Effects of Laser and Ozone Therapies on Bone Healing in the Calvarial Defects

Hakki Oguz Kazancioglu, PhD, DDS, Seref Ezirganli, PhD, DDS,* and Mehmet Serif Aydin, DDS†*

Abstract: This study aims to analyze the effect of the low-level laser therapy (LLLT) and ozone therapy on the bone healing of critical size defect (CSD) in rat calvaria. A total of 30 Wistar male rats were used. A 5-mm-diameter trephine bur was used to create CSD on the right side of the parietal bone of each rat calvarium. Once the bone was excised, a synthetic biphasic calcium phosphate graft material was implanted to all the bone defect sites. The animals were randomly divided into 3 groups as follows: the control group (n = 10), which received no LLLT or ozone therapy; the LLLT group (n = 10), which received only LLLT (120 seconds, 3 times a week for 2 weeks); and the ozone therapy group (n = 10) (120 seconds, 3 times a week for 2 weeks). After 1 month, all the rats were killed, and the sections were examined to evaluate the presence of inflammatory infiltrate, connective tissue, and new bone formation areas. Histomorphometric analyses showed that in the LLLT and ozone groups, the new bone areas were significantly higher than in the control group ($P < 0.05$). In the LLLT group, higher new bone areas were found than in the ozone group ($P < 0.05$). This study demonstrated that both ozone and laser therapies had a positive effect on bone formation in rat calvarial defect, compared with the control group; however, ozone therapy was more effective than LLLT (808 nm; 0.1 W; 4 J/cm^2; 0.028 cm^2, continuous wave mode).

Key Words: Bone healing, low-level laser, ozone, calvarial defects, rat

(*J Craniofac Surg* 2013;24: 2141–2146)

The need to regenerate large bone defects has a significant component of clinical dental practice. Bone loss may be caused by trauma, congenital defects, bone atrophy, and tumor excision. In addition, the treatment of some fractures and the restoration of extensive bone defects may require the use of bone regeneration procedures to help enhance the possibility of a successful treatment.[1]

Bone grafting materials aim to facilitate the growth of new bone into severe alveolar resorption areas. Various types of graft materials have been described for alveolar bone augmentation procedures. Because of their osteogenic, osteoinductive, and osteoconductive properties, autogenous grafts still remain the gold-standard reconstructive materials for augmentation. However, because of the limitation of supply, additional surgical time, and the risk of morbidity, transplantation of autograft bone is not the optimal choice. This has led to the development of various alternative graft materials.[2]

Critical size defect (CSD) can be defined as the smallest size bone defect that will not completely heal over the natural lifetime of an animal.[1] Fibrous connective tissue fills the defect and aggregates on the margins of the defect; therefore, limited bone regeneration could occur. The rat calvarium defect model has been used for experiments in bone regeneration.[3,4]

The repair of bone defects has attracted the interest of researchers in several fields of health. Currently, bone healing stimulation has been achieved with the application of chemical stimuli such as biomaterials, and bone morphogenetic proteins as well as by the use of physical stimuli, such as ultrasound, electromagnetic fields and, more recently, low-level laser therapy (LLLT) and ozone therapy.[5,6]

Several studies have demonstrated that LLLT can biomodulate and accelerate new bone formation by inducing proliferation and differentiation of osteoblasts, and stimulating cell proliferation and vascularization in injured tissues.[7,8] In addition, LLLT may also provide collagen fiber organization, elevate adenosine triphosphate levels, increase collagen synthesis by fibroblasts, and activate the lymphatic system as well as the proliferation of epithelial cells and fibroblasts.[9–11] Although both in vitro and in vivo experiments have produced positive results, other studies show contradictory effects of LLLT on bone healing.[12]

Another physical stimuli method used for wound and bone healing is ozone therapy.[6,13] Ozone (3 atoms of oxygen instead of 2) is normally present as a gas. It can react with components of the vascular system (erythrocytes, leukocytes, platelets, and endothelial cells) and positively affect oxygen metabolism, cell energy, the immunomodular property, the antioxidant defence system, and microcirculation in tissues. Such effects resemble the biostimulatory property of LLLT.[13] Most of the published articles considering the use of ozone in dentistry have been in relation to its antimicrobial effects.[14,15] Adequate evidence is lacking for the application of ozone in oral and maxillofacial region.[13]

Based on the individual effects of LLLT and ozone therapy on osteoblastic activity and trabecular bone volume, the purpose of this study was to analyze histologically the effect of the association of these two therapies on bone healing of CSD in rat calvaria.

