

Effect of Low-Level Laser Therapy and Calcitonin on Bone Repair in Castrated Rats: A Densitometric Study

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Abstract

Objective: To investigate the healing of bone defects in male rats treated with salmon calcitonin, low-level laser therapy (LLLT), or both. **Background:** Healing of bone defects still represents a challenge to health professionals in several areas. In this article, the effect of calcitonin in combination with LLLT on bone repair was studied. Densitometry was used as a valuable tool for the measurement of bone regeneration. **Methods:** Sixty male Wistar rats underwent bilateral castration surgery before the creation of a surgical bone defect. The animals were randomly divided into four groups: control, treated with calcitonin (Ca), treated with LLLT (La), and treated with calcitonin and LLLT (CaLa). Groups Ca and CaLa received 2 IU/kg of synthetic salmon calcitonin intramuscularly three times a week. Groups La and CaLa received laser therapy using a gallium-aluminum-arsenide laser (10 mW, 20 J/cm², wavelength 830 nm). Control animals were submitted to sham irradiation. The animals were sacrificed 7, 14, and 21 days after surgery, and bone defects were analyzed using densitometry. **Results:** The CaLa group had a higher degree of bone regeneration 14 and 21 days after surgery. **Conclusions:** The La and CaLa had significantly higher bone mineral density than the control and Ca groups.

Introduction

THE STUDY OF BONE REPAIR is important because the healing of bone defects represents a challenge to health professionals. Over the last 30 years, the number of studies on bone regeneration conducted to obtain new knowledge that could be applied to surgical reconstruction procedures, to bone repair, and to the use of implants has increased markedly.^{1–4} A study using calcitonin showed faster bone repair in female castrated rats than in a control group;³ different results were obtained for bone repair in male rats.⁴ Bone turnover is a complex process in which bone formation and bone resorption are tightly linked in time and space. These processes are essential for the development, maturation, maintenance, and repair of bones.⁵ Osteoporosis is the most frequent systemic disease when hormonal variations are present.^{3,6}

Several studies have investigated the ability of salmon calcitonin to inhibit bone resorption in patients with bone metabolism disorders.^{3,4,6,7} This hormone favors bone formation, inhibits osteoclastic activity, and prevents osteope-

nia.^{4,6,7} *In vitro* and *in vivo* studies have shown that this hormone stimulates the growth of bone tissue,^{3,8} demonstrated by a decrease in inflammatory cell infiltration, tissue flattening, reduced number of lymphoid follicles, and other histological effects.⁸ Since 1994, the World Health Organization (WHO) has recommended bone densitometry as the criterion standard for the diagnosis of osteoporosis. Densitometric studies have been performed to evaluate bone regeneration in osteoporosis.^{3,9} This method has been shown to produce results similar to those of histological studies.¹⁰

In the early 1960s, low doses of different light wavelengths were used for the treatment of specific diseases, with the main areas of application being the reduction of acute inflammation, stimulation of the tissue repair process, and pain relief. Laser therapy uses different wavelengths of the visible and near-infrared spectra, for example, helium–neon (632.8 nm), gallium-aluminum-arsenide (Ga-Al-As; 805 or 650 nm), and gallium-arsenide (904 nm).^{11–13} Laser therapy has been shown to positively stimulate bone growth *in vivo* and *in vitro*. The results indicate that photophysical and

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photochemical properties of some wavelengths are primarily responsible for the tissue responses. The use of correct and appropriate parameters promotes a positive biomodulatory effect on bone healing.¹²

The hypothesis of this densitometric study was that calcitonin in combination with Ga-Al-As LLLT (830 nm) would yield better results in surgical defects created in the femurs of castrated male rats.

Materials and Methods

Animals

All experiments were conducted according to the guidelines for animal care of Vale do Paraíba University, São José dos Campos, Brazil (L035/2003/CEP). For the experiment, 60 male Wistar rats (*Rattus norvegicus* var. *albinos*) 60 days old and weighing 200 to 250 g were used. The animals were maintained under standard conditions of temperature (22–25°C), relative humidity (40–60%), and light/dark cycle, with food and water available *ad libitum*. The rats were placed in a collective box and randomly divided into the following four groups of 15 animals each: control, animals treated with synthetic salmon calcitonin (Ca), animals treated with Ga-Al-As LLLT (830 nm, 20 J/cm²) (La), and animals treated with calcitonin plus Ga-Al-As LLLT (830 nm) (CaLa).

