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*Research Article*

# Low-Level Laser Therapy and Calcitonin in Bone Repair: Densitometric Analysis

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The aim of this work was to evaluate the association of low-level laser therapy (LLLT, 830 nm) and calcitonin in bone repair considering that bone healing remains a challenge to health professionals. Calcitonin has antiosteoclastic action and LLLT is a treatment that uses low-level lasers or light-emitting diodes to alter cellular function. Both are used to improve bone healing. Densitometry is a clinical noninvasive valuable tool used to evaluate bone mineral density (BMD). Sixty male rats were submitted to bone defect with a trephine bur, randomly divided into four groups of 15 animals each: control (C); synthetic salmon calcitonin (Ca); LLLT (La); LLLT combined with calcitonin (LaCa). Animals from Ca and LaCa received 2 UI/Kg synthetic salmon calcitonin intramuscularly on alternate days after surgery. Animals from groups La and LaCa were treated with infrared LLLT (830 nm, 10 mW, 20 J/cm$^2$, 6 s, contact mode). Five animals from each group were euthanized 7, 14, and 21 days after surgery and bone defects were analyzed by densitometry. Statistical analysis showed a significant difference in BMD values in LaCa group at 7 and 21 days ($P = 0,005$). The results of the densitometric study showed that LLLT (830 nm) combined with calcitonin improved bone repair.

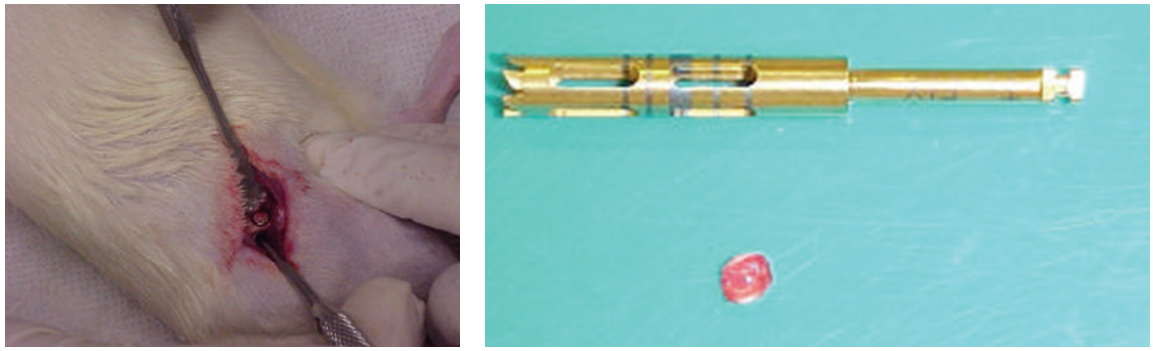
## 1. Introduction

Bone healing of large defects is still a challenge to health professionals [1–3]. During the remodeling process, several cytokines, peptides, and growth factors are released locally. Bone-formation markers include serum osteocalcin, bone-specific alkaline phosphatase, and procollagen I carboxyterminal propeptide [4].

Calcitonin has analgesic, anti-inflammatory, and antiosteoclastic actions, therefore, it is used to treat clinical and biological diseases characterized by excessive human bone remodeling [1, 2, 5]. The treatment with calcitonin helps to accelerate healing of bone defects in rats [2, 5]. Calcitonin action becomes more evident during repair in the initial phases of osteogenesis by stimulating bone formation [2, 3, 5]. Salmon calcitonin cannot only increase bone mineral density (BMD) in osteoporotic bone but also enhance the bone biomechanical properties and improve the healing process in fractured osteoporotic bone [6].

Different techniques have been used in dentistry with an ultimate aim of improving the bone quality. Low-level laser therapy (LLLT) has been used to improve bone healing in several conditions such as in alveolus of dental extraction, bone fractures, [7] dental implants, [8–10] orthodontic treatment, and orthognatic surgery [11].

