Original Article

Stimulatory Effects of Low Intensity Laser Therapy on the Healing of Rabbit Tibial Defects

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Abstract

The present study was to investigate the effects of low intensity laser therapy (LILT) on the healing of rabbit tibial defects. Circular defects were perforated at the medial site of tibial crests on both hind legs of 18 male New Zealand White rabbits. The defects were randomly allocated into 3 groups (n=12): 1) defects irradiated with LILT, at 4 J/cm^2 (LS4); 2) defects irradiated with LILT, at 20 J/cm^2 (LS20), and 3) defects receiving sham treatment (control group). Irradiation began 24 h after surgery, and repeated every 48 h, with a total of 8 sessions. In the 3rd and 6th weeks after surgery, the rabbits were euthanized, and the healing of defects was assessed by cone beam computed tomography, qualitative and quantitative histological techniques. Results showed that the 2 dosages of LILT similarly and significantly enhanced bone healing by increasing the bone area fraction in the 3rd week and the density of newly formed bone tissues in the 6th week after surgery. In conclusion, our findings support the hypothesis that LILT could improve the healing of the rabbit tibial defects via increased bone formation. The equal positive effects of the 2 settings suggest that the lower dosage of LILT may be more preferable and practical than the higher one in bio-stimulation of healing of bone fractures/defects.

Keywords: bone area fraction, bone density, bone healing, LILT, rabbits

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Introduction

Bone healing is the result of complex interactions among many elements including various cell types, cytokines and signaling proteins. Adjunctive treatments of bone fractures have involved bone grafts (Flierl et al., 2013), stem cells (Schmitt et al., 2012) and growth factors (Ronga et al., 2013). Although majority of treated fractures can reach union, non-union may occur in 10% of fractures (Zimmermann and Moghaddam, 2010). In this context, the use of low intensity laser therapy (LILT) in bio-stimulation of bone regeneration has been drawing substantial attention.

Laser light, once absorbed by photoreceptors including cytochrome c oxidase located in the irradiated cells, can modulate many physiological activities at cellular levels (Karu, 2010). Changes occurring in mitochondria are listed as nitric oxide release from cytochrome c oxidase's catalytic center, generation of superoxide and singlet oxygen and alteration of redox state. Those alterations resulted in increment of adenosine triphotphate production, and DNA and RNA synthesis (Simunovic, 2000).

Moreover, LILT has been shown to cause several effects on osteogenic cells. Such effects include increased osteoblast mitochondrial activity (Pires-Oliveira et al., 2008), increased expression of osteopontin, osteocalcin and alkaline phosphatase (Stein et al., 2008; Petri et al., 2010). LILT stimulates bone cells to produce insulin-like growth factor-1 (Shimizu et al., 2007), transforming growth factor-β, bone morphogenetic proteins, type I collagen (Saracino et al., 2009), cyclo-oxygenase-2, core-binding factor α-1, and vascular endothelial growth factor (Bossini et al., 2012). Increase in DNA and RNA syntheses in bone cells is also attributable to stimulation of LILT (Yamamoto et al., 2001; Stein et al., 2008). Furthermore, laser irradiation resulted in improvement on proliferation and differentiation of bone cells via expression of receptor activator of nuclear factor κB and its ligand (Aihara et al., 2006; Fujita et al., 2008). An increasing number of *in vivo* studies demonstrate that LILT stimulates bone formation (Adel et al., 2011), bone density (Kazem-Shakouri et al., 2010), collagen organization (Bossini et al., 2012) and bone mechanical strength (Son et al., 2012), and decreases duration of fracture treatment (Thanoon and Ibrahim, 2010).

Despite the positive effects of LILT on bone cells and tissues, its use as an approach for bone fracture treatment is still debatable since there has not been an established optimal dosage (Tajali et al., 2010). Energy density used in this therapy has been reported to vary from as low as 0.72 J/cm^2 (Nissan et al., 2006) to 300 J/cm^2 (Guzzardella et al., 2003). The variety of laser dosage may cause skepticism about its use in treatment of orthopedic intervention. In the present study, hypotheses that LILT could stimulate bone healing, and different dosages of laser do not express the same effects were tested. To this end, influences of 2 dosages of an 830 nm LILT on the healing of rabbit tibial defects were evaluated with the use of cone beam computed tomography and histological assessments.