Surgery

All animals underwent bilateral castration surgery (Fig. 1A) 60 days before the creation of a surgical bone defect in the distal epiphysis of the right femur. The animals were weighed, pre-anesthetized with butorphanol (0.01 mL/kg, intramuscularly) plus acepromazine (0.02 mL/kg, intramuscularly), and anesthetized with Zoletil® 125 mg (Virbac, São Paulo, Brazil). After anesthesia, local asepsis was performed using polyvinylpyrrolidone iodine. A longitudinal incision

was made in the midline of the scrotal region, and one of the testes was exposed. Next, the vascular pedicle was ligated and sectioned. After removal of the testis, the skin was sutured with No. 4 silk thread (Ethicon, Johnson & Johnson, São José dos Campos, SP, Brazil).

Surgical procedure for bone defect creation

Surgery for exposure of the distal epiphysis of the right femur was performed after anesthesia using the same protocol as described above. An incision was made in the skin, followed by plane-by-plane muscle dissection and incision of the periosteum. In this region, a bone defect was created using a spear-shaped bur measuring 2.8 mm in diameter coupled to a low-rotation motor at 1100 rpm. During the surgical procedure (Fig. 1B), the region was abundantly irrigated with saline. Immediately after creation of the bone defect (Fig. 1C), the animals were treated according to the protocol described below.

Animals of the Ca and CaLa groups received 2 IU/kg of synthetic salmon calcitonin (Sandoz AG, Nürnberg, Germany), corresponding to the human therapeutic dose. The medication was diluted in physiological saline (0.9% sodium chloride (NaCl)), corresponding to a concentration of 0.6 IU, and administered intramuscularly immediately after the procedure and on alternate days until the day of sacrifice. Control animals received physiological saline (0.9% NaCl) by the same route and at the same time interval.

Groups La and CaLa received laser therapy using a continuous-wave diode laser (Ga-Al-As); diode laser wavelength 830 nm, with mean optical output power of 10 mW, energy density of 20 J/cm², time irradiated 3 min 33 s, and area 0.1 cm² (model: Thera Laser, D. M. C. Equipamentos Ltda., São Carlos, SP, Brazil) were used. Spectroscopic measurements showed no thermal drift for the 830-nm laser. The 830-nm laser showed a 0.4-nm wavelength drift from the

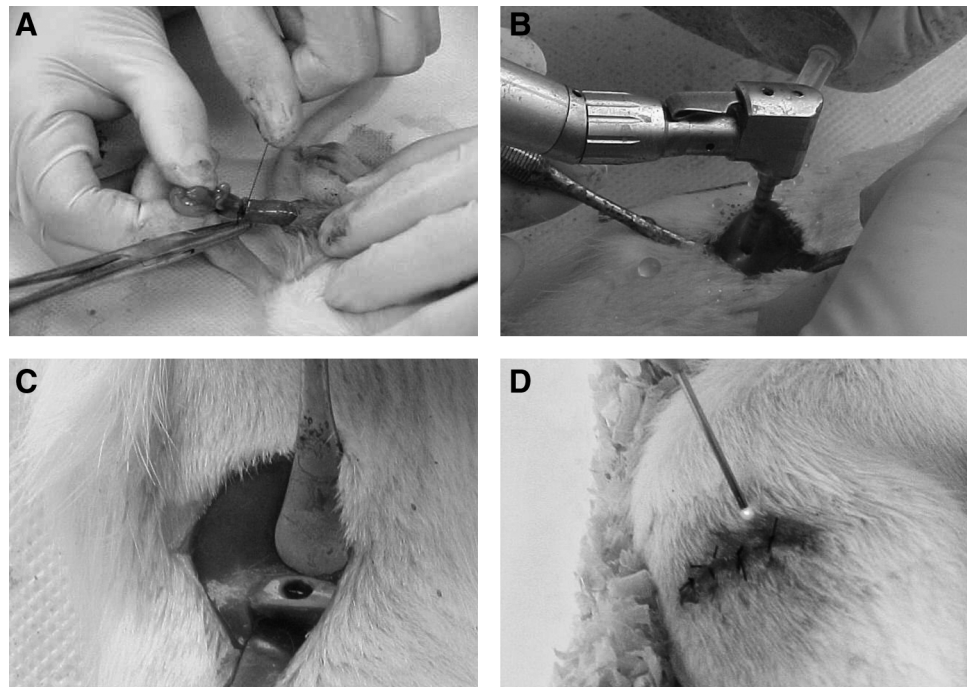


FIG. 1. (A) Exposure of the vascular pedicle of the scrotum and ligament. (B) Creation of the bone defect. (C) Surgical bone defect. (D) Suture and laser therapy (application immediately after surgery).