Several studies have demonstrated that the nonsurgical near-infrared laser is more suitable for bone repair, due to deeper penetration in bone tissue when compared to



(a) (b)

Figure 1

Table 1: Parameters of laser protocol.

| Groups | Wavelenght | Energy density | Potency | Time irradiated | Area ($cm^2$) |
|---|---|---|---|---|---|
| La and LaCa | 830 nm | 20 J/$cm^2$ | $10 \times 10^{-3}$ W | 6 s | 0.002827 |

visible laser [12]. Although the use of LLLT on bone healing biomodulation has been growing steadily and several studies have demonstrated positive results on the healing of bone tissue, there are no reports on the combination of LLLT and calcitonin [3].

Prado-Filho and Sterman [13] used radiographic optical densitometry (ROD) in the evaluation of mineral bone density in thoroughbred horses in an initial training program. ROD is a quantitative analysis used to quantify bone mineral density (BMD) in equivalence to aluminum millimeters (mmAL) [2, 3, 9].

In this study, ROD was used to evaluate BMD after LLLT (830 nm) combined with calcitonin to measure the improvement in bone repair in surgical defects in rats.

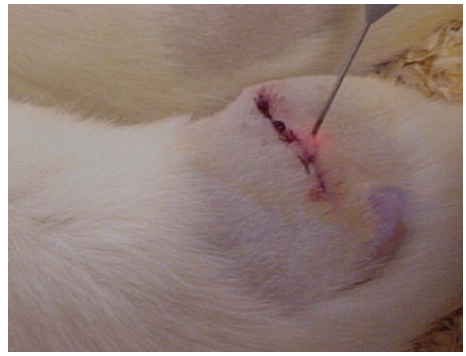


Figure 2

## 2. Materials and Methods

Sixty male Wistar rats, weighting an average of 250 g, were used in this study and were maintained in accordance to the guidelines of the committee on care and use of laboratory animals of the Brazilian National Research Council and the Research Ethic Committee at the Universidade do Vale do Paraíba, Brazil (L036/2003/CEP). The rats had water and food available ad libitum. The animals were randomly distributed into four groups of 15 animals each, control group (C); synthetic salmon calcitonin (Ca); LLLT (La); LLLT combined with calcitonin (LaCa). These groups were subdivided into three subgroups of 5 animals according to the time of euthanasia.

Surgical bone defects in the right femur were made under general anesthetic, using Acepromazine 1 mg/kg (Acepran 0,2%, Univet S.A), Butorfanol 1 mg/kg (Lab. Strong Dodge Ltda) and Zolazepan/Tiletamine 15 mg/Kg (Zoletil, Lab.Virbac S.A). A longitudinal incision on the skin and subcutaneous tissue was made. After femur exposure, a mechanical bone defect was created by a low-speed drill with a 2.8 mm trephine bur (Figure 1) kept under constant refrigeration with a sterile 0.9% saline solution. The muscle and subcutaneous tissue was sutured using catgut 4.0 (Cirumédica, Cotia, SP, Brazil), and the skin was sutured with silk 4.0 (Ethicon, Johnson & Johnson, São José dos Campos, SP, Brazil).

The animals from the Ca and LaCa groups received 2 UI/Kg synthetic salmon calcitonin (Miacalcic, Novartis SA) intramuscularly immediately after the surgical procedure and then on alternate days until euthanasia. Groups La and LaCa were irradiated in a contact mode with a continuous wave (CW) 830 nm diode laser, with mean optical output power of 10 mW, energy density of 20 J/$cm^2$, time irradiated 3 min 33 s, and area 0.1 $cm^2$ (model: thera Laser, D. M. C. Equipamentos Ltda., São Carlos, SP, Brazil). The parameters of laser protocol used in this study are presented in Table 1. The LLLT was applied transcutaneously, with the hand piece perpendicularly positioned to the wound (Figure 2). The 830 nm laser showed a 0.4 nm wavelength drift from the 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

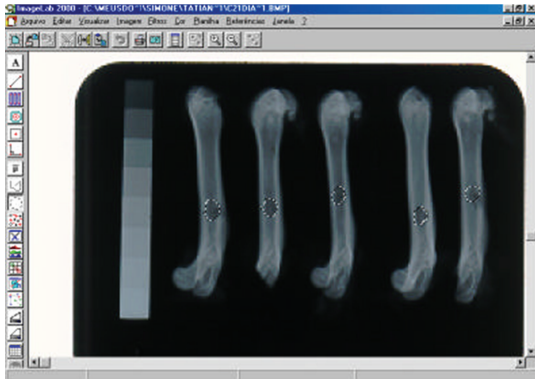


Figure 3

was calibrated using a Newport Multifunction Optical Meter (model 1835C).

