Photomedicine and Laser Surgery
Volume 25, Number 3, 2007

Pp. 197–204
DOI: 10.1089/pho.2007.2002

Effect of Laser Therapy on Bone Tissue Submitted to Radiotherapy: Experimental Study in Rats

SAMANTHA SEARA DA CUNHA, D.D.S., Ph.D.,[1] VIVIANE SARMENTO, D.D.S., Ph.D.,[1] LUCIANA MARIA PEDREIRA RAMALHO, D.D.S., Ph.D.,[1] DARCY DE ALMEIDA, D.D.S., Ph.D.,[1] ELAINE BAUER VEECK, D.D.S., Ph.D.,[2] NILZA PEREIRA DA COSTA, D.D.S., Ph.D.,[2] ALESSANDRA MATTOS, D.D.S., Ph.D.,[1] APARECIDA MARIA MARQUES, D.D.S., Ph.D.,[1] MARLENY GERBI, D.D.S., Ph.D.,[1] and ANDRÉ CARLOS FREITAS, D.D.S., M.S., Ph.D.[1]

ABSTRACT

Objective: **The aim of this study was to investigate the effect of laser therapy ($\lambda = 780$ nm) on bone tissue submitted to ionizing radiation.** ***Background Data:*** **The biostimulation effect of laser in normal bone tissue has already been demonstrated successfully; however its effect on bone tissue submitted to radiotherapy has not yet been studied.** ***Methods:*** **Twenty-two *Wistar* rats were randomly divided into four groups: group I, control ($n = 4$), submitted only to radiotherapy; group II, laser starting 1 day prior to radiotherapy ($n = 6$); group III, laser started immediately after radiotherapy ($n = 6$); group IV, laser 4 weeks after radiotherapy ($n = 6$). The source of ionizing radiation used was Cobalt 60, which was applied in a single dose of 3000 cGy on the femur. The laser groups received seven applications with a 48-h interval in four points per session of DE = 4 J/cm^2, P = 40 mW, t = 100 sec, and beam diameter of 0.04 cm^2. All animals were killed 6 weeks after radiotherapy.** ***Results:*** **Clinical examination revealed cutaneous erosions on experimental groups (II, III, and IV) starting at the 6th week after radiotherapy. The radiographic findings showed higher bone density in groups II and IV ($p < 0.05$) compared to the control group. The results further showed an increase of bone marrow cells, and number of osteocytes and Haversian canals in experimental groups II and IV ($p < 0.05$). It was also found an increase of osteoblastic activity, in groups II, III, and IV ($p < 0.05$).** ***Conclusion:*** **Laser therapy on bone tissue in rats presented a positive biostimulative effect, especially when applied before or 4 weeks after radiotherapy. However, the use of laser in the parameters above should be used with caution due to epithelial erosions.**

INTRODUCTION

High doses of radiation are commonly used in the treatment of malignant tumors. However, side effects occur due to the fact that ionizing radiation cannot distinguish tumor cells from healthy ones.[1,2] As a consequence, the destruction of healthy tissue limits the broad use of radiotherapy.

Like other tissues, the bone is subjected to the effects of radiation, complicating its regenerative capacity.[2,3] One alteration is a disturbance of osteoblastic and osteoclastic activity, in favor of a destructive process. It can also be observed a reduction in the number of osteocytes and osteoblasts, leading to a decline in collagen synthesis and alkaline phosphatase activity, causing the bone matrix formation process to stop, blocking the mineralization progress and leading to spontaneous fractures.[4,5] The endothelial cells are also affected, and vascular fibrosis results in a reduction of vascularization, affecting the vitality of the bone and medullar cells, making the area susceptible to infection or necrosis, even after a small trauma.[3,5] For this reason, dental extractions are contraindicated up to 1 year after radiotherapy.[6]

Bone regeneration is of huge interest for dentistry, due to the high number of surgeries in this tissue. In order to support the process of bone regeneration, laser therapy has been studied as

[1]Federal University of Bahia, Salvador, Bahia, Brazil.
[2]Catholic University of Rio Grande do Sul-Porto Alegre, Rio Grande do Sul, Brazil.

Table 1. Distribution of Groups

Groups	*Animals*	*Description*
I	4	Control (radiotherapy)
II	6	Radiotherapy + laser starting 1 day prior to ionizing radiation
III	6	Radiotherapy + laser starting the same day of ionizing radiation
IV	6	Radiotherapy + laser starting 4 weeks after ionizing radiation

an alternative to normal treatment. It is believed that the laser can act on biomodulation of non-differentiated mesenquimal cells, transforming them into osteoblasts and osteocytes.[7]

Laser Therapy is based on tissue biostimulation by monochromatic light. Over the past few decades, laser light has been used and tested in treatment of lesions in hard and soft tissue. Scientific studies since 1971 have shown a positive effect of laser light on soft tissues,[8] regeneration of injured nerves,[9,10] an increase of capillary formation by a liberation of growth factor hormones,[11] stimulation of cellular DNA and RNA,[12–14] and an increase of cellular growth and protein synthesis. The growth factor β_1 (TGF-β_1) is directly connected to regulation of bone remodeling and healing process. TGF-β_1 stimulates the proliferation of precursor cells of osteoblastic lineage and collagen formation.[15]

The aim of the present study was to investigate the effect of laser therapy ($\lambda = 780$ nm) on bone tissue submitted to ionizing radiation in three different moments: on 1 day before radiotherapy, on the same day as radiotherapy, and at 4 weeks after radiotherapy.