MATERIALS AND METHODS

Sample

The experimental protocol of this study was approved by the Institutional Review Board and Animal Use Committee of the Bezmialem Vakif University (protocol no. 2012/405). In total, thirty 3-month-old male rats (*Rattus norvegicus albinus*; Wistar) were used. At the beginning of the study, the body weight of the rats was between 300 and 330 g. The study was conducted in accordance with the accepted guidelines for the care and use of laboratory animals in

From the *Department of Oral and Maxillofacial Surgery, Faculty of Dentistry; and †Department of Histology, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey.

Received May 30, 2013.
Accepted for publication June 15, 2013.

Address correspondence and reprint requests to Hakki Oguz Kazancioglu, PhD, DDS, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Bezmialem Vakif University, 34093, Fatih, Istanbul, Turkey; E-mail: dt_oguz@yahoo.com

This work is supported by the Scientific Research Project Fund of Bezmialem Vakif University under the project number 12.2011/2.
The authors report no conflicts of interest.

Copyright © 2013 by Mutaz B. Habal, MD

ISSN: 1049-2275
DOI: 10.1097/SCS.0b013e3182a244ae

Copyright © 2013 Mutaz B. Habal, MD. Unauthorized reproduction of this article is prohibited.

research. The rats were kept in individual plastic cages in an experimental animal room (21°C, 55%–70% humidity, 1 atm pressure, with a 12-hour day/night cycle). The animals were fed a standard laboratory pellet diet, and drinking water was available ad libitum throughout the experiment.

Experimental Design

All surgical applications were conducted under sterile conditions. Animals were anesthetized by intramuscular injection of 3 mg/kg of xylazine hydrochloride (Rompuns; Bayer, Leverkusen, Germany) and 35 mg/kg of ketamine hydrochloride (10% Ketasol; Richter Pharma AG, Wels, Austria). Before each surgery, the dorsal part of the cranium was shaved and disinfected with povidone iodine solution. To allow reflection of a full-thickness flap, an approximately 20-mm-long incision was made through in the scalp along the sagittal suture and skin, musculature, and periosteum. After the calvarium was exposed, a 5-mm-diameter CSD was created with a trephine burr on the right side of the parietal bone without damaging the underlying dura mater (Fig. 1). Because the dura may play an important role in bone healing and regeneration, the bone is not completely penetrated by the trephine to avoid damage to the underlying tissues.[16] Instead, the bone is thinned considerably and elevated using blunt instruments to separate the bone. The released bone was removed and a synthetic biphasic calcium phosphate graft material (Osscream; Bredent Medical GmbH & Co KG, Senden, Germany) was implanted into the defects. The skin was then carefully sutured with resorbable 4/0 poliglactin 910 sutures (Vicryl, Ethicon Inc., Somerville, NJ). Animals were then randomly divided into 3 groups as follows:

- The control group (n = 10), receiving no LLLT or ozone therapy;
- The LLLT group (n = 10), receiving only LLLT (120 seconds, 3 times a week for 2 weeks); and
- The ozone therapy group (n = 10) (120 seconds, 3 times a week for 2 weeks).

Starting immediately after the operation, intramuscular injections of ceftriaxone were given to each animal to prevent postoperative infection (3 d, 25 mg/kg) and 4 mg/kg of the intramuscular analgesic carprofen (Rimadyl, Pfizer, New York, NY) was administered every 24 hours for 3 days. In all animals, healing progressed uneventfully and no postoperative complications were noticed. All surgical procedures were performed by the same operator (H.O.K.).

Ozone Therapy

Ozone therapy was performed using an ozone generator (Biozonix GMbH, Munich, Germany) with a sterile tissue probe (AL probe), attached to the hand-piece, hand-guided over the whole defect area like to clinical procedure. It was applied with 80% oxygen for 120 seconds per day, 3 days per week, for 2 weeks.

Laser Therapy

Animals in the LLLT group were treated by laser irradiation (energy density, 4 J/cm^2 per session; 0.028 cm^2 beam diameter). The light source was a diode laser (Fotona XD-2; Fotona, Ljubljana, Slovenia; 808 nm, 0.1 W, continuous wave mode). The wounds and 0.5 cm of their surrounding marginal zones were illuminated with a spot size of 1 cm^2. The laser never contacted the tissues in any session. The application distance was 0.5 to 1 cm, a distance that did not affect the spot size with the hand piece that was used. Irradiation was applied with for 120 seconds per day, 3 days per week, for 2 weeks.