Five animals from each group were euthanized at 7, 14, and 21 days after surgery. The femurs were fixed in formol 10%. Radiographic images of the specimens were obtained using occlusal film (Kodak Ektaspeed EO-41 P, São José dos Campos, SP, Brazil). The following parameters were standardized for all radiographs: film-focus distance of 40 cm, exposure time of 0.25 s, 10 mA, 65 kVp. The digitalized images were measured using a computer program (ImageLab, Softium Sistema de Informática Ltda).

The average density of the femur was compared with the average density of an aluminum scale to determine the equivalence in millimeters of aluminum. An area of the bone defect related to the area of bone neoformation was bounded (Figure 3). An image pattern was chosen to compare with the X-Ray images. The calibration system eliminates the variations in radiographic processing. The area corresponding to the surgical defect resulted in a value of optical density (OD) that refers to BMD. The protocol was based on the study of Prado-Filho and Sterman [13].

## 3. Statistical Analysis

The densitometric results were compared between groups by analysis of variance (ANOVA) and the Tukey test, with the level of significance set at 5%, yielding an $F$ value equal to 21.84, greater than the critical $F$ (1.99), implying a statistically significant difference between groups and time points.

## 4. Results

The absolute values of the BMD analysis allowed the assessment of bone healing by comparing the C, Ca, La, and LaCa groups in relation to the experimental periods (Table 2). This analysis showed that bone repair occurred gradually in all groups. At seven days, the LaCa group had the highest BMD values (Table 2). At 14 days, the results were similar in all groups. At 21 days, the values of BMD for the LaCa group were statistically significant compared to other groups. During this period, the values of La group were lower than observed in Ca and LaCa groups (Figure 4).

Table 2: Average and standard deviation of BMD (mm/Al).

| Groups | 07 days | 14 days | 21 days |
|---|---|---|---|
| Control (C) | 8 ± 2 | 14 ± 3 | 18 ± 5 |
| Calcitonin (Ca) | 10 ± 3 | 16 ± 1 | 24 ± 4 |
| Laser (La) | 9 ± 2 | 15 ± 2 | 21 ± 2 |
| LLLT and Calcitonin (LaCa) | 14 ± 3* | 15 ± 2 | 28 ± 1* |

* Statistically significant values, $P \leq 0,005$.

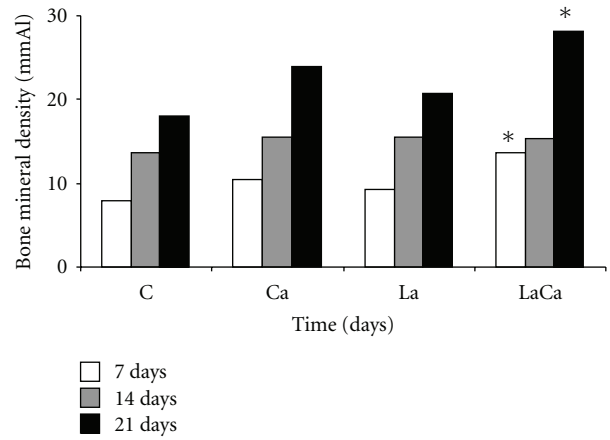



Figure 4

BMD gradually increased over time in all groups. However, compared with treated groups, significantly higher values of BMD were obtained for the LaCa (7 and 21 days). The average densitometric values in the control group were consistently lower than in all other groups, indicating a lower amount of mineralized tissue in the bone defect area (Table 2; Figure 4).

## 5. Discussion

In this study, authors evaluated densitometrically the effects of LLLT and calcitonin in rat femurs. Bone repair, regardless of how the injury occurred, results from a series of events that are similar to soft tissue repair. These include inflammation, cell proliferation, and tissue remodeling, the latter being characteristic of bone tissue [2, 3].

