Key words: low level laser therapy, bone, fractures, osteoblasts.

Low level laser therapy for bone regeneration.

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ABSTRACT
Since 1960, low level laser therapy (LLLT) has been used to stimulate a series of biologic tissues. Some authors have showed, trough experimental and clinics studies, the biostimulatory effects of LLLT on bone both in vivo and in vitro. Although the effects of LLLT have been demonstrated in many studies, the regulatory mechanisms of laser on tissues are poorly understood. Also, it has been postulated that there is an existence of a curve dose-response which means that the use of the appropriate parameters is effective on promoting an acceleration of bone healing. Then, the aim of this study was to show the state of the art with about the osteogenic effects of LLLT on bone cells and fracture consolidation. It was made a review in the databases including MEDLINE, EMBASE, Pubmed and Cochrane and the articles that met the inclusion criteria stated below were selected: papers published until December 2010 including the words "LLLT and bone and fracture", in the title, abstract, or keywords. In all studies, a fracture in tibiae or femur was induced and this injury was irradiated with LLLT. It was observed a wide variety of the parameters of LLLT used in the studies analyzed. Authors used different kinds of laser and different wave lengths, power, doses and time of application. Results obtained showed that LLLT can stimulate osteoblastic proliferation and can accelerate the consolidation of bone fracture. Although, a lot of studies state that LLLT contributes to accelerate the consolidation of bone fracture, further studies are necessary to investigate the effects of LLLT on bone tissue and to determine the best parameters to use.

INTRODUCTION
Laser is an acronym for "Light Amplification by stimulated Emission of Radiation" [1]. The first laser was demonstrated in 1960 and since then, it has been used for surgery, diagnostics and therapeutic medical applications. It is an electromagnetic energy and its physiological effects occur at the cellular level, stimulating or inhibiting biochemical and physiological proliferation activities by altering intercellular communication [2]. The action of LLLT is based on the absorption of the light by tissues, which will generate a series of modifications in cell metabolism. When the LLLT is applied to tissue, the light is absorbed by photoreceptors located in the cells, called chomophores. Once absorbed, the light can modulate cell chemical reactions and stimulate mitochondrial respiration, the production of molecular oxygen and ATP synthesis [3]. These effects can increase the synthesis of DNA, RNA and cell-cycle regulatory proteins, stimulating cell proliferation [4-6].
A significant body of evidence has now accumulated demonstrating that low level laser therapy (LLLT) is effective in reducing post-injury inflammatory processes, accelerating soft tissue healing and stimulating the formation of new blood vessels [4,5].
Since the decade of 70, some authors investigated the osteogenic potential of low level laser therapy (LLLT) and its use on healing of different connective tissues, including bone [7]. In 1971, a short report by Chekurov stated that laser was an effective modality in bone healing acceleration. Subsequently, other researchers studied the effects of LLLT osteoblast cell proliferation and bone healing after laser irradiation using histological, histochemical and radiographic measures [6, 8-10].
Many in vitro studies using osteoblastic cells showed that LLLT is capable of increasing mitochondrial activity [11,12], osteoblast DNA and RNA synthesis, bone nodule formation, osteocalcin and osteopontin gene expression and ALP activity, increasing osteoblast proliferation [13]. Ozawa et al (1998) [14] found a significant increase in the DNA synthesis in osteoblast cells after 830 nm laser irradiation. In a study investigating the effects of different dosages and wavelengths on osteoblast cells, our group observed that there was an increase in osteoblast proliferation and phosphatase activity after the irradiation of 830nm laser [6]. Kiyosaki et al (2010) [15] examined the effects of LLLT on osteoblasts via insulin-like growth factor I (IGF-I) signal transduction. The authors observed that laser therapy significantly increased the expression of IGF-I, Runx2 and calcium content in the mineralized nodules. Also, Aleksic et al (2010) [16] observed that low-level Er:YAG laser irradiation produced a significantly higher proliferation in laser-irradiated MC3T3-E1 cells at a fluence of 1 J/cm2, through the phosphorylation of extracellular signal-regulated protein kinase (MAPK/ERK).
Pires-Oliveira et al (2008) [17] observed that 830nm gallium-aluminium-arsenide diode laser (50 mW, 3 J/cm2) produced an intense grouping of mitochondria in the perinuclear region in osteoblasts cells, which culminated on the increase of cell proliferation. Xu et al (2009) [18] demonstrated that laser irradiation of 1.14 J/cm2, produced an increase of osteoblasts, stimulated alkaline phosphatase activity and indirectly inhibit osteoclast differentiation, by downregulating the RANKL:OPG mRNA ratio in osteoblasts. These authors suggest