Specimen Preparation

One month after the surgery, the animals were killed with an overdose of 200 mg/kg, iv, pentobarbital (Pentothal; Abbott Diagnostic Division, Abbott Park, IL). For the histopathologic analysis, the skin was dissected and the area of the original surgical defect were removed en bloc with the surrounding tissues from the animals' calvarium bone, rinsed in physiologic saline, fixed in 10% buffer formaldehyde for 24 hours, rinsed with water, and decalcified in 18% ethylediaminetetraacetic acid (EDTA) solution. Each specimen was divided longitudinally into 2 blocks in the sagittal direction and embedded in paraffin. Serial sections were cut longitudinally, beginning at the center of the surgical defect. Under a light microscope (Nikon Eclipse E 600; Nikon Instruments Inc, Tokyo, Japan), the sections were stained with hematoxylin and eosin for analysis.

Histologic and Histomorphometric Assessment

A single examiner (M.S.A.) who was also blinded to the identity of the samples performed the histologic analysis. The sections were evaluated for the presence of inflammatory infiltrate, connective tissue formation, and new bone formation. Using an automated image analysis system, computer-assisted histomorphometic measurements were carried out. A photomicroscope (Nikon Eclipse i5) coupled with a video camera on a light microscope (Nikon, DS-Fi1c) with an original magnification of ×40 was used for examining of the sections. NIS Elements version 4.0 image analysis systems (Nikon Instruments Inc) were used for calculating of the new regenerated bone area (mm^2).

Statistical Analysis

All statistical analyses were performed using a commercially available software program IBM SPSS 20.0 (SPSS Inc, Chicago, IL). The amounts of regenerated bone were calculated by mean values and SDs. For detection of any statistically significant difference

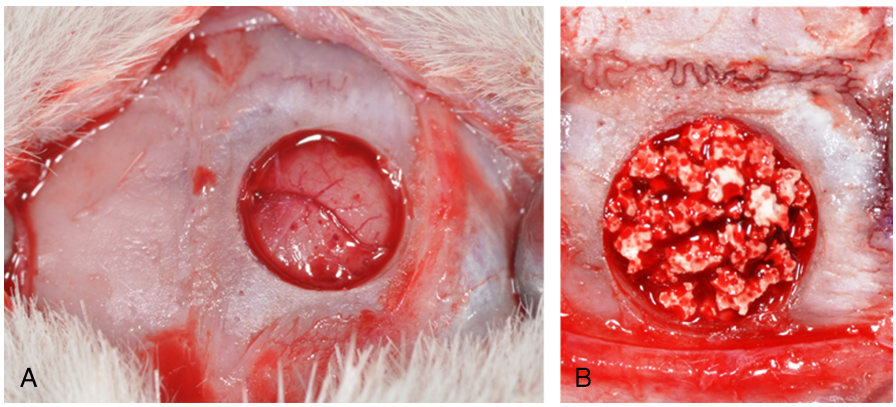

FIGURE 1. A, Critical-size defect (5 mm diameter) created on the parietal part of the rat calvarium. B, Graft material applied to the defect.

© 2013 Mutaz B. Habal, MD

Copyright © 2013 Mutaz B. Habal, MD. Unauthorized reproduction of this article is prohibited.

between groups, Kruskal-Wallis test with Dunn's post hoc multiple comparison test was used. P values less than 0.05 were considered statistically significant. Results are reported as means ± SD and medians for each group.

RESULTS

Animal Findings

All rats survived at the end of the study, and no postoperative complications were noticed such as inflammatory tissue responses, exposure of graft material, or allergic reaction.

Histologic Analysis

Damage to dura mater was not seen histopathologically in any of the specimens because of the creation of calvarial defects. None of the defects was completely filled with the new bone. No inflammatory process was noticed in all the specimens because no acute inflammatory cells were present. Almost all of the surgical defects were filled up with connective tissue with collagen fibers parallel to the wound surface and a moderate number of fibroblasts. Newly regenerated bone tissue surrounded by a small number of osteoblasts was restricted to areas close to the borders of the surgical defects (Fig. 2). In all groups, an increase was observed in the amount of regenerated bone and the osteoblastic activity (Fig. 3).