Experimental studies have shown that the femur is a possible anatomical site for the creation of bone defects because it provides adequate irrigation to supply nutrition to the injured tissue and has mechanical stability [14]. Thus, surgical circumscribed bone defects in the femur are recommended with a standard diameter of 3.7 mm, considered critical size [1–3].

The optical densitometry is an effective technique to evaluate BMD. It is a safe, easily applied method, which determines higher mineral density (BMD) by the increase of radiopacity in the radiographic image [9, 13]. Studies have reported that BMD values show results similar to those of histological studies [2].

Nonsurgical laser treatment is an excellent alternative to reduce the period of the healing process, considering that it

is a noninvasive therapy of simple application [8]. Currently, there are experimental and clinical studies showing favorable results with nonsurgical laser application in bone tissue [3, 14–16]. However, no reports of the association of LLLT and medications such as calcitonin were found in the literature [3].

Calcitonin is a potent inhibitor of osteoclastic bone resorption. It is also capable to stimulate osteoblast proliferation and to induce osteogenic effect in cell studies. Calcitonin has been used in the treatment of many bone disorders such as osteoporosis due to its direct inhibitory effect on osteoclasts and capacity to promote bone formation [2, 17].

A study of ovariectomized rats treated with calcitonin showed a decrease in time of bone healing when compared to the control group, confirmed by assessment of BMD, histomorphometric, and histological analysis [2]. However, the results of this study highlight the possible effect of calcitonin in rats without hormone deficiency, differing from the results of Almeida et al. [1] that also studied male rats.

Calcitonin acts at all stages of bone repair. In the early stages, it probably induces the cell differentiation into osteoblasts of precursor cells present in the granulation tissue. In the late period of bone neoformation, it stimulates osteoblastic activity and inhibits osteoclastic action, through antiresorptive properties. Thus, the newly formed bone tissue has a more compact and organized trabeculae [1, 2, 14, 16].

In addition, several studies have reported that the use of LLLT in the early stages of bone repair decreases the total period of osteoid formation. It aids the organization of clot and promotes granulation tissue formation. LLLT also supplies deficient energy to damaged cells, reducing vascular permeability and inflammatory infiltrate, allowing formation of granulation tissue [7, 10].

In order to establish a protocol to compare our results with other studies, an attempt to quantify the amount of energy applied and the irradiation time was made, according to the parameters used by Nascimento et al. [3] and Lopes et al. [8]. All variations of these factors were eliminated, since small doses of energy are not able to stimulate the repair and excessively high doses can inhibit this process [16].

The irradiation protocol used in this study is similar to those used on previous reports [3, 8, 14, 16]. Furthermore, the punctual distribution of energy was aimed to irradiate the entire area of the surgical bone defect [3, 12, 14, 18].

The combination of calcitonin and LLLT provided the best results, particularly within 21 days, showing higher rates of BMD than the other experimental groups. A study conducted with male rats with hormonal deficiency observed similar results, when using the combination of LLLT and calcitonin [3]. The results of this study validate the hypothesis that the combination of these therapies is effective in bone repair and allows greater bone formation in reduced time. Although administration of calcitonin is indicated to treat a hormone deficiency, its use in the treatment of experimental bone defects in normal rats showed that this medication contributed to the significant increase in BMD in all periods. Consequently, administration of calcitonin could be recommended in situations where there is need to reduce the time of bone healing and increase of bone density, frequently observed in cases of dental and orthopedic treatments.

It is possible that the effect of LLLT on bone regeneration depends not only on the total dose of irradiation, but also on the time and mode of irradiation [3, 16]. Many studies have indicated that irradiated bone, mostly with infrared wavelengths, has increased osteoblastic proliferation, collagen deposition, and bone neoformation, when compared to nonirradiated bone [16, 19].

## 6. Conclusion

Densitometric analysis of bone experimental defects in rats showed that LLLT (830 nm) combined with calcitonin improved bone repair and reduced the duration of the bone repair process.

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