that lasertherapy may play an important role in bone remodeling and should be valuable for the treatment of bone diseases such as osteoporosis. Stein et al (2008) [13] investigated the initial effect of low-level laser therapy on growth and differentiation of human osteoblast-like cells. SaOS-2 cells were irradiated with 670 nm laser. Over the observation period, cell viability, alkaline phosphatase activity and the expression of osteopontin and collagen type I mRNA were slightly enhanced in the irradiated cells compared with untreated control cells. Simizu et al (2007) [19] observed that osteoblast-like cells irradiated with a low-intensity Ga-Al-As laser (830 nm) presented a higher concentration of rIGF-I and area of bone nodules.

Also, many authors demonstrated that LLLT stimulates neoangiogenes at the site of fracture, increase collagen fiber deposition and promote higher bone cell proliferation, accelerating callus formation and fracture healing. Tajali et al (2010) [2] in a meta-analysis, stated that several studies in the literature indicates that LLLT can enhance biomechanical properties of bone during fracture healing in animal models.

However, LLLT needs better parameterization of variables to obtain the most appropriate stimulus, because many of the actual effects and limitations are not yet entirely clear and there is much controversy about its mechanism of action on tissues [6]. Moreover, many studies have produced no scientific validity for the low reliability of data because of methodological problems [20, 21]. However, excellent studies open this field, which in future will lead the individual with bone injuries to a faster return to their normal functions [22], avoiding the consequences of a prolonged immobilization, such as muscle mass loss and decrease in bone mineral density.

METHODS

A systematic search of four electronic databases including MEDLINE, EMBASE, Pubmed and Cochrane was performed and all the articles that met the inclusion criteria stated below were selected: papers published until December 2010 including the words "LLLT and bone and fracture", in the title, abstract, or keywords. Thus, ten studies were obtained, ranging between the years 2003 to 2010. In all studies, the authors produced bone defects in rats or rabbits and this injury was irradiated with LLLT.

LLLT IN OSTEOGENESIS

Several authors have demonstrated in experimental studies an acceleration of bone repair by the use of LLLT [23-25]. Mester et al. (1985) [26], suggest that the metabolic pathways are responsible for this healing effect, mainly due to the increased bioavailability of chemical energy (ATP) in cells.

Garavello-Freitas et al (2003) [23] examined the influence of daily energy doses of 0.03, 0.3 and 0.9 J of He-Ne laser irradiation on the repair of surgically produced tibia damage in Wistar rats. Laser treatment was initiated 24 h after the trauma and continued daily for 7 or 14 days. After 7 days, there was a significant increase in the area of neoformed trabeculae in tibiae irradiated with 0.3 and 0.9 J compared to the controls. At a daily dose of 0.9 J (15 min of irradiation per day) the 7-day group showed a significant increase in trabecular bone growth compared to the 14-day group. The Picrosirius-polarization method revealed bands of parallel collagen fibers (parallel-fibered bone) at the repair site of 14-day-irradiated tibiae, regardless of the dose. This organization improved when compared to 7-day-irradiated tibiae and control tibiae. These results show that low-level laser therapy stimulated the growth of the trabecular area and the concomitant invasion of osteoclasts during the first week, and hastened the organization of matrix collagen (parallel alignment of the fibers) in a second phase not seen in control, non-irradiated tibiae at the same period.