Control Group

Healing was characterized by the remaining bone grafts and thin loose fibrous connective tissue in which a large number of collagen fibers and fibroblasts were oriented parallel to the wound surface and some blood vessels in the defect areas (Fig. 4). The poorly mineralized new bone areas were beginning inside the bone defect margins minimally, after 30 days from the surgery.

Experimental Groups

Histologic results were found similarly. The center part of the bone defect was filled by fibrous connective tissue and remaining bone grafts in both the ozone and LLLT groups. A layer of newly formed bone was evident on the surface of some remaining bone grafts. A fibrous connective tissue surrounded the remaining bone grafts containing a large number of collagen fibers with a moderate amount of fibroblasts and numerous blood vessels (Fig. 4). The experimental groups had a higher amount of newly formed bone tissue than the control group.

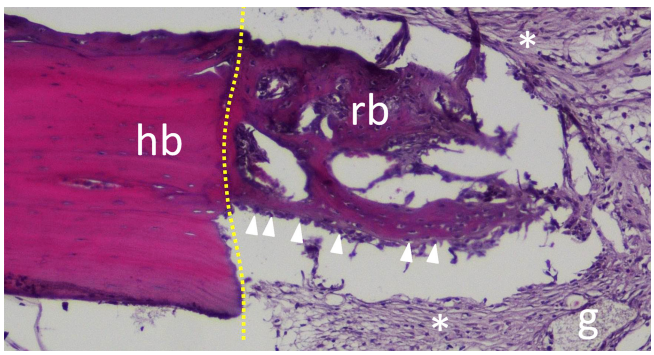

FIGURE 2. Regenerated bone area and host bone area boundaries are observed (yellow line). There are many osteoblasts on new bone tissue matrix (arrowheads). * indicates fibrocellular connective tissue (star): hb, host bone; rb, regenerated bone; g, graft material; –, the border of host bone tissue and regenerated tissues (hematoxylin-eosin staining, original magnification ×100).

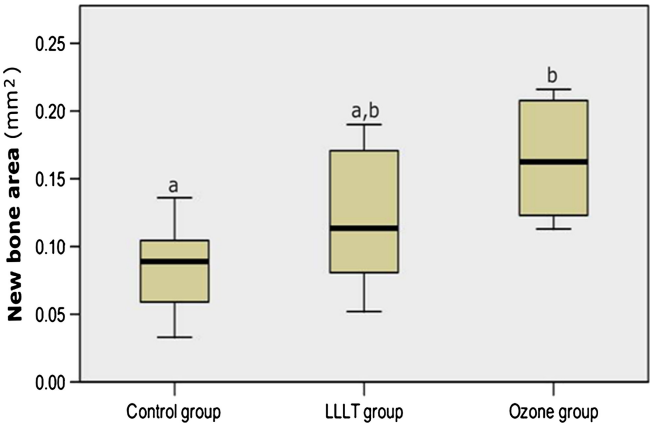

FIGURE 3. Graphs showing the regenerated bone analysis for each group; Dunn test $P < 0.05$: area (mm^2, Kruskal Wallis test $- \chi^2 = 9.613$, $P < 0.008$, control group vs ozone group).

In the experimental groups, the connective tissue presented a moderate number of fibroblasts and numerous collagen fibers. Newly formed bone surrounded by a small number of osteoblasts was restricted to areas close to the borders of the surgical defect.

Histomorphometric Analysis

The total new bone areas were significantly greater in the ozone group than in the control and LLLT groups ($P < 0.05$) (Fig. 4). In addition, the LLLT group had higher amount of total newly formed bone area than the control group, and it was not significant ($P > 0.05$). In the ozonated group, newly organized bone areas are covered with osteoblast cells and regenerated bone areas contain higher amount of osteocytes (Fig. 5).