METHODS

Animals

Twenty-two young adult healthy male *Wistar albinus* rats (weighing 220–270 g) were kept individually under natural conditions of light, humidity, and temperature (22°C) at the Animal Experimentation Laboratory, UNIME University, Bahia, Brazil. The animals were fed with laboratory diet and water *ad libitum*. The rats were selected at this age due to their rapid increase of skeletal size, providing a favorable model for studying normal bone healing. The rats were randomly divided into four groups (Table 1): group I, control ($n = 4$), submitted only to radiotherapy; group II, laser treatment starting 1 day prior to radiotherapy ($n = 6$); group III, laser treatment starting immediately after radiotherapy ($n = 6$); and group IV, laser treatment starting 4 weeks after radiotherapy ($n = 6$).

Ionizing irradiation procedure

The rats were anesthetized by intraperitoneal administration of thiopental sodium (0.2 mL/100 g). The animals were immobilized in an acrylic device manufactured especially for this procedure. Irradiation was performed with Co-60 source (*Theratron 780*®) delivering a total dose of 3000 cGy in an area of 2×2 cm on the femur. The rest of the body was protected by lead blocks positioned on top of the Co-60 device (Fig. 1).

Laser protocol

The animals of group II, III, and IV were submitted to seven sessions of laser therapy ($\lambda = 780$ nm, 40 mW, $\phi \sim 0.04$ cm^2, t = 100 sec, continuous wave [CW]; Twin Laser, MM Optics, São Carlos, Brazil), at 48-h intervals. A dose of 4 J/cm^2 was applied to four points (direct contact) around the ionized irradiated area. The laser application points were 1 cm^2 apart from each other (Fig. 2).

The body weight of each animal was recorded before radiotherapy and on the day it was killed. All animals were killed 6 weeks after ionizing radiation with anesthetic overdose, and their irradiated legs were completely removed and kept on 4% buffered paraformaldeyde solution.

Radiographic procedure

The specimens were placed directly over an image plate for cephalometric radiograph of *DenOptix*® digital system (300 dpi, pixel of 85 μm). An aluminum step wedge (five steps, 1-cm increment) was added to the whole. The radiographic device

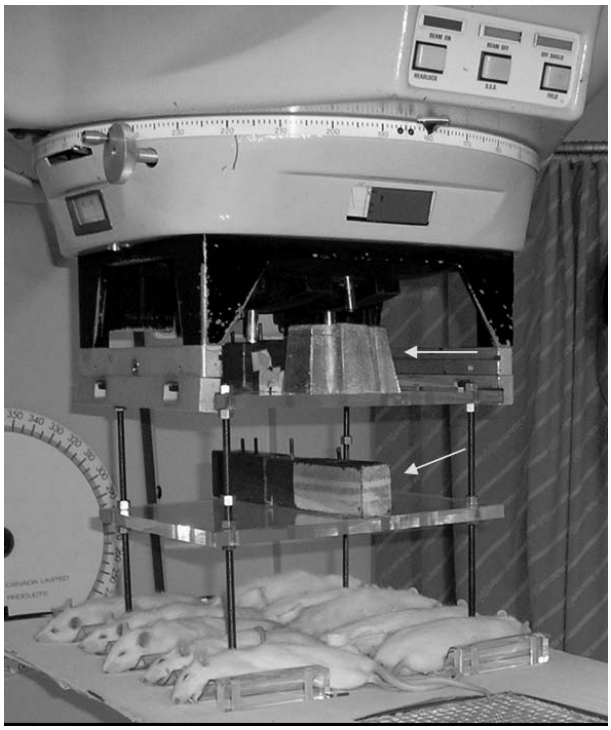

FIG. 1. Organization of animals during radiotherapy session; arrow points to lead blocks.