cold to warm operation conditions. Stabilization at 834 nm was achieved within a period of less than 30 s after turning on the diode laser device at room temperature. The optical power was calibrated using a Newport Multifunction Optical Meter model 1835C (Newport corp., Irvine, CA, USA).

The laser beam was applied at two sites around the lesion for 6 s immediately after creation of the bone defect (Fig. 1D) and on alternate days until the day of sacrifice. Control animals were submitted to sham irradiation.

Experimental periods

Five animals from each group were killed at 7, 14, and 21 days after surgery. The bone containing the surgical defect was removed *en bloc*, fixed in 10% formalin, and submitted to radiographic analysis for densitometry.

Optical Densitometry

Radiographic images of the specimens were obtained using periapical film (Kodak Ektaspeed EO-41 P, São José dos Campos, SP, Brazil). The following parameters were standardized for all radiographs: a film-focus distance of 40 cm, exposure time of 0.25 s, 10 mA, and 65 kVp. The femurs obtained from control and experimental animals (Ca, La, and CaLa) at the same observation time were placed on the same film. An aluminum reference step wedge consisting of eight intervals of 7°, each 5 mm long and 0.4 mm thick, was placed between the specimens. The first degree measured 0.5 mm in height, and the remaining degrees were increased at an interval of 0.5 mm until reaching a height of 4 cm, corresponding to the seventh degree. The radiographs were processed at 27°C in an automatic processing machine (Gender GXP Dental X-ray Processor, Des Plaines, IL, USA) at 4-min cycles. Freshly mixed developer and fixer solutions were used to ensure proper processing. Optical densitometry was performed to evaluate the surgical bone defects in each specimen.^{4,8} The radiographic images were digitalized, and bone mineral density (BMD) was reported as aluminum thickness.⁴ Three measurements were performed per selected region, and the mean values obtained were multiplied by 0.5 (Fig. 2).

Statistical analysis

The densitometric results were compared between groups using analysis of variance and the Tukey test, with the level of significance set at 5%.

Results

Control group

In the sample studied, BMD gradually increased over time in the control, although significantly higher BMD values were obtained for the treated groups (La at 7 and 21 days and CaLa at 21 days). The average densitometric values obtained for the control group were consistently lower than those observed for all other groups, indicating a smaller amount of mineralized tissue in the bone defect area (Fig. 3A–C).

Ca group

The use of calcitonin led to a gradual increase in BMD, with a significant difference at 7 and 21 days from the control group (Fig. 3A, C).

La group

Densitometric analysis 7 days after surgery showed higher BMD in the La group than in the other groups (Fig. 3A). BMD remained stable until 14 days and increased between 14 and 21 day. Statistically significantly different results were observed at 7 and 21 days (Fig. 3B, C).

CaLa group

At 14 days, the CaLa group had higher absolute BMD values than the other groups (Fig. 3B). Statistically significant differences were observed between the CaLa and the other groups at 21 days (Fig. 3C).

Comparison between all groups

At 21 days, the Ca, La, and CaLa groups had significantly higher BMD than the control group. In absolute values, the La group also had increased values of BMD. Comparison of BMD between all groups at the different experimental times is shown in Figure 3A, B, and C.

Discussion

The findings of this densitometric study support the research hypothesis that calcitonin in combination with Ga-Al-As LLLT (830 nm) yields better results in surgical defects created in the femurs of castrated male rats.