DISCUSSION

The present study aimed to investigate and compare (from a histopathologic perspective) the effects of ozone therapy and LLLT on bone formation during the process of bone healing. In this study, in the CSD model was used to evaluate the efficacy of LLLT and ozone therapy on bone formation because it allows standardized production of defects that enable convenient analysis of the newly formed bone. In addition, observations can be focused on the healing process of the bone, preparation of tissue specimens is easy, parameters can be simply and accurately measured in each specimen, and spontaneous healing would not occur at the CSD model. Rats were chosen as the experimental animal because surgical procedures on the rat calvarial bone are relatively simple to perform because the lateral borders of the rat calvaria have no major nerves or blood vessels that cause extensive bleeding. In addition, the calvarial defect model has many similarities to the human maxillofacial region (similar to the mandible, anatomically the calvaria consists of 2 cortical plates with a region of intervening cancellous bone, and physiologically, the cortical bone in the calvaria resembles an atrophic mandible).[17–19]

In the present study, a 5-mm CSD was created in the rat calvaria because spontaneous bone healing does not occur in adult rats at this size.[6] This was confirmed by the lack of bone regeneration at the control defects in the current experiment.

Histologic evaluation showed that the creation of calvarial defects caused no damage to the underlying dura. Healing was characterized by thin fibrous connective tissue in which a large amount of collagen fibers and fibroblasts were oriented parallel to the wound

© 2013 Mutaz B. Habal, MD

Copyright © 2013 Mutaz B. Habal, MD. Unauthorized reproduction of this article is prohibited.

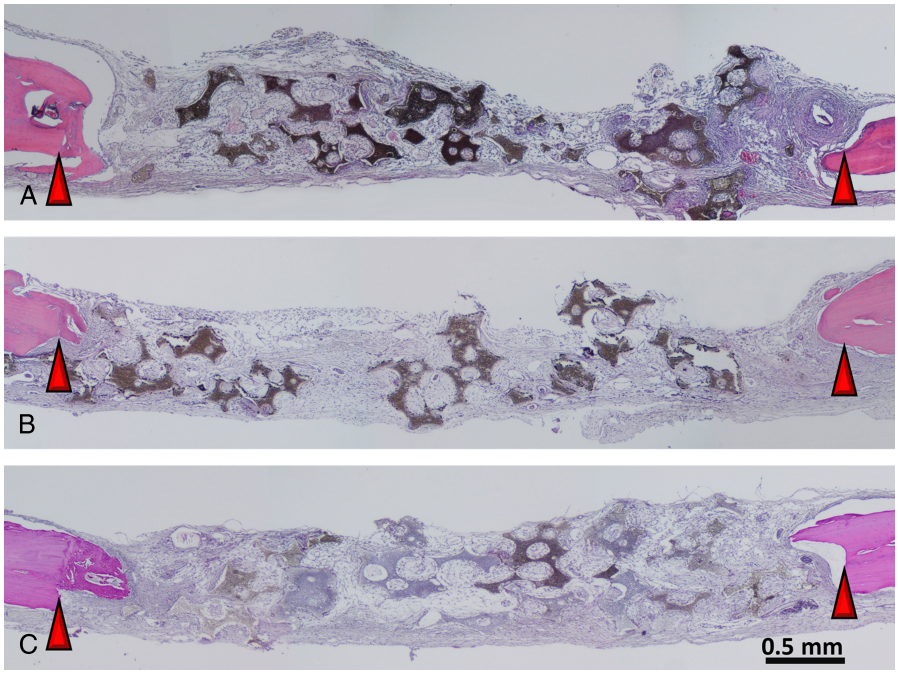

FIGURE 4. Panoramic views of the defect areas showing regenerated bone (red arrowheads) in all groups. A, Control group. B, LLLT group. C, Ozone therapy group (hematoxylin-eosin staining, original magnification ×40) with 0.5 mm bar.

surface in the control group. In addition, the calvarial bone defects were filled by some blood vessels.

For enhancing bone healing, several treatment modalities such as bisphosphonates, LLLT, ozone, and hyperbaric oxygen therapy have been researched.[5,6] The use of laser for the biostimulation of bone regeneration has been on the rise in medicine, and many researchers report positive results regarding the healing of bone defects with LLLT application.[11,14] Possible mechanisms of LLLT on tissue include stimulation of the absorption of ascorbic acid by cells, increase of mitochondrial adenosine triphosphate production, stabilization of the cell membrane, stimulation of lymphocytes, mast cell activation, and proliferation of several types of cells, thus promoting antiinflammatory effects. In addition, it helps to improve local circulation, cell proliferation, and collagen synthesis.[20]

Several studies have reported that a laser contributes to the acceleration of the bone healing process in bone defects.[11,14,21]

