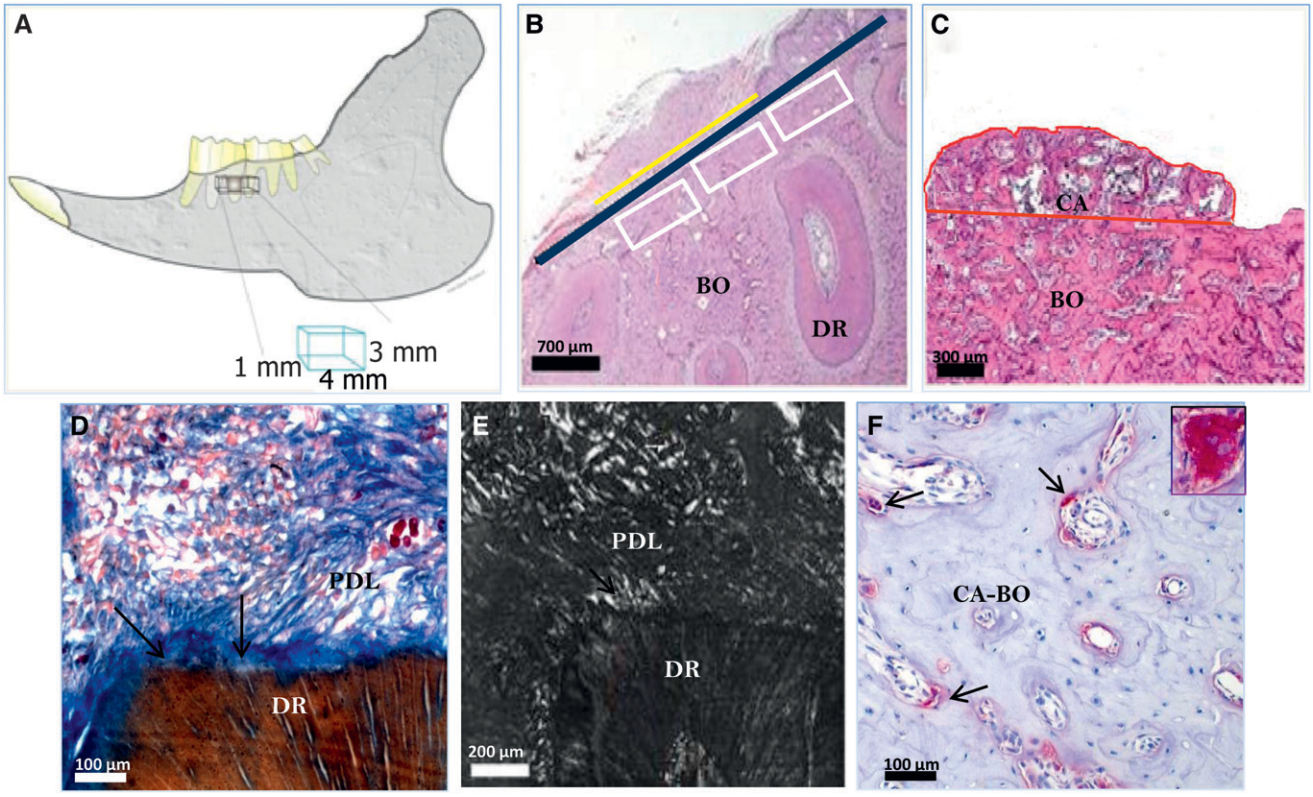

Figure 1.
A) *Schematic representation of the fenestration defect created in the rat hemimandibles. The defects were generated at the buccal aspect of the distal root from the mandibular right first molar.* ***B through F)*** *Photomicrographs of transverse tissue sections (4 µm) obtained from the middle region of the periodontal defects unstained (E), stained with H&E (B and C) or Mallory trichrome (D), or stained for tartrate-resistant acid phosphatase (TRAP) detection (F). Note the regions from which measurements were derived: extension of the initial defect (B, blue), NFBD (B, white), RBDE (B, yellow), and TCA (C, red). D) Black arrows indicate NFC, which was measured in a 620-µm (mesio-distal direction) extension from the instrumented root. E) Black arrow indicates PDL fiber reattachment in the NFC, as observed by polarizing light microscopy. F) Black arrows indicate TRAP-positive cells in the bone TCA; TRAP-positive osteoclast can be observed under high magnification. BO = bone; DR = distal root from the mandibular right first molar; CA = callus area; CA-BO = callus bone area.*