Bone is a living tissue, and new bone formation coexists with bone resorption throughout life. Although a large

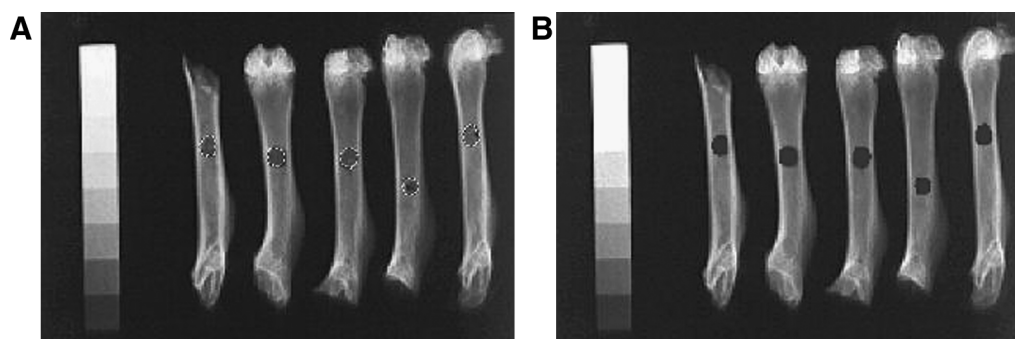


FIG. 2. (A) Radiographs showing the delimitation of the bone defect areas. (B) Radiographs showing the selection of the bone defect areas and optical density reading.