Materials and Methods

Experimental animals: The present study was conducted in the agreement with the Guide for Care and Use of Laboratory Animals approved by Animal Ethics Committee of Khon Kaen University (AEKKU26/2556). Twenty 3-month-old male New Zealand White rabbits were purchased from National Laboratory Animal Center, Mahidol University, and housed at the Northeastern Laboratory Animal Center, Khon Kaen University. The animals were raised in individual metal cages, and offered food and water *ad libitum*. When the animal reached 7 months old, physical examination was performed, and blood samples were collected for hematological and biochemical analyses. Two rabbits with increased alanine aminotransferase were excluded from the experiment.

Surgical procedure: Eighteen animals (7 months old, weighing 3.5-4.5 kg) were subjected to the operation. Anesthesia was induced with an injection of ketamine (Calypsol, Gedeon Ritcher, Hugary, 20 mg/kg) combined with xylazine (X-ZINE, L.B.S Laboratory Ltd, Thailand, 3 mg/kg), and maintained with 0.5-2% isoflurane (Baxter Healthcare of Puerto Rico, USA). Intravenous administration of diazepam (Diapine, Atlantic Laboratories Corporation Ltd, Thailand, 0.5 mg/kg) was used to induce muscular relaxation. To minimize the risk of infection in rabbits, preoperative prophylaxis antibiotic was applied during the preparation of surgical sites (Enrofloxacin, Baytril, Bayer, Korea Ltd, Ansan, Korea, 10 mg/kg). Preoperative pain control was performed using tramadol hydrochloride (VESNON-V100, Vesco Pharmaceuticals Ltd, Thailand, 4 mg/kg). Regions from groin to metatarsal joint were shaved, and disinfected with povidone iodine. Both tibial crests were exposed by 2-cm long incisions from the skin at their medial sites. At each tibial crest, 2 circular full cortical defects, 3.5 mm in diameter, 4 mm apart, were punctured with a carbon bur under continuous irrigation of normal saline. Periostea and subcutaneous tissues were closed with absorbable polyglactin 910 suture (3-Vicryl, Johnson & Johnson Intl, Belgium), and skin was closed with polyamide 6.6 suture (Supramid 3.0, Kruuse, Belgium). During the first week after operation the animals were daily injected with tolfenamic acid (Tolfedine, Vétoquinol Ireland Ltd, Ireland, 4 mg/kg) and enrofloxacin (Baytril, Bayer, Korea Ltd, Ansan, Korea, 10 mg/kg) for pain relief and infection control.

Low intensity laser therapy treatment: Rabbits' legs were randomly allocated into 3 groups (12 legs/group): 1) LS4 group: defects were treated with a GaAlAs laser (BTL-5000, Hungragy) (830 mn, 200 mW, 4 J/cm^2, 20 seconds, continuous wave, irradiated area = 1 cm^2); 2) LS20 group: defects received the same treatment protocol as in group A, however, at 20 J/cm^2 and for 100 seconds; and 3) control group: defects served as sham control. The LILT treatment started 24 h after operation, with a 48-hour interval for 15 d. During the treatment, the laser probe was moved gently and evenly on the surface of the defects in a

contact manner. The rabbits were euthanized in the 3rd and 6th weeks post-operation, nine rabbits each time, by administration of an overdose of pentobarbital (Nembutal, CEVA Santé Animale-LIBOURNE, France, 100 mg/kg) through their ear veins. Tibiae were removed for evaluation of bone healing with cone beam computed tomography, and qualitative and quantitative histology.

***Cone beam computed tomography analysis*:** All defects were scanned with a cone beam computed tomography (105 kvA, 9 mA and 64.8 mAs, WhiteFox, Italy). Twelve slices, 300 µm in thickness, at sagittal view were used for evaluation of the healing of each defect with criteria modified from a previous study (Santic et al., 2009) (Table 1). Accordingly, 2 parameters were assessed, i.e. amount and radiodensity of newly formed bone. Grades of the 2 defects on the same leg were averaged to generate a final grade that was subjected to statistical analysis.