Pretel et al (2007) [8] evaluated bone repair in defects created in rat lower jaws after stimulation with infrared LLLT directly on the injured tissue. Bone defects were prepared on the mandibles of 30 rats allocated in two groups which were divided in three evaluation periods (15, 45, and 60 days), with five animals each: control group-no treatment of the defect; laser group-single laser irradiation with a GaAlAs (780nm, 35 mW; 178 J/cm²) directly on the defect area. The histological results showed bone formation in both groups. However, the laser group exhibited an advanced tissue response compared to the control group, abbreviating the initial inflammatory reaction and promoting rapid new bone matrix formation at 15 and 45 days. On the other hand, there were no significant differences between the groups at 60 days. The use of infrared LLLT directly to the injured tissue showed a biostimulating effect on bone remodeling by stimulating the modulation of the initial inflammatory response and anticipating the resolution to normal conditions at the earlier periods.

Liu et al (2007) [27] investigated the biological effects of LLLT on tibial fractures using radiographic, histological, and bone density examinations. Fourteen rabbits with surgically induced mid-tibial osteotomies were included in the study. Seven were assigned to a group receiving LLLT (LLLT-A) and the remaining seven served as a sham-treated control group (LLLT-C). A low-energy laser apparatus with a wavelength of 830 nm, and a sham laser (a similar design without laser diodes) were used for the study. Continuous outflow irradiation with a total energy density of 40 J/cm² and a power level of 200 mW/cm² was directly delivered to the skin for 50 seconds at four points along the tibial fracture site. Treatment commenced immediately post-surgery and continued once daily for 4 weeks. The results demonstrated that LLLT may accelerate the process of fracture repair or cause increases in callus volume and bone mineral density, especially in the early stages of absorbing the hematoma and bone remodeling.

Lirani-Galvão et al (2006) [28] compared the effects of LLLT and low-intensity pulsed ultrasound (LIPUS) on bone repair in rats. One group had the osteotomized limb treated with LLLT (GaAlAs laser, 780 nm, 30 mW, 112.5 J/cm²) and the second group with LIPUS (1.5 MHz, 30 mW/cm2, both for 12 sessions (five times

per week); a third group was the control. In the bending test, maximum load at failure of LLLT group was significantly higher. Bone histomorphometry revealed a significant increase in osteoblast number and surface, and osteoid volume in the LLLT group, and a significant increase in eroded and osteoclast surfaces in the LIPUS group. LIPUS enhanced bone repair by promoting bone resorption in the osteotomy area, while LLLT accelerated this process through bone formation.

Ribeiro and Matsumoto (2008) [29] studied the action of anti-COX-2 selective drug (celecoxib) on bone repair associated with laser therapy (735nm, 16J/cm²). A total of 64 rats underwent surgical bone defects in their tibias, being randomly distributed into four groups: negative control, animals treated with celecoxib, animals treated with LLLT and animals treated with celecoxib and LLLT. The animals were killed after 48 h, 7, 14 and 21 days. Statistical significant differences were observed in the quality of bone repair and quantity of formed bone between groups at 14 days after surgery for irradiated animals. COX-2 immunoreactivity was more intense in bone cells for intermediate periods evaluated in the laser-exposed groups. Taken together, such results suggest that low-level laser therapy is able to improve bone repair in the tibia of rats as a result of an up-regulation for cyclooxygenase-2 expression in bone cells.

Blaya et al. (2008) [24] evaluated the laser biomodulation of bone repair in cavities made in the femurs of rats. In Group I the complete surgical protocol to produce a bone defect was followed but without laser radiation (control). In Group II a continuous wave 830 nm infrared laser was used at 10 J/cm2 and 50 mW at each point of the surgical site. In Group III a continuous wave 685 nm infrared laser at 10J/cm2 and 35 mW was used at each point of surgical site. The animals were irradiated at intervals of 48 hours beginning immediately after the preparation of the defect and were sacrificed on the 15th, 21st, and 30th days. Greater degrees of new bone formation and vertical regeneration were found in the irradiated groups than in the control group. The authors concluded that laser therapy in this study protocol was efficient in promoting bone repair.