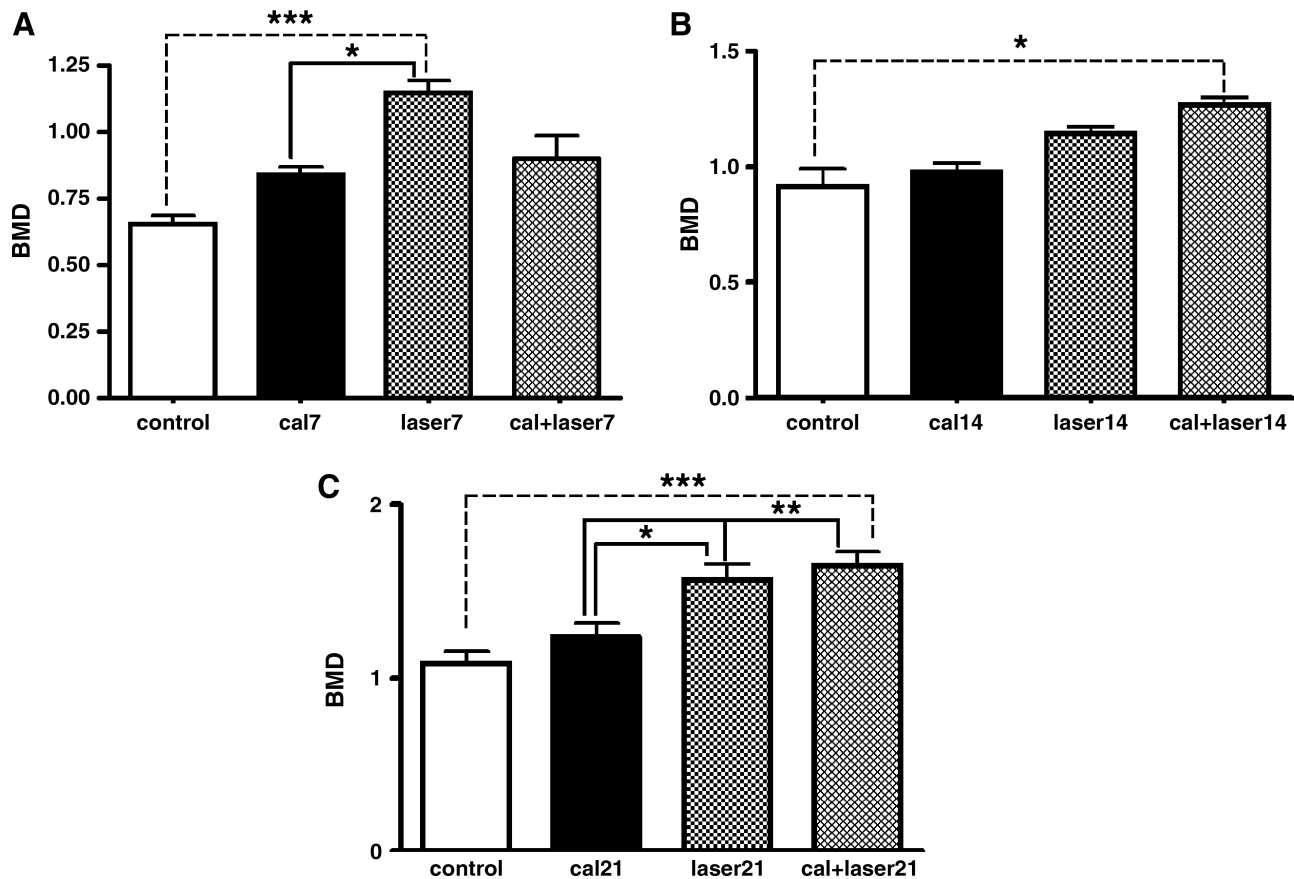


FIG. 3. Effect of low-level laser therapy and calcitonin on bone mineral density. (A) at 7 days; (B) at 14 days; (C) at 21 days. *Statistical analysis of variance and the Tukey test indicated that $p < 0.05$.

number of hormones and cytokines modulate osteoblast and osteoclast function, osteoporosis results from any disorder in which bone formation becomes uncoupled from bone resorption. The most common condition is the loss of action of gonadal steroids on bone, as observed in menopause or in male and female hypogonadism not associated with menopause.^{3,6,8-10,14,15} Osteoporosis is a skeletal disorder characterized by compromised bone strength, which predisposes the individual to a greater risk of fracture. In the United States, 26% of women aged 65 and older and more than 50% of women aged 85 and older have osteoporosis.¹⁴

Several studies have demonstrated the stimulatory effect of calcitonin on bone growth as a result of the inhibition of osteoclast activity, emphasizing the importance of investigating the effect of this drug on bone repair.^{3-4,15}

The biomodulatory effects of LLLT mediated using non-thermal mechanisms result from the interaction between light and tissue and thus from photochemical interactions.¹¹⁻¹³ According to Schindl et al.,¹⁶ its ability to induce athermal, nondestructive photobiological processes characterize LLLT. *In vivo* and *in vitro* studies have shown the biological effects of laser therapy such as an increase in local microcirculation, activation of the lymphatic system, and proliferation of epithelial cells and fibroblasts.¹⁷⁻²⁰

Pinheiro and Gerbi¹² discussed the positive effects of laser irradiation on the cell membrane and mitochondria and its influence on adenosine triphosphate (ATP) production. Bone tissue is a major source of angiogenic and endothelial factors,

as well as bone morphogenetic proteins, which are essential for osteogenesis and angiogenesis. Furthermore, LLLT increases the levels of growth factors, such as fibroblast growth factor, which are also found in healing bone tissue. These chemical mediators act on differentiated cells, increasing the rate of proliferation and stimulating maturation and the secretion of bone matrix. It has also been accepted that acceleration of the repair process may be a result of the effect of laser therapy on the synthesis of bone matrix due to increased vascularization and early onset of the inflammatory response.¹² Laser therapy can biomodulate metabolic activities within tissues and cells. One measurable effect is the increase of local tissue blood flow in the microcirculation. This might explain some successful treatments of wound healing with laser-induced biomodulation.²¹

Depending on its wavelength, electromagnetic radiation in the form of light can stimulate macromolecules, initiate conformational changes in proteins, or transfer energy to electrons. Low-intensity laser radiation of the red and near-infrared region corresponds to the characteristic energy and absorption levels of the relevant components of the respiratory chain. This laser stimulation vitalizes the cell by increasing mitochondrial ATP production.²²

The present results agree with those reported by Silva Junior et al.¹⁸ and Lopes et al.²⁰ in terms of the wavelength used but differ from the findings of Dortbudak et al.,¹⁷ who used a longer wavelength.

LLLT may exert a direct effect on cell activity by biostimulating bone repair, promoting the acceleration of osteoblast

activity. This may explain the results observed for the CaLa group, which had a higher level of bone regeneration 14 and 21 days after bone defect creation. The results suggest that calcitonin inactivates the action of osteoclasts, whereas LLLT acts on osteoblasts, mediating an increase in osteogenesis.

The results obtained with the administration of calcitonin suggest that, in castrated male rats, this hormone acts more effectively from the first week, with its action being maintained until the end of the third week, corresponding to the end of the experimental period in this study.

The present findings demonstrate that BMD was greater in the treated groups. The use of laser therapy alone or in combination with calcitonin yielded significant results. In this respect, the results indicate that the combination of calcitonin and laser therapy accelerated and favored the repair of surgical bone defects.

Conclusion

The LLLT and Ca groups had significantly higher BMD than the control and Ca groups.

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