Enzymohistochemistry

An enzymohistochemical assay, commonly used to detect tartrate-resistant acid phosphatase (TRAP), was performed to detect osteoclasts.[17] Five sections (4 µm) obtained from the middle portion of the defect for each animal were incubated at 37°C for 15 minutes with a solution containing 4 mg naphthol AS-BI phosphate‡‡‡‡ as substrate, 24 mg red violet salt§§§§ diluted in 30 mL acetate buffer (pH 5.2), and 0.3 mmol/L tartrate‖‖‖‖ (pH 5.0). For negative control, the substrate was excluded. Sections were counterstained with Harris hematoxylin,¶¶¶¶ and the TRAP-positive cells (Fig. 1F) with at least three nuclei were counted in the TCA by one masked, previously calibrated examiner (DFPV) using image analysis software.####

Statistical Analyses

Sample size was calculated using statistical software. ***** A sample size of eight per group was required for detection of a significant difference (80% power, 5% significance level). The values obtained from the histometric measurements (extension of the initial bone defect, NFBD, RBDE, NFC, and TCA) and TRAP histochemistry were analyzed via one-way analysis of variance (ANOVA) and Tukey multiple comparisons tests.

RESULTS

Histometric Analyses and Polarizing Light Microscopy

Spontaneous periodontal repair was evaluated to verify whether intermittent hPTH 1-34 administration would affect periodontal healing/regeneration. Histologic analysis indicated newly formed bone, NFC, and PDL within the healing area. Whereas tissue

‡‡‡‡ Sigma-Aldrich.
§§§§ Sigma-Aldrich.
‖‖‖‖ Sigma-Aldrich.
¶¶¶¶ MPL.
Image-Pro Plus, Media Cybernetics.
***** SAS/STAT v.9.3, SAS Institute, Cary, NC.

sections stained with H&E showed newly formed PDL in all operated rats, qualitative birefringence analysis revealed dense PDL fiber reattachment to the NFC only in group 2 animals treated with hPTH 1-34 for 21 days (T21) (Fig. 2). Histometric analysis revealed that the initial extension (C14, 4.56 ± 0.16 mm; T14, 4.65 ± 0.17 mm; C21, 4.69 ± 0.35 mm; T21, 4.65 ± 0.07 mm) of the bone defect was similar in all groups (P >0.05). In a comparison among the experimental and control groups treated for the same periods (C14 and T14; C21 and T21), intermittent hPTH 1-34 administration was found to increase bone formation, reducing RBDE (P <0.01) (C14, 1.72 ± 0.37 mm; T14, 0.87 ± 0.13 mm; C21, 0.7 ± 0.18 mm; T21, 0.33 ± 0.21 mm) and increasing both NFBD (P <0.01) (C14, 42.50 ± 3.50%; T14, 59.62 ± 6.09%; C21, 51.87 ± 5.71%; T21, 72.12 ± 5.38%) and TCA (P <0.01) (C14, 0.51 ± 0.10 mm^2; T14, 3.21 ± 0.31 mm^2; C21, 0.96 ± 0.15 mm^2; T21, 2.20 ± 0.24 mm^2).

TRAP-positive cells identified in the callus region were quantified (Fig. 3). Significant increases (P <0.01) in osteoclast number (ON) were evident in the groups treated with hPTH 1-34, considering groups treated at the same periods (C14 and T14; C21 and T21) (C14, 17.62 ± 2.38 cells/0.5 mm^2; T14, 28.62 ± 2.7738 cells/0.5 mm^2; C21, 15.75 ± 2.37cells/0.5 mm^2; T21, 25.50 ± 3.92 cells/0.5 mm^2).

The obtained values also showed that hPTH 1-34 treatment increased the deposition of NFC (P <0.01) (C14, 5,564.10 ± 293.42 μm^2/620 μm of the root; T14, 11,884.30 ± 973.43 μm^2/620 μm of the root; C21, 6,421.84 ± 446.87 μm^2/620 μm of root; T21, 15,259.42 ± 1,879.78 μm^2/620 μm of root).