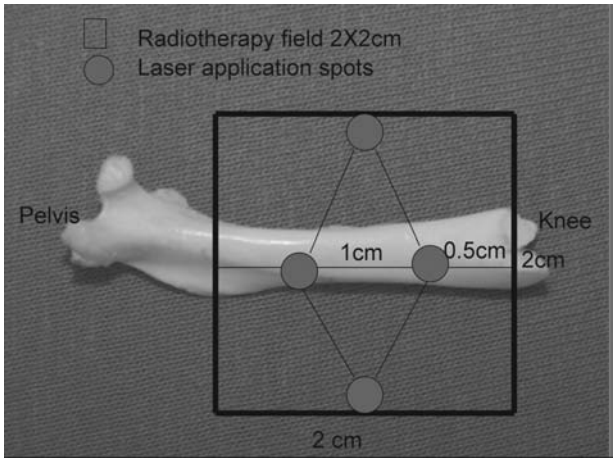

FIG. 2. Laser application spots and radiotherapy field.

(70 kV and 7 mA) was programmed with exposure time of 0.06 sec and focal distance of 1.20 cm, with beam perpendicular to the object. After radiographic exposure, the plate was taken to the reading unit of *DenOptix*® digital system, where the digital images were saved in bmp format. The images were opened in *Photoshop*® (Adobe Systems Inc., Mountain View, CA), and the brightness was corrected by means of the aluminum step wedge. Then, the images were opened in *Image Tool*® (University of Texas Health Science Center, San Antonio, TX), and the gray level average of the irradiated area was recorded.

Histological procedure

After the radiographic procedure, the legs were incised and the soft tissue totally removed. The femur was disarticulated, decalcified with 10% nitric acid, and routinely stained with hematoxylin and eosin (HE) and Picrosirius.

The descriptive and semiquantitative analysis included the presence of collagen fibers, degree of osteoblastic activity in periosteum, degree of bone resorption in marrow, and degree of fat cells in marrow. The parameters used for degree evaluation were numerical values: 1 (discrete presence), 2 (moderate), 3 (intense).

Moreover, by means of a light microscope, 10 fields were randomly chosen along the bone cortical, at magnification of 20×. These fields were photographed with the *Vidcap*® digital system and submitted to a quantitative analysis of the number of osteocytes and Harversian canals with the computer program *Image Tool*®.

Statistical analysis

The data were tabulated and analyzed through the non-parametric tests of Kruskal-Wallis, chi-square, Mann-Whitney and parametric test, Student *t*-test (significance level of 5% in all analyses).

RESULTS

General inspection

All animals remained healthy during the experiment, except one that died right after radiotherapy. The alopecia was noted from the first week in all the animals, being more obvious in the experimental groups II, III, and IV. Clinical exam revealed slight cutaneous erosions on all animals of experimental groups (II, III, and IV) that started at the 6th week after radiotherapy. In two animals (one of group II, and one of group IV), these lesions were severe (Fig. 3). The erosions were situated in the internal face of the leg and not in the external surface, which was nearer to the light source of the radiation. In the control, receiving only radiotherapy, such erosions could not be observed.

Body weight

The animals of group I (control) presented a body weight gain of 30.5%. The rats of experimental groups gained more weight. Group II presented the higher percentage gain (59.1%); group III gained 52.6% and group IV, 58.6% (Table 2).

When the body weight was compared between day 0 and week 6 in the group control, an increase of body weight was noted, but it was statistically nonsignificant ($p = 0.026$, test Mann-Whitney). In the experimental groups, body weight gain was more pronounced in groups II, III, and IV, and the p value was less than 0.0001 (non-paired t-test).

Radiographic and histological results

The radiographic findings revealed that group I (highest) presented the least amount of bone tissue. The higher bone densities were observed in groups II and IV ($p < 0.05$, Student t-test) compared to control group. This did not occur to group III ($p > 0.05$). These results are represented in Figure 4.

In histological evaluation, by means of Kruskal-Wallis test, the results showed an increase of number of osteocytes and Haversian canals in laser groups II and IV ($p < 0.05$) compared to control group; however, in group III, the difference was nonsignificant ($p > 0.05$; Figs. 5 and 6).

The presence of collagen fibers, degree of osteoblastic activity in periosteum, degree of bone resorption in marrow, and degree of fat cells in marrow cells were evaluated with nonparametric chi-square test. An increase of osteoblastic activity was observed in the experimental groups II, III, and IV

FIG. 3. Severe cutaneous erosion.

Table 2. Body Weight Gain on Day 0 and Week 6

	Body weight (g)				
	Day 0		*Week 6*		
	Mean	*SD*	*Mean*	*SD*	*% of gain*
Control group	256	6.13	334	21.7	30.5
Group II	225	10.9	358	26.48	59.1
Group III	234	8.52	357	14.88	52.6
Group IV	244	10.4	387	24.16	58.6

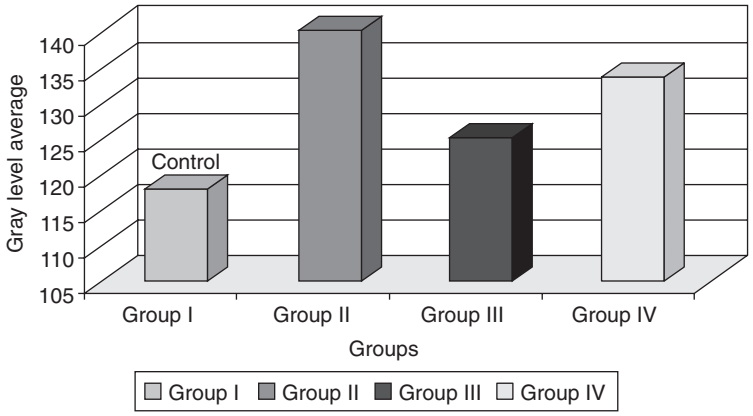

FIG. 4. Average of gray level.