Histological analysis: Bone samples were fixed in 10% buffered formalin (VWR BDH, *Prolabo*®, Belgium), decalcified in 10% Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA, VWR BDH, *Prolabo*®, Belgium), processed in tissue processor (Shandon Citadel 2000 Tissue Processor, USA) and embedded in paraffin blocks. The samples were cut into 5 µm sections in a transverse plane, and stained with Hematoxylin and Eosin (Bio-Optica, Italy).

A score system modified from Santic et al. (2009) was employed for qualitative histological evaluation of bone healing (Table 2). Two parameters, i.e. bridging of the defects and maturation of newly formed bone tissue were assessed. The assessment was conducted under a light microscope (Primo star, Zeizz, Germany) in a blind fashion. At least 4 sections per defect were evaluated.

Criteria for quantitative histological evaluations were in accordance with those of Barushka et al. (1995) and de Almeida et al. (2013). Two parameters were examined. Bone area fraction as the area occupied by bone tissues (including mineralized, un-mineralized osteoid, bone cells buried within osteoid, and bone marrow spaces) was taken as the percentage of the original gap, and bone density as the area occupied by mineralized, un-mineralized osteoid, and bone cells buried within osteoid was taken as the percentage of bone tissue. Two sections per defect were evaluated.

To calculate the bone area fraction, stained sections were subjected to a slide scanner (VS 120, Olympus, Japan). Then, virtual slides were viewed with OlyVIA program (OlyVIA ver.2.6, Olympus, Japan), and one picture that covered the whole defect was taken from each section at 2 x 10 magnification. The pictures were then opened in AxioVision program (AxioVs40 V 4.8.2.0, Carl Zeiss Micro Imaging GmbH, Germany), and the areas (in square pixel) of bone tissues and original gap were determined by using the outline tool of the program. The bone area fraction was calculated by dividing the area of new bone by the area of the original defect.

Table 1 Criteria for cone beam computed tomography evaluation of bone healing

Radiographic appearances	**Grade**
Amount of newly formed bone	
No trace of radiodense materials in the defect	0
Trace of radiodense materials at only margins of defect	1
Substantial radiodense materials in the defect, but no bridging	2
Defects are bridged and radiodense materials fill in < 25% area of defect	3
Defects are bridged and radiodense materials fill in 25 - 50% area of defect	4
Defects are bridged and radiodense materials fill in 50 - 75% area of defect	5
Defects are bridged and radiodense materials fill in ≥ 75% area of defect	6
Radiodensity of newly formed bone	
Lower than that of native bone	0
Same as that of native bone	1

Table 2 Criteria for histological evaluation of bone healing

Histological morphological criteria	**Grade**
Bridging of defects	
No bridging	0
Bridging with fibrous tissue	1
Bridging with fibrous and cartilaginous tissue	2
Bone defect closure with bony tissue	3
Maturation of newly formed bone tissue	
No newly formed bone tissue	0
Osteoblast accumulation	1
Woven bone	2
Lamellar bone	3

To calculate density of new bone, 4 pictures (each covered 880 x 655 µm bone tissue area) of each section were captured at 10 x 10 magnification with a digital camera (AxioCam ERc 5s, Carl Zeiss MicroImaging GmbH, Germany). Subsequently, the pictures were mounted on ImageJ program (ImageJ 1.47V, http://rsb.info.nih.gov/nih-imageJ), and each picture was covered by a grid (58 x 43 crossed points). Density of new bone was calculated by dividing the total number of crossed points falling onto mineralized and un-mineralized osteoid, and bone cells buried within osteoid in the 4 pictures by the number of crossed points falling onto osteoid, bone cells buried within osteoid, and bone marrow spaces in the same 4 pictures.

Statistical analysis: Data were subjected to Shapiro-Wilk test for checking normal distribution, then difference among groups at the same time points was analyzed by using Linear Mixed Models, in which treatments were the fixed effect and rabbits were the random effect. In case significant difference among groups was detected, Least Significant Difference test (LSD) was performed for comparison of groups in pairs. Moreover, independent t-tests and Mann-Whitney U tests were used to compare difference within each group between 2 time points. All tests were conducted in SPSS program (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp).