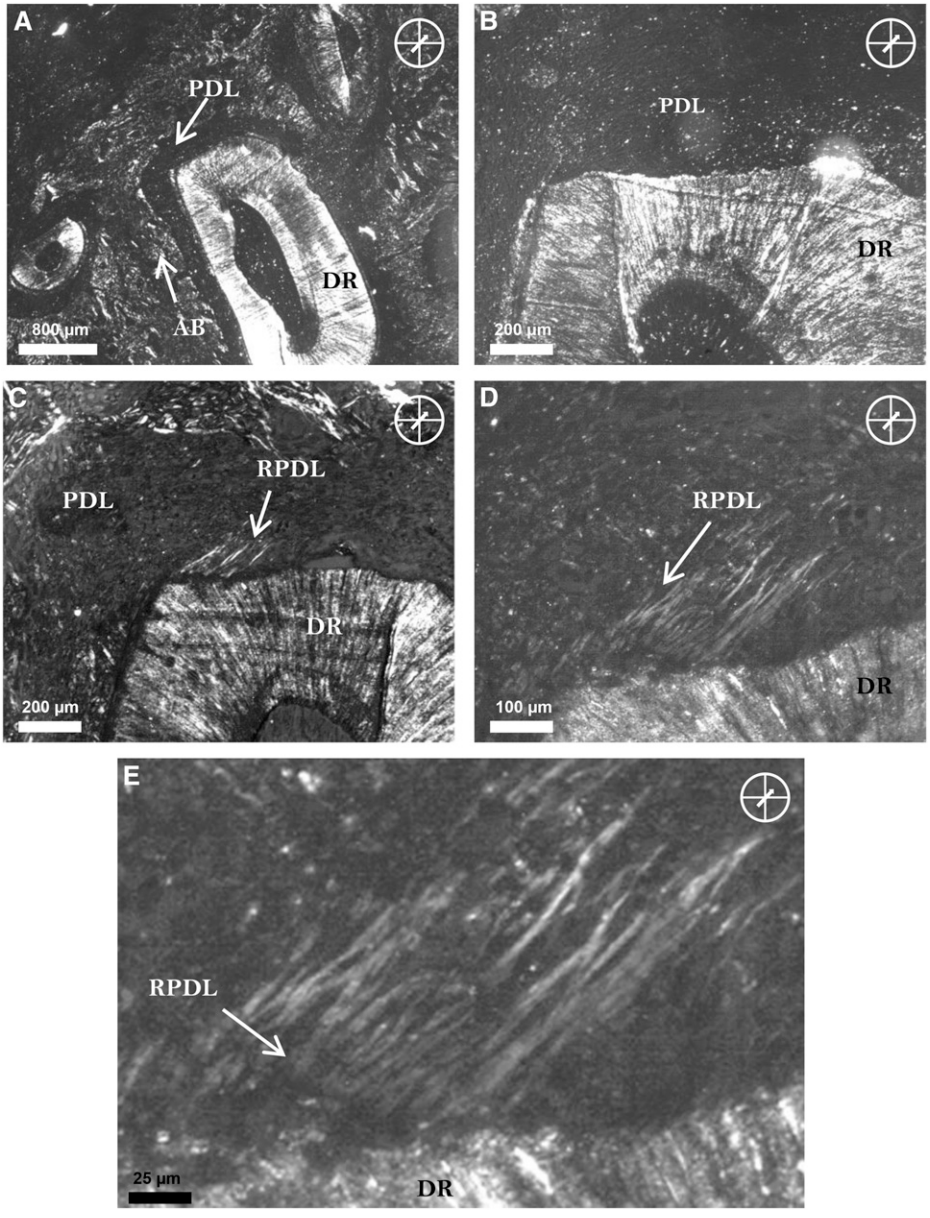

Figure 2.
Polarizing photomicrographs of the transverse tissue sections (4 μm) of the middle region of the periodontal defects. The analyzer and polarizer are denoted by crossed bars. The arrow at 45° with the polarizer and analyzer indicates the position of maximum birefringence. ***A)*** *Photomicrography at low magnification to localize the region analyzed by polarizing light microscopy.* ***B)*** *Photomicrography showing a representative histologic section from an animal of the C21 group.* ***C through E)*** *Photomicrographs showing representative histologic sections from an animal from the T21 group at low, medium, and high magnification, respectively. Note the reattachment of PDL in root for the T21 sample. AB = alveolar bone; DR = distal root from the mandibular right first molar; RPDL = reattachment of PDL.*