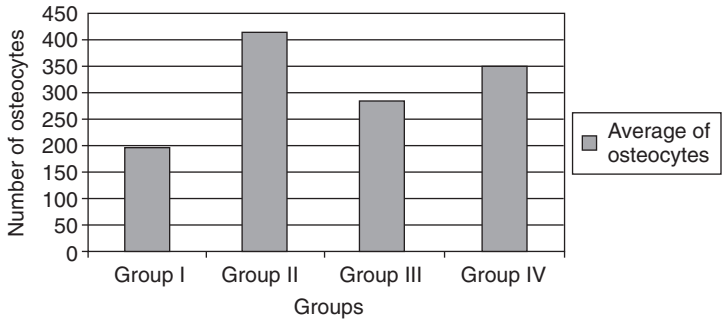

FIG. 5. Average of osteocytes, in 10 fields.

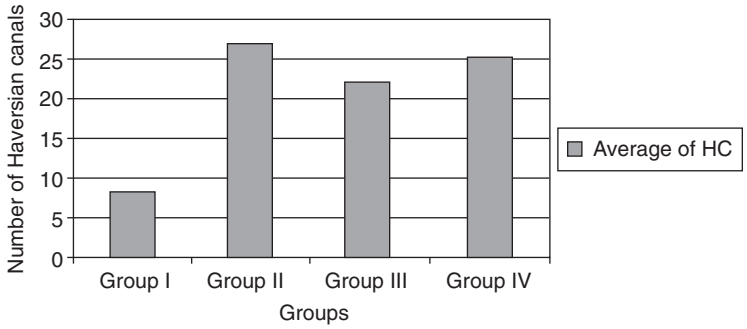

FIG. 6. Average of Haversian canals, in 10 fields.

TABLE 3. HISTOLOGICAL FINDINGS ACCORDING TO THE GROUP STUDIED

Groups	*Degree of fat cells in marrow (1, 2, or 3)*	*Presence of collagen fibers (yes/no)*	*Bone resportion in marrow (1, 2, or 3)*	*Degree of osteoblastic activity in periosteum (1, 2, or 3)*
Control group				
Rat 1	3	Y	2	1
Rat 2	2	N	2	1
Rat 3	3	N	2	1
Rat 4	3	Y	3	1
Group II				
Rat 1	1	Y	2	3
Rat 2	2	Y	3	3
Rat 3	2	Y	3	2
Rat 4	1	Y	3	3
Rat 5	2	N	2	3
Rat 6	2	N	1	2
Group III				
Rat 1	2	Y	1	2
Rat 2	2	N	2	2
Rat 3	2	Y	1	2
Rat 4	2	N	1	3
Rat 5	2	N	2	3
Rat 6	2	Y	3	1
Group IV				
Rat 1	2	Y	2	2
Rat 2	2	N	3	3
Rat 3	2	N	1	3
Rat 4	2	N	2	3
Rat 5	Died	Died	Died	Died
Rat 6	1	Y	3	3

($p < 0.05$) when compared to the control group. Collagen fibers and bone resorption in marrow was not statistically significant among the groups ($p > 0.05$). An increase of bone marrow cells could be observed in experimental groups II and IV ($p < 0.05$); in group III, this difference was non-significant ($p > 0.05$). These results are shown in Table 3 and Figures 7–14.

DISCUSSION

In this study, the animal model chosen was the rat because it is so often used for evaluation of side effects of radiotherapy.[2,5,16,17] Because experimental rats cannot survive frequent anesthesia, rats were exposed to a single irradiation dose that was sufficient to produce trabecular bone changes. This single dose has been used in previous studies, varying from 25 to 35 Gy.[2,5,16–18]

The cobalt source was chosen because it is well known to cause more side effects than other sources of radiation[19] and was more accessible. In addition, its build-up starts at 0.5 cm, whereas linear accelerators start around 1.2 cm.[20] Due to the fact that the femur of the rat is thin, this source of radiation was considered appropriate.

The presence of alopecia, epithelial desquamation, and cutaneous lesions had been mentioned in the past by Wurzler et al.[5] as a consequence of radiotherapy. However, in their experiment, cutaneous lesions started right after radiotherapy and regressed on the third week. In this study, the erosions were observed only on the sixth week and occurred only on the animals that received laser therapy.