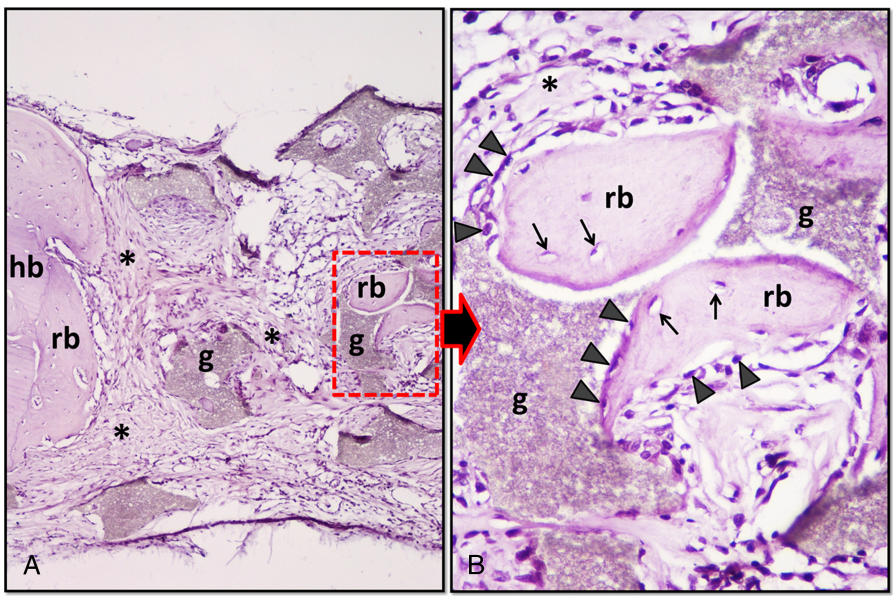

FIGURE 5. A, Minor regenerated bone areas with host bone area, connective tissue, and graft materials were observed in the ozonated group (hematoxylin-eosin staining, original magnification ×100). B, Regenerated bone areas, covered with osteoblast cells, within the graft materials are shown, and these bone areas contain several osteocytes (hematoxylin-eosin staining, original magnification ×400). Arrows indicates osteocytes; arrowheads, osteoblasts; g, graft material; hb, host bone area; rb, regenerated bone area; *, fibrocellular connective tissue.

© 2013 Mutaz B. Habal, MD

Copyright © 2013 Mutaz B. Habal, MD. Unauthorized reproduction of this article is prohibited.

Garcia et al[21] found that 660-nm diode laser (24 J, 0.4285 W/cm^2) was effective for stimulating bone formation in CSD in the calvaria of osteoporotic rats. In other studies such as that by Pretel et al[22] (As-Ga-Al, 830 nm, continuous, 40 mW, 57.6 J/cm^2) and Fávaro-Pípi et al[23] (Ga-As-Al, 830 nm, continuous, 50 J/cm^2), similar results were reported about the acceleration of the bone healing process by laser irradiation. In addition, the laser seems to accelerate the fracture repair process and cause an increase on the callus volume and bone mineral density. Renno et al[24] reported that 830-nm laser irradiation resulted in a significant increase in the proliferation of osteoblasts and another study by Pinheiro et al[25] showed that the laser (830 nm, 40 mW, 4.8 J/cm^2) was able to increase the amount of mineralized bone tissue in induced fractures in rats' femurs. On the contrary, Silva et al[26] aimed to histologically evaluate the response of epithelial, bone, and connective tissues subjected to LLLT (660 and 780 nm, 7.5 energy densities, 15 J/cm^2), and they found that epithelial and connective tissues responded to laser irradiation with constant cell renewal, while there was an acceleration of regenerative bone formation within normal limits in bone tissues.

The dose (wavelength, power, frequency, fluency or dose, and energy parameters) applied during laser treatment is an important benefit of LLLT for obtaining good results. However, a precisely determined dose has not been determined for each indication. In the literature, biostimulation has been reported with doses between 0.001 and 10 J/cm^2 as a therapeutic window.[27] Although an applied dose is within the therapeutic window range, it might be too low or too high for the desired effect.[28] The laser parameter settings used in this study were chosen based on the positive results obtained in a previous study on wound healing after wisdom tooth extraction.[13]