DISCUSSION

Regeneration comprises the reconstruction of lost or injured tissues in such a way that both the original structures and their function are completely restored. Procedures aimed at restoring lost periodontal tissues favor the creation of new attachment, including the formation of a new PDL with fibers that penetrate into NFC and alveolar bone. The main new

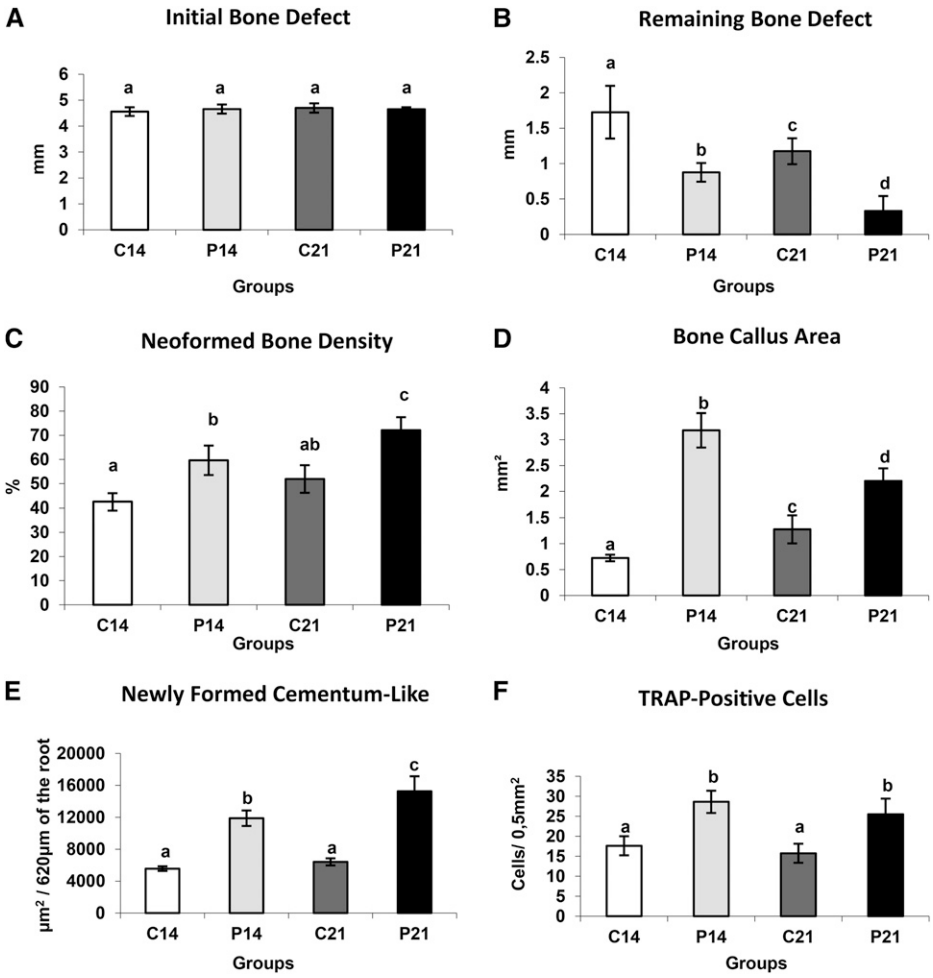

Figure 3.
*Values represent the mean ± SD of the initial extension of the bone defect **(A)**, RBDE **(B)**, NFBD **(C)**, TCA **(D)**, NFC **(E)**, and TRAP-positive cells in the bone TCA **(F)**. Different lowercase letters (a through d) represent significant intergroup differences in each graphic (as determined by ANOVA and the Tukey test for intergroup comparisons).*

finding of this study is that intermittent hPTH 1-34 administration increases NFC formation.