Regarding body weight gain, the animals in the control group gained less body weight than the animals that received laser therapy (groups II, III, and IV). The fact that radiotherapy causes a reduction of body weight had already been noted by other studies.[5,17] However, the groups that were submitted to laser therapy gained more weight than the control group. It is possible to suggest that laser therapy produced higher activity

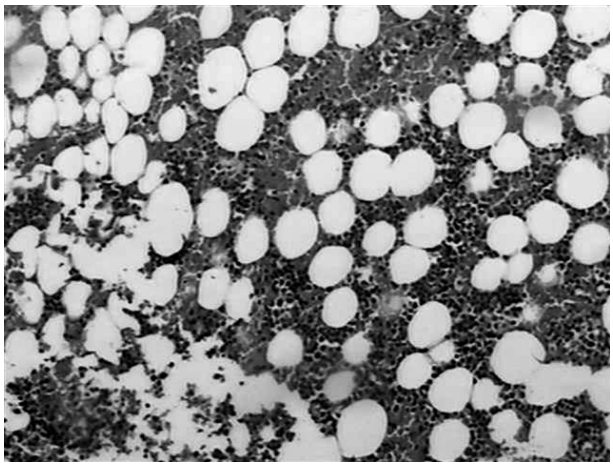

FIG. 7. Fat cells on medulla, group I. ×100.

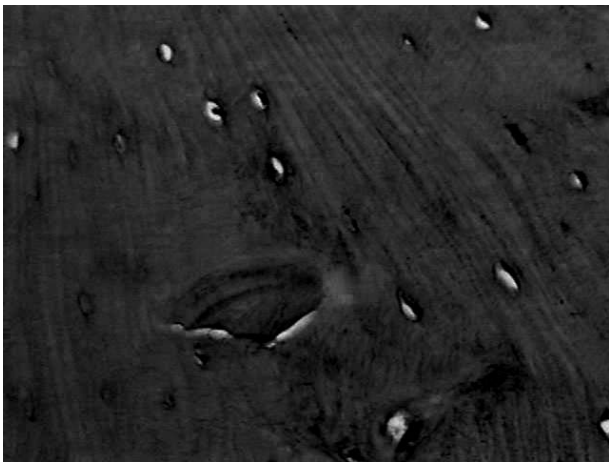

FIG. 8. Osteocytes in cortical bone, group I. ×100.

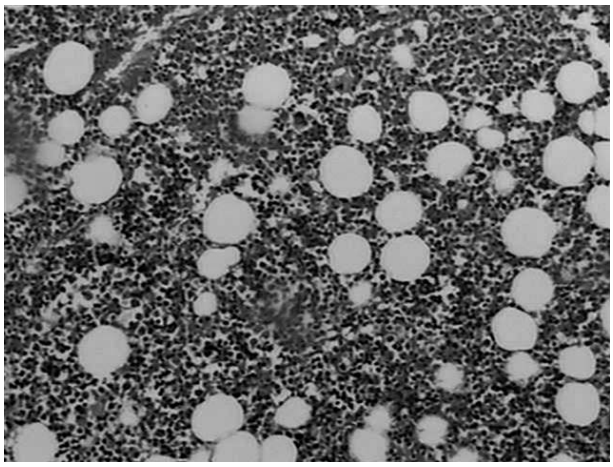

FIG. 11. Moderate amounts of fat cells on medulla, group III. ×100.

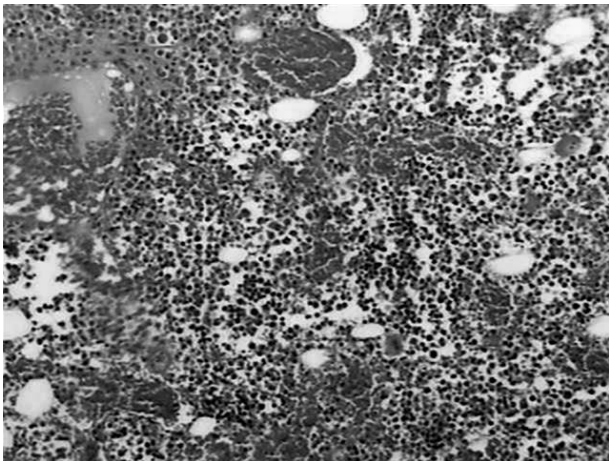

FIG. 9. Small number of fat cells on medulla, group II. ×100.