The evidence for the clinical application of ozone therapy in dentistry is not extensive. Ozone can react with composition of the vascular system and positively affect oxygen metabolism, cell energy, the immunomodulator property, antioxidant defense system, and microcirculation.[13] Based on these findings, some authors suggest that ozone therapy may be useful in the management of bone necrosis or in extractive sites during and after oral surgery in patients treated with bisphosphonates because it might stimulate cell proliferation and soft tissue healing.[29] Ozdemir et al[6] analyzed the effect of ozone therapy in combination with autogenous bone grafts on bone healing in CSD in rat calvaria. The animals were divided into 3 groups: the autogenous bone graft group, the autogenous bone graft with ozone therapy group (80%, 30 seconds, 3 days for 2 weeks), and the control group. They reported that ozone therapy increased bone formation in autogenous bone grafts in the rat calvarial defect model. Application of gaseous ozone has also been shown effective in facilitating oral wound healing after high-dose radiotherapy.[30] Petrucci et al[31] reported that application of 15 days ozone therapy was beneficial on the treatment of bisphosphonate-related osteonecrosis of the jaws. Agrillo et al[29] performed 20 tooth extraction from 15 patients with avascular bisphosphonate-related jaw osteonecrosis and they reported that ozone was effective when used 7 days before and after the tooth extraction. In contrast, Matsumura et al[32] reported that ozone does not have a major impact on stimulation of gingival cells for osteoblastic activity in the regeneration of the periodontium around implants. In this study, it was found that ozone therapy has a positive effect on bone healing. As a possible explanation of this, the ozone has excellent bactericidal and antioxidant properties; and as a result, it improves wound healing, modulate immune system, and act as an antibacterial agent.

The conclusion of the present study is that both ozone and laser therapies increase bone formation vs the control group in the rat calvarial defect model. However, the ozone therapy was more effective than the LLLT (808 nm; 0.1 W; 4 J/cm^2; 0.028 cm^2, continuous wave mode) on bone healing. Our data suggest that both ozone and laser therapies provide additional new insights into therapeutic strategies in improving bone regeneration in dentistry, but further experimental and clinical evaluations are needed.

ACKNOWLEDGMENTS

The authors thank S. Delacroix for improving the English of this article and Dr. Omer Uysal for his expertise in conducting the statistical analysis.

REFERENCES

1. Spicer PP, Kretlow JD, Young S, et al. evaluation of bone regeneration using the rat critical size calvarial defect. *Nat Protoc* 2012;7:1918–1929
2. Jensen SS, Broggini N, Hjørting-Hansen E, et al. Bone healing and graft resorption of autograft, anorganic bovine bone and beta-tricalcium phosphate. A histologic and histomorphometric study in the mandibles of minipigs. *Clin Oral Implants Res* 2006;17:237–243
3. Linde A, Thorén C, Dahlin C, et al. Creation of new bone by an osteopromotive membrane technique: an experimental study in rats. *J Oral Maxillofac Surg* 1993;51:892–897
4. Zambuzzi WF, Coelho PG, Alves GG, et al. Intracellular signal transduction as a factor in the development of "smart" biomaterials for bone tissue engineering. *Biotechnol Bioeng* 2011;108:1246–1250
5. Nagata MJ, Santinoni CS, Pola NM, et al. Bone marrow aspirate combined with low-level laser therapy: a new therapeutic approach to enhance bone healing. *J Photochem Photobiol B* 2013;5:6–14
6. Ozdemir H, Toker H, Balcı H, et al. Effect of ozone therapy on autogenous bone graft healing in calvarial defects: a histologic and histometric study in rats. *J Periodontal Res* [published online ahead of print March 3, 2013] doi: 10.1111/jre.12060
7. Dortbudak O, Haas R, Mallath-Pokorny G. Biostimulation of bone marrow cells with a diode soft laser. *Clin Oral Implants Res* 2000;11:540–545
8. Garavello-Freitas I, Baranauskas V, Joazeiro PP, et al. Low-power laser irradiation improves histomorphometrical parameters and bone matrix organization during tibia wound healing in rats. *J Photochem Photobiol B* 2003;70:81–89
9. Karu T, Pyatibrat L, Kalendo G. Irradiation with He-Ne laser increases ATP level in cells cultivated in vitro. *J Photochem Photobiol* 1995;27:219–223
10. Alghamdi KM, Kumar A, Moussa NA. Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells. *Lasers Med Sci* 2011;27:237–249
11. García-Morales JM, Tortamano-Neto P, Todescan FF, et al. Stability of dental implants after irradiation with an 830-nm low-level laser: a double-blind randomized clinical study. *Lasers Med Sci* 2011;27:703–711
12. Jakse N, Payer M, Tangl S, et al. Influence of low-level laser treatment on bone regeneration and osseointegration of dental implants following sinus augmentation. An experimental study on sheep. *Clin Oral Implan Res* 2007;18:517–524
13. Kazancioglu HO, Ezirganli S, Demirtas N. Comparison of the influence of ozone and laser therapies on pain, swelling, and trismus following impacted third-molar surgery. *Lasers Med Sci* [published online ahead of print March 14, 2013] doi:10.1007/s10103-013-1300-y
14. Polydorou O, Halili A, Wittmer A, et al. The antibacterial effect of gas ozone after 2 months of in vitro evaluation. *Clin Oral Investig* 2012;16:545–550
15. Baysan A, Lynch E. Effect of ozone on the oral microbiota and clinical severity of primary root caries. *Am J Dent* 2004;17:56–60
16. Hobar PC, Masson JA, Wilson R, et al. The importance of the dura in craniofacial surgery. *Plast Reconstr Surg* 1996;98:217–225
17. Frame JW. A convenient animal model for testing bone substitute materials. *J Oral Surg* 1980;38:176–180
18. Schmitz JP, Hollinger JO. The critical size defect as an experimental model for craniomandibulofacial nonunions. *Clin Orthop Relat Res* 1986;205:299–308