Although the number of animals used in this study is relatively small, no significant differences in the initial extension of the bone defect were evident among any of the groups. This initial analysis verified that the surgeries were performed similarly in all animals and thus justified subsequent comparative statistical analyses among groups with regard to the other experimental variables investigated. The experimental model used in the present study has been described previously,[13] and it has been considered by numerous groups to be a good tool for evaluating the effect of bioactive molecules.[16,18,19] This model has also proved informative in the investigation of systemic changes in the context of periodontal regeneration in an aseptic environment,[14,20,21] because it does not directly expose the relevant tissues to the oral cavity. The use of a model derived from the literature facilitates valid comparisons among results from different studies. For example, Huang et al.[16] used synthetic bone morphogenetic protein-6 in the same model of periodontal repair and reported a similar rate of NFC generation as that observed in the present study after administration of hPTH 1-34.

Birefringence is the anisotropy caused by the difference between the two refractive indices of a substance, and it occurs as a consequence of ordered polymerization of macromolecules, such as organic matrix of enamel and collagen fibers in the periodontium.[22,23] One of the key functional characteristics of the acellular cementum is the insertion of collagen fibers from the PDL, the so-called Sharpey fibers.[23] In the present study, PDL fiber attachment to the new cementum was verified via polarizing light microscopy, which allowed the observation of functionality of the restored periodontium in the defects created in animals from the T21 group.

Intermittent hPTH 1-34 administration promoted an increase in bone formation in the defective area for both of the time periods investigated (≈20% and ≈11% for 14 and 21 days, respectively), and an increase in the TCA was also evident in both of the hPTH 1-34-treated groups. In some animal models of bone repair, PTH (or analogs, peptides) administration can improve bone formation at the repair site. These increases are commonly associated with enhanced callus mechanical properties, and similar results have been replicated in rats,[24-27] mice,[28] and non-humans.[29,30] In addition, similar to the present study, previous studies have reported that increased callus formation caused by PTH treatment is accompanied by an increase in ON (TRAP-positive cells) in the area of bone repair.[31]

Skeletal repair in non-human models used to investigate the incorporation of intermittent hPTH 1-34 therapy have reported some efficacy with regard to

long-bone fracture healing, critical-sized bone defects, spinal arthrodesis, and distraction osteogenesis.[32] The efficacy of hPTH 1-34 therapy has also been tested in impaired bone healing models of aging, estrogen deficiency, inflammatory-erosive arthritis, and steroid use.[29,33-36]

Data obtained by the present authors from healthy and estrogen-deficient rats, with similarly aged animals used in the present study, suggest that intermittent PTH administration has the ability to protect against periodontitis-associated bone loss.[9,10] Similarly, a recent clinical study showed the benefit of intermittent PTH administration with regard to improving the outcome of periodontal surgery in patients suffering from severe chronic periodontitis. The authors of that study followed a clinically established protocol for the systemic administration of PTH with an approved dosing regimen.[12,37] Although these studies have yielded informative results, the precise mechanisms by which PTH acts on the periodontium are not yet completely understood.

Cementoblasts are mesenchymal cells with phenotypic features similar to osteoblastic cells in vitro, but in vivo, they perform different functions.[38] It has been reported that cementoblasts express PTH 1 receptor (PTH1R).[38] PTH is the main calcium metabolism regulator, and in vitro and in vivo evidence indicates that transient activation of the PTH1R activates multiple interconnected pathways leading to increased survival signaling.[39]

Currently available evidence indicates that the cyclic adenosine monophosphate–protein kinase A response to PTH1R activation is the main mechanism that drives the anabolic action of PTH in bone.[40-44] Activation of the PTH receptor in osteoblasts directly induces canonical Wnt (wingless-type MMTV integration site family) signaling, which is essential for osteoblast proliferation and differentiation. This pro-osteoblastic signal is further amplified by suppression of the Wnt antagonist sclerostin in osteocytes.[43,44] Conversely, PTH also reportedly modulates the expression of the Wnt antagonist Dickkopf-related protein 1.[43-45]