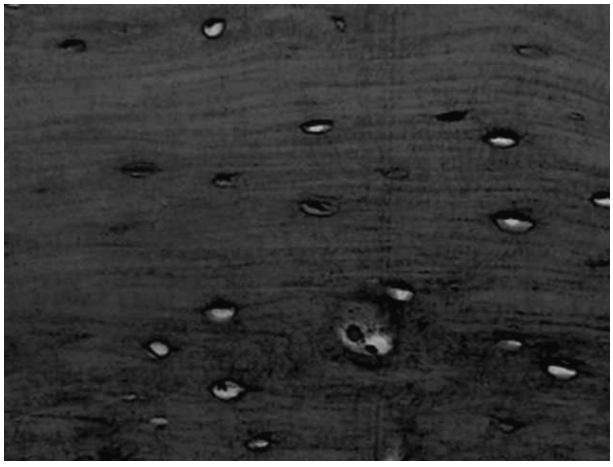

FIG. 12. Few number of osteocytes and Haversian canals, group III. ×200.

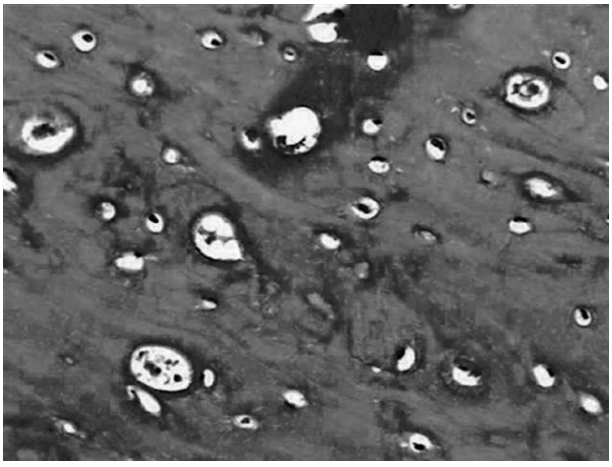

FIG. 10. High number of Haversian canals and osteocytes, group II. ×200.

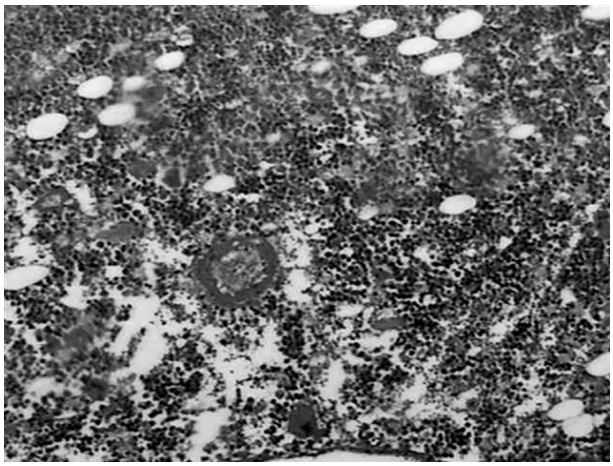

FIG. 13. Small number of fat cells on medulla, group IV. ×100.

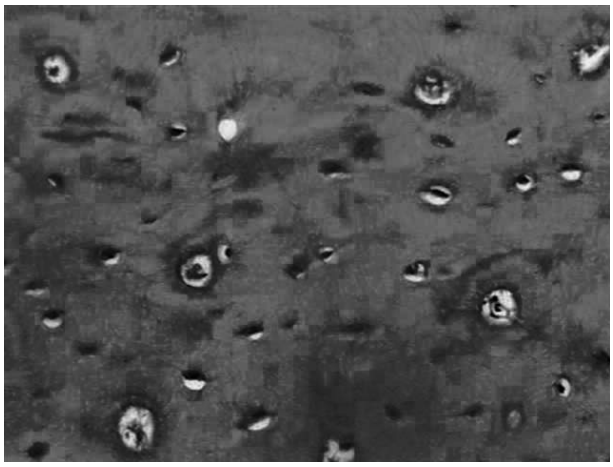

FIG. 14. High number of Haversian canals and osteocytes, group IV. ×200.

in the animals, thus increasing their appetite and consequently body weight.

With reference to histological analysis, the osseous alterations of group I were directly related to a reduction of osteocytes, Haversian canals, and osteoblastic activity, and an increase of fat cells, when compared to experimental groups. Other authors have found a smaller number of osteocytes[17] and a reduction of local vascularization[21] in an irradiated bone when compared to a non-irradiated bone. It is suggested that ionizing radiation leads to terminal differentiation of precursor bone cells, or osteoblasts.[22–24]

Radiographic results corroborated the histological findings, where the control group presented a lower amount of bone tissue when compared to experimental groups. The high sensitivity of digital radiographic system had already been documented by other authors.[25,26]

When laser therapy is discussed, it is known that this treatment modality has been used successfully in the past years and its applications are innumerous. It has already been proved its effect in orthodontics treatment,[27] in bone defects,[28,29] in bone fractures,[30] and after implant placement.[31] In this study, the laser protocol used (energy density (DE) = 4J/cm^2, p = 40 mW, E = 4J, spot of 0.04 cm^2, t = 100 sec, per point) was similar to other studies where there was a positive effect on bone tissue.[28,32–34] The cutaneous lesions observed in several groups (II, III, and IV) were unexpected; they may have been due to an accumulation of energy caused by radiotherapy with laser therapy, exceeding the tissue threshold of energy. However, the bone tissue was healthier and more vital in groups where the laser was applied, suggesting that laser therapy can reduce the side effects caused by ionizing radiation. The number of osteocytes was statistically higher in groups II and IV, but not in group III. These results are in agreement with researchers who suggest that laser therapy acts mainly via stimulation of cellular differentiation, especially precursor cells, and osteoblats,[35] and in this case, the cells of group III were probably small in number, given that the first laser therapy treatment started right after ionizing radiation.