© 2013 Mutaz B. Habal, MD

Copyright © 2013 Mutaz B. Habal, MD. Unauthorized reproduction of this article is prohibited.

19. Choi JY, Jung UW, Kim CS, et al. The effects of newly formed synthetic peptide on bone regeneration in rat calvarial defects. *J Periodontal Implant Sci* 2010;40:11–18
20. Conlan MJ, Rapley JW, Cobb CM. Biostimulation of wound healing by low-energy laser irradiation. A review. *J Clin Periodontol* 1996;23:492–496
21. Garcia VG, da Conceição JM, Fernandes LA, et al. Effects of LLLT in combination with bisphosphonate on bone healing in critical size defects: a histological and histometric study in rat calvaria. *Lasers Med Sci* 2013;28:407–414
22. Pretel H, Lizarelli RF, Ramalho LT. Effect of low-level laser therapy on bone repair: histological study in rats. *Lasers Surg Med* 2007;39:788–796
23. Fávaro-Pípi E, Feitosa SM, Ribeiro DA, et al. Comparative study of the effects of low-intensity pulsed ultrasound and low-level laser therapy on bone defects in tibias of rats. *Lasers Med Sci* 2010;25:727–732
24. Renno ACM, McDonnell PA, Parizotto NA, et al. The effects of laser irradiation on osteoblast and osteosarcoma cell proliferation and differentiation in vitro. *Photomed Laser Surg* 2007;25:275–280
25. Pinheiro ALB, Oliveira MG, Martins PPM, et al. Biomodulatory effects of LLLT on bone regeneration. *Laser Therapy* 2001;13:73–79
26. Silva EM, Gomes SP, Ulbrich LM, et al. Avaliação histológica da laserterapia de baixa intensidade na cicatrização de tecidos epitelial, conjuntivo e ósseo: estudo experimental em ratos. *Rev Sul-Bras Odontol* 2007;4:29–35
27. Ishii J, Fujita K, Komori T. Laser surgery as a treatment for oral leukoplakia. *Oral Oncol* 2003;39:759–769
28. Mester E, Spiry T, Szende B, et al. Effect of laser rays on wound healing. *Am J Surg* 1971;122:532–535
29. Agrillo A, Ungari C, Filiaci F, et al. Ozone therapy in the treatment of avascular bisphosphonate-related jaw osteonecrosis. *J Craniofac Surg* 2007;18:1071–1075
30. Saini R. Ozone therapy in dentistry: A strategic review. *J Nat Sci Biol Med* 2011;2:151–153
31. Petrucci MT, Gallucci C, Agrillo A, et al. Role of ozone therapy in the treatment of osteonecrosis of the jaws in multiple myeloma patients. *Haematologica* 2007;92:1289–1290
32. Matsumura K, Hyon SH, Nakajima N, et al. Effects on gingival cells of hydroxyapatite immobilized on poly (ethylene-co-vinyl alcohol). *J Biomed Mater Res* 2007;82:288–295

© 2013 Mutaz B. Habal, MD

Copyright © 2013 Mutaz B. Habal, MD. Unauthorized reproduction of this article is prohibited.