Javadieh et al (2009) [30] examined the effects of LLLT on a bone defect model in streptozotocin-induced diabetic rats. Twenty-eight rats were divided into five groups: 1 (diabetes, no LLLT), 2 (diabetes, LLLT high dose), 3 (diabetes, LLLT low dose), 4 (no diabetes, no LLLT), and 5 (no diabetes, LLLT low dose). A bone defect was made in the right tibia of rats in all groups. The defect in groups 2, 3, and 5 was treated with LLLT (890 nm, 70 W, 3000 Hz). Doses of 23.3 J/cm² for group 2 and 11.6 J/cm² for groups 3 and 5 were applied three times a week. The authors showed that LLLT with 11.6 J/cm² significantly increased bending stiffness and maximum force in diabetic rats compared with group 1.

Our group, in a study investigating the effects of LLLT during the process of bone healing, demonstrated that lasertherapy had a positive effect on bone consolidation. We used a 830nm laser, at 50J/cm2, during 7, 13 and 25 days to treat tibial bone defects in rats. The results pointed out intense new bone formation surrounded by highly vascularized connective tissue presenting a slight osteogenic activity, primary bone deposition was observed in the group exposed to laser in the intermediary (13 days) and late stages of repair (25 days). This was confirmed by morphometric analysis, in which statistically significant differences ($p<0.05$) were noticed when compared to control. Taken together, our results indicate that laser therapy improves bone repair in rats as depicted by histopathological and morphometric analysis, mainly at the late stages of recovery [31a].

Also, Favaro-Pipi et al (2010) [32] showed that 830nm laser (50 W/cm2, 50 J/cm², 30 mW) produced an increase in the expression of genes related to bone differentiation. The authors showed that laser irradiation produced an upregulation of BMP4, ALP and Runx 2 on day 25 after surgery, stating that laser therapy improves bone repair in rats as depicted by differential histopathological and osteogenic genes expression.

Pires-Oliveira et al (2010) [25] investigated the action of AsGA laser irradiation (904nm, 50mW, 50mJ/cm²) on bone repair in the tibia of osteopenic rats. The animals were randomly divided into eight experimental groups according to the presence of ovarian hormone (sham group) or the absence of the hormone (ovariectomy group), as well as being irradiated or non-irradiated. Low-level 904-nm laser accelerated the repair process of osteopenic fractures, especially in the initial phase of bone regeneration.

The same results were found by Kazem Shakouri et al (2010) [22] in a study showing an increased rate of bone mineral density and higher biomechanical properties in rabbits after laser irradiation (780nm, 4J/cm²). These authors stated that the use of laser could enhance callus development in the early stage of healing process and it should be recommended as an additional treatment in non-union fractures in humans.

It can be concluded that low level laser therapy acts as a proliferative stimulus on osteoblast cells and may accelerate bone metabolism and fracture healing. However, the mechanism by which LLLT acts on bone tissue is not fully understood [6]. Thus, there is a clear clinical need to understand the molecular details of the pathways that control bone formation after laser irradiation, which might allow to accelerate the healing of fractures and to treat the 5%–10% of fractures that fail to heal satisfactorily.

CONCLUSION

Many studies have demonstrated the positive effects of LLLT on bone metabolism, mainly in producing an increase in bone formation and accelerating fracture repair. Although the osteogenic effects of LLLT, there is no established protocol and there is a wide range of doses used by different authors, which are difficult to compare published results. Therefore, before LLLT can be used with confidence as a treatment within the clinical, it is necessary to investigate the mechanisms of action of this therapy to determine its safety and efficacy.

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