Besides the high number of number of osteocytes, groups II and IV presented a high number of Haversian canals and medullar cells. Other studies corroborated our findings, where laser therapy increased the number of osteocytes and cell proliferation.[36]

CONCLUSION

In this study, laser therapy presented a positive biostimulative effect on rat bone tissue, especially when applied before radiotherapy (as prevention) or 4 weeks after radiotherapy (as for treatment). However, the use of laser at the parameters described above should be handled with caution due to epithelial erosions.

ACKNOWLEDGMENTS

To be accomplished, this work was done in several different institutions (Federal University of Bahia, UNIME, Santa Izabel Hospital, and PUCRS) and has required the help of many people and specialists in the area, including a radiotherapist (Maria Eulina Tavares) and a physicist (Jaílton Caetano Souza), who gave us assistance during the radiotherapy procedure.

REFERENCES

1. Calhoun, K.H., Shapiro, R.D., Stiernber, C.M., et al. (1988). Osteomyelitis of the mandible. Arch. Otolaryngol. Head Neck Surg. 114, 1157–1162.
2. Kiyohara, S., Sakurai, T., and Kashima, I. (2003). Early detection of radiation-induced structural changes in rat trabecular bone. Dentomaxillofac. Radiol. 32, 30–38.
3. Bras, J., De Jonge, H.K., and Van Merkesteyn, J.P. (1990). Osteoradionecrosis of the mandible: pathogenesis. Am. J. Otolaryngol. 11, 244–250.
4. Aitasalo, K. (1986). Bone tissue response to irradiation and treatment model of mandibular irradiation injury. An experimental and clinical study. Acta Otolaryngol. Suppl. 428, 1–54.
5. Wurzler, K.K., DeWeese, T.L., Sebald, W., et al. (1998). Radiation-induced impairment of bone healing can be overcome by recombinant human. J. Craniofac. Surg. 9, 131–137.
6. Németh, Z., Somogyi, A., Takácsi-Nagy, Z., et al. (2000). Possibilities of preventing osteoradionecrosis during complex therapy of tumors of the oral cavity. Pathol. Oncol. Res. 6, 53–58.
7. Tunér, J., and Hode, L. (2004). *The Laser Therapy Handbook.* Grängesberg, Sweden: Prima Books.
8. Mester, E., Spiry, T., Szende, B., et al. (1971). Effects of laser rays on wound healing. Am. J. Surg. 122, 532–535.
9. Rochkind, S., Nissan, M., Razon, N., et al. (1986). Electrophysiological effect of HeNe laser on normal and injured sciatic nerve in the rat. Acta Neurochir. 83, 125–130.
10. Anders, J.J., Borke, R.C., Woolery, S.K., et al. (1993). Low-power laser irradiation alters the rate of regeneration of the rat facial nerve. Lasers Surg. Med. 13, 72–82.
11. Kovacs, I.B., Mester, E., and Gorog, P. (1974). Laser-induced stimulation of the vascularization of the healing wound. An ear chamber experiment. Experientia. 30, 341–343.
12. Karu, T.I., Kalendo, G.S., Ketokhov, V.S., et al. (1982). Biostimulation of HeLa cells by low-intensity visible light. Nuovo Cimento 1, 828–840.
13. Karu, T., Smolyaninova, N., and Selenin, A. (1991). Long-term and short-term responses of human lymphocytes to He-Ne laser irradiation. Lasers Life Sci. 4, 167–178.