PDL cells respond to intermittent hPTH 1-34 administration in an osteoblast-like manner with changes in proliferation, apoptosis, and differentiation.[37,46,47] In addition, intermittent PTH administration leads to osteoblastic differentiation of human PDL cells and helps biomineralization when these cells are transplanted into immunocompromised mice. In an in vivo study of periodontal repair in the context of a rat model of tooth-root resorption, Lossdörfer et al.[48] reported that PDL cells could be potent regulators of periodontal repair via modification of the local microenvironment, and the authors suggested that the anabolic potential of intermittent PTH administration could contribute to reparative processes of the periodontium. Based on the findings of these previous studies, the authors of the present study hypothesize that the increase in the cementum-like deposition during periodontal repair seen in this study may have been related, at least in part, to the action of hPTH 1-34 in precursor cells from residual PDL, which are the main cells responsible for cementum regeneration.[49]

CONCLUSIONS

Together, the results from the present study indicate that intermittent hPTH 1-34 administration may accelerate periodontal healing in rats.

ACKNOWLEDGMENTS

This study was supported by Coordination for Enhancement of Higher Education Personnel, Brazil. The authors thank Mariana Ribeiro, Department of Morphology, Piracicaba Dental School, São Paulo, São Paulo, Brazil, for editing images. The authors report no conflicts of interest related to this study.

REFERENCES

1. Murakami S. Periodontal tissue regeneration by signaling molecule(s): What role does basic fibroblast growth factor (FGF-2) have in periodontal therapy? *Periodontol 2000* 2011;56:188-208.
2. Ramseier CA, Rasperini G, Batia S, Giannobile WV. Advanced reconstructive technologies for periodontal tissue repair. *Periodontol 2000* 2012;59:185-202.
3. Chen FM, Zhang J, Zhang M, An Y, Chen F, Wu ZF. A review on endogenous regenerative technology in periodontal regenerative medicine. *Biomaterials* 2010; 31:7892-7927.
4. 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.
5. Hanyu R, Wehbi VL, Hayata T, et al. Anabolic action of parathyroid hormone regulated by the β2-adrenergic receptor. *Proc Natl Acad Sci USA* 2012;109:7433-7438.
6. Komatsubara S, Mori S, Mashiba T, et al. Human parathyroid hormone (1-34) accelerates the fracture healing process of woven to lamellar bone replacement and new cortical shell formation in rat femora. *Bone* 2005;36:678-687.
7. Komrakova M, Stuermer EK, Werner C, et al. Effect of human parathyroid hormone hPTH (1-34) applied at different regimes on fracture healing and muscle in ovariectomized and healthy rats. *Bone* 2010;47:480-492.
8. Aspenberg P, Genant HK, Johansson T, et al. Teriparatide for acceleration of fracture repair in humans: A prospective, randomized, double-blind study of 102 postmenopausal women with distal radial fractures. *J Bone Miner Res* 2010;25:404-414.
9. Barros SP, Silva MA, Somerman MJ, Nociti FH Jr. Parathyroid hormone protects against periodontitis-associated bone loss. *J Dent Res* 2003;82:791-795.

10. Marques MR, da Silva MA, Manzi FR, Cesar-Neto JB, Nociti FH Jr., Barros SP. Effect of intermittent PTH administration in the periodontitis-associated bone loss in ovariectomized rats. *Arch Oral Biol* 2005;50: 421-429.
11. Marques MR, dos Santos MC, da Silva AF, Nociti FH Jr., Barros SP. Parathyroid hormone administration may modulate periodontal tissue levels of interleukin-6, matrix metalloproteinase-2 and matrix metalloproteinase-9 in experimental periodontitis. *J Periodontal Res* 2009;44:744-750.
12. Bashutski JD, Eber RM, Kinney JS, et al. Teriparatide and osseous regeneration in the oral cavity. *N Engl J Med* 2010;363:2396-2405.
13. King GN, King N, Cruchley AT, Wozney JM, Hughes FJ. Recombinant human bone morphogenetic protein-2 promotes wound healing in rat periodontal fenestration defects. *J Dent Res* 1997;76:1460-1470.
14. Benatti BB, César-Neto JB, Gonçalves PF, Sallum EA, Nociti FH Jr. Smoking affects the self-healing capacity of periodontal tissues. A histological study in the rat. *Eur J Oral Sci* 2005;113:400-403.
15. Guimarães GN, Cardoso GB, Naves LZ, Correr-Sobrinho L, Line SR, Marques MR. Short-term PTH administration increases dentine apposition and microhardness in mice. *Arch Oral Biol* 2012;57:1313-1319.
16. Huang KK, Shen C, Chiang CY, Hsieh YD, Fu E. Effects of bone morphogenetic protein-6 on periodontal wound healing in a fenestration defect of rats. *J Periodontal Res* 2005;40:1-10.
17. Feitosa DS, Marques MR, Casati MZ, Sallum EA, Nociti FH Jr., de Toledo S. The influence of thyroid hormones on periodontitis-related bone loss and tooth-supporting alveolar bone: A histological study in rats. *J Periodontal Res* 2009;44:472-478.
18. King GN, Hughes FJ. Bone morphogenetic protein-2 stimulates cell recruitment and cementogenesis during early wound healing. *J Clin Periodontol* 2001; 28:465-475.
19. Killeen AC, Rakes PA, Schmid MJ, et al. Impact of local and systemic alendronate on simvastatin-induced new bone around periodontal defects. *J Periodontol* 2012; 83:1463-1471.
20. Corrêa MG, Campos ML, Benatti BB, et al. The impact of cigarette smoke inhalation on the outcome of enamel matrix derivative treatment in rats: Histometric analysis. *J Periodontol* 2010;81:1820-1828.
21. Corrêa MG, Gomes Campos ML, Marques MR, Casati MZ, Nociti FH Jr., Sallum EA. Histometric analysis of the effect of enamel matrix derivative on the healing of periodontal defects in rats with diabetes. *J Periodontol* 2013;84:1309-1318.
22. do Espírito Santo AR, Frozoni MR, Ramos-Perez FM, Novaes PD, Line SR. Birefringence of the secretory-stage enamel organic extracellular matrix from rats submitted to successive injections of bisphosphonates. *Connect Tissue Res* 2010;51:208-215.
23. Foster BL. Methods for studying tooth root cementum by light microscopy. *Int J Oral Sci* 2012;4:119-128.
24. Nakajima A, Shimoji N, Shiomi K, et al. Mechanisms for the enhancement of fracture healing in rats treated with intermittent low-dose human parathyroid hormone (1-34). *J Bone Miner Res* 2002;17:2038-2047.
25. Alkhiary YM, Gerstenfeld LC, Krall E, et al. Enhancement of experimental fracture-healing by systemic administration of recombinant human parathyroid hormone (PTH 1-34). *J Bone Joint Surg Am* 2005;87: 731-741.
26. Warden SJ, Komatsu DE, Rydberg J, Bond JL, Hassett SM. Recombinant human parathyroid hormone (PTH 1-34) and low-intensity pulsed ultrasound have contrasting additive effects during fracture healing. *Bone* 2009;44:485-494.
27. Tägil M, McDonald MM, Morse A, et al. Intermittent PTH (1-34) does not increase union rates in open rat femoral fractures and exhibits attenuated anabolic effects compared to closed fractures. *Bone* 2010;46: 852-859.
28. Kakar S, Einhorn TA, Vora S, et al. Enhanced chondrogenesis and Wnt signaling in PTH-treated fractures. *J Bone Miner Res* 2007;22:1903-1912.
29. Manabe T, Mori S, Mashiba T, et al. Human parathyroid hormone (1-34) accelerates natural fracture healing process in the femoral osteotomy model of cynomolgus monkeys. *Bone* 2007;40:1475-1482.
30. Struillou X, Boutigny H, Soueidan A, Layrolle P. Experimental animal models in periodontology: A review. *Open Dent J* 2010;4:37-47.
31. Gardner MJ, van der Meulen MC, Carson J, et al. Role of parathyroid hormone in the mechanosensitivity of fracture healing. *J Orthop Res* 2007;25:1474-1480.
32. Aleksyniene R, Thomsen JS, Eckardt H, Bundgaard KG, Lind M, Hvid I. Parathyroid hormone PTH(1-34) increases the volume, mineral content, and mechanical properties of regenerated mineralizing tissue after distraction osteogenesis in rabbits. *Acta Orthop* 2009; 80:716-723.
33. Andreassen TT, Willick GE, Morley P, Whitfield JF. Treatment with parathyroid hormone hPTH(1-34), hPTH(1-31), and monocyclic hPTH(1-31) enhances fracture strength and callus amount after withdrawal fracture strength and callus mechanical quality continue to increase. *Calcif Tissue Int* 2004;74:351-356.
34. Redlich K, Görtz B, Hayer S, et al. Repair of local bone erosions and reversal of systemic bone loss upon therapy with anti-tumor necrosis factor in combination with osteoprotegerin or parathyroid hormone in tumor necrosis factor-mediated arthritis. *Am J Pathol* 2004; 164:543-555.
35. Takahata M, Awad HA, O'Keefe RJ, Bukata SV, Schwarz EM. Endogenous tissue engineering: PTH therapy for skeletal repair. *Cell Tissue Res* 2012;347: 545-552.
36. Ali MN, Kobayashi T, Tanaka M, Ohshima H, Ejiri S, Saito C. Effects of intermittent parathyroid hormone treatment on new bone formation during distraction osteogenesis in the rat mandible. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2012;114:e36-e42.
37. Wolf M, Lossdörfer S, Abuduwali N, et al. Effect of intermittent PTH(1-34) on human periodontal ligament cells transplanted into immunocompromised mice. *Tissue Eng Part A* 2012;18:1849-1856.
38. Berry JE, Ealba EL, Pettway GJ, et al. JunB as a downstream mediator of PTHrP actions in cementoblasts. *J Bone Miner Res* 2006;21:246-257.
39. Jilka RL. Molecular and cellular mechanisms of the anabolic effect of intermittent PTH. *Bone* 2007;40: 1434-1446.
40. Swarthout JT, D'Alonzo RC, Selvamurugan N, Partridge NC. Parathyroid hormone-dependent signaling pathways regulating genes in bone cells. *Gene* 2002;282: 1-17.