14. Saperia, D., Glassberg, E., Lyons, R.F., et al. (1986). Demonstration of elevated type I and type III procollagen mRNA levels in cutaneous wounds treated with helium-neon laser. Proposed mechanism for enhanced wound healing. Biochem. Biophys. Res. Commun. 138, 1123–1128.
15. Bonewald, L.F., and Mundy, G.R. (1990). Role of transforming growth factor-beta in bone remodeling. Clin. Orthop. Rel. Res. 250, 261–276.
16. Arnold, M., Stas, P., Kummermehr, J., et al. (1998). Radiation-induced impairment of bone healing in the rat femur: effects of radiation dose, sequence and interval between surgery and irradiation. Radiother. Oncol. 48, 259–265.
17. Maeda, M., Bryant M.H., Yamagata, M., et al. (1998). Effects of irradiation on cortical bone and their time-related changes. A biomechanical and histomorphological study. J. Bone Joint Surg. Am. 70, 392–399.
18. Jacobsson, M., Jonsson, A., Albrektsson, T., et al. (1985). Dose-response for bone regeneration after single doses of ^{60}Co irradiation. Int. J. Radiat. Oncol. Biol. Phys. 11, 1963–1969.
19. Malicki, J., Kosicka, G., Stryczynska, G., et al. (2001). Cobalt 60 versus 15 MeV photons during total body irradiation: doses in the critical organs and complexicity of the procedure. Ann. Transplant. 6, 18–22.
20. Schmitt, G., Glaeser, L., and Kotsch, E. (1978). [Advantages and disadvantages of a 5.7 MeV linear accelerator compared to a ^{60}Co unit] [in German]. Strahlentherapie 154, 257–260.
21. Morales, M.J., Marx, R.E., and Gottlieb, C.F. (1987). Effects of pre- and postoperative irradiation on the healing of bone graft in the rabbit. J. Oral Maxillofac. Surg. 45, 34–41.
22. Matsumura, S., Jikko, A., Hiranuma, H., et al. (1996). Effect of x-ray irradiation on proliferation and differentiation of osteoblasts. Calcif. Tissue. Int. 59, 307.
23. Dare, A., Hachisu, R., Yamaguchi, A., et al. (1997). Effects of ionizing radiation on proliferation and differentiation of osteoblast-like cells. J. Dent. Res. 76, 658.
24. Dudziak, M., Saadeh, P., Mehrara, B., et al. (2000). The effects of ionizing radiation on osteoblast-like cells in vitro. Plast. Reconst. Surg. 106, 1049–1061.
25. Sarmento, V.A., and Rubira, I.R.F. (1998). Mensuração da densidade óptica apical—uma proposta para diagnóstico diferencial em endodontia. J. Bras. Odontol. Clín. 12, 65–68.
26. Sarmento, V.A., and Pretto, S.M. (2003). Diagnóstico radiográfico de alteraç es periapicais de origem endodôntica através da determinação do nível de cinza em imagens digitais—estudo experimental em ratos. Rev. Pós Grad. Fac. Odontol. Univ. São Paulo Braz. 10, 333–345.
27. Kawasaki, K., and Shimizu, N. (2000). Effects of low-energy laser irradiation on bone remodeling during experimental tooth movement in rats. Laser Surg. Med. 26, 282–291.
28. Garavello-Freitas, I., Baranauskas, V., Joazeiro, P.P., et al. (2003). Low-power laser irradiation improves histomorphometrical parameters and bone matrix organization during tibia wound healing in rats. J. Photochem. Photobiol. B 70, 81–89.
29. Rochkind, S., Kogan, G., Luger, E.G., et al. (2004). Molecular structure of the bony tissue after experimental trauma to the mandibular region followed by laser therapy. Photomed. Laser Surg. 22, 249–253.
30. Luger, E.J., Rochkind, S., Wollman, Y., et al. (1998). Effect of low-power laser irradiation on the mechanical properties of bone fracture healing in rats. Laser Surg. Med. 22, 97–102.
31. Lopes, C., Pinheiro, A., Sathaiah, S., et al. (2005). Infrared laser light reduces loading time of dental implants: a Raman spectoscopic study. Photomed. Laser Surg. 23, 27–31.
32. Lirani, A. (2004). Estudo comparativo dos efeitos do ultra-som e do laser de baixa intensidade no reparo ósseo de tíbia de rato [M.S. thesis]. São Carlos da Universidade de São Paulo, Brazil.
33. Khadra, M., Lyngstadaas, S.P., Haanaes, H.R., et al. (2005). Effect of laser therapy on attachment, proliferation and differentiation of human osteoblasts-like cells cultured on titanium implant material. Biomaterials 26, 3503–3509.
34. Gerbi, M., Pinheiro, A., Marzola, C., et al. (2005). Assessment of bone repair associated with the use of organic bovine bone and membrane irradiated at 830 nm. Photomed. Laser Surg. 23, 382–388.
35. Ozawa, Y., Shimizu, N., Kariya, G., et al. (1998). Low-energy laser irradiation stimulates bone nodule formation at early stages of cell culture in rat calvarial cells. Bone 22, 347–354.
36. Dortbudak, O., Haas, R., and Mailath-Pokorny, G. (2002). Effect of low-power laser irradiation on bony implant sites. Clin. Oral Implants Res. 13, 288–292.

Adrress reprint requests to:
Samantha Seara Da Cunha, D.D.S., Ph.D.
Alameda Praia de Jaguaripe 76
Villas do Atlântico
Lauro de Freitas-Bahia 42700-000, Brazil

E-mail: samanthadacunha@hotmail.com