41. Silver J, Bushinsky D. Harnessing the parathyroids to create stronger bones. *Curr Opin Nephrol Hypertens* 2004;13:471-476.
42. Yang D, Guo J, Divieti P, Bringhurst FR. Parathyroid hormone activates PKC-delta and regulates osteoblastic differentiation via a PLC-independent pathway. *Bone* 2006;38:485-496.
43. Kousteni S, Bilezikian JP. The cell biology of parathyroid hormone in osteoblasts. *Curr Osteoporos Rep* 2008;6:72-76.
44. Kraenzlin ME, Meier C. Parathyroid hormone analogues in the treatment of osteoporosis. *Nat Rev Endocrinol* 2011;7:647-656.
45. Anastasilakis AD, Polyzos SA, Avramidis A, Toulis KA, Papatheodorou A, Terpos E. The effect of teriparatide on serum Dickkopf-1 levels in postmenopausal women with established osteoporosis. *Clin Endocrinol (Oxf)* 2010;72:752-757.
46. Lossdörfer S, Götz W, Jäger A. PTH(1-34) affects osteoprotegerin production in human PDL cells in vitro. *J Dent Res* 2005;84:634-638.
47. Lossdörfer S, Götz W, Rath-Deschner B, Jäger A. Parathyroid hormone(1-34) mediates proliferative and apoptotic signaling in human periodontal ligament cells in vitro via protein kinase C-dependent and protein kinase A-dependent pathways. *Cell Tissue Res* 2006;325:469-479.
48. Lossdörfer S, Yildiz F, Götz W, Kheralla Y, Jäger A. Anabolic effect of intermittent PTH(1-34) on the local microenvironment during the late phase of periodontal repair in a rat model of tooth root resorption. *Clin Oral Investig* 2010;14:89-98.
49. Ohta S, Yamada S, Matuzaka K, Inoue T. The behavior of stem cells and progenitor cells in the periodontal ligament during wound healing as observed using immunohistochemical methods. *J Periodontal Res* 2008;43:595-603.

Correspondence: Dr. Pedro Duarte Novaes, State University of Campinas, School of Dentistry at Piracicaba, Department of Morphology, Division of Histology, Av. Limeira 901, Caixa Postal 052, CEP 13414-903, Piracicaba, São Paulo, Brazil. E-mail: novaes@fop.unicamp.br.

Submitted March 4, 2013; accepted for publication June 19, 2013.