Results

General findings: Abnormal clinical signs were not detected in any rabbits throughout the course of experiment. No rabbits suffered from postoperative complication or exhibited behavioral change. Their physiological conditions restored rapidly, and they returned to normal diet without signs of weight loss. No rabbits died during the experiment.

Cone beam computed tomography analysis: In the 3rd week after operation, 6 out of 12 defects in the control group bridged, and those in both laser groups were 8 out of 12. The lower radiodensity compared to that of native bone were observed in the defects. Interestingly, in the 6th week, all of the defects in the control group showed the bridging of their ends, while there were gaps in 1 and 2 defects in the LS4 and LS20 groups, respectively. Noticeably, all of the 3 un-bridged gaps were in the same rabbit. At this time point, newly formed bone was radiologically denser than that in the 3rd week after surgery, and its density was close to that of the native bone (Fig 1). Statistical analysis demonstrated that healing of defects among the 3 groups at the same time points and within each group between 2 time points was not significantly different (Table 3, $P > 0.05$).

Histological analysis: In the 3rd week after surgery, all defects in the 3 groups were dominantly filled with woven bone which was mostly in trabecular form. Trabeculae were separated by bone marrow spaces (Fig 2, a-c). Lamellar bone was more frequently seen in the 2 laser groups than in the control group. There was an increase in the mean of bone healing score in the 2 laser groups (5.05 ± 1.20 and 5.00 ± 1.05 in LS4 and LS20 groups, respectively, versus 4.25 ± 1.37 in the control group), however, the remarkable positive effects of LILT were not approved by statistical analysis (Table 4).

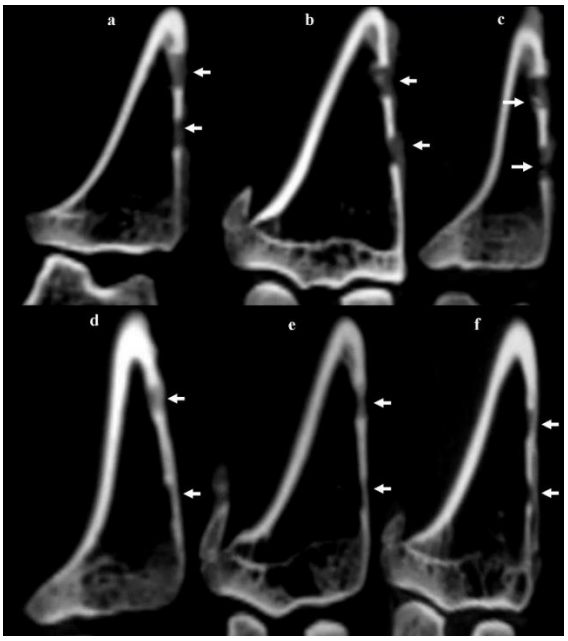

Figure 1 Representative CBCT images at sagittal view of defects (arrows). a, b, c: pictures taken from groups LS4, LS20 and control in the 3rd week, respectively; d, e, f: pictures taken from groups LS4, LS20 and control in the 6th week, respectively. In the 3rd week after surgery, radiodensity of the newly formed bone was remarkably lower than that of the native bone. In the 6th week, radiodensity was close to that of the native bone.

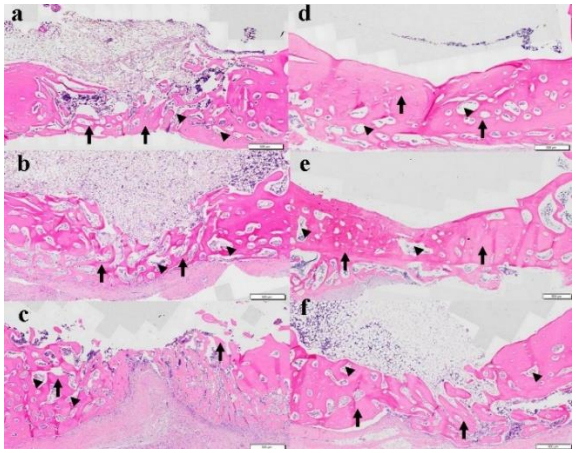

Figure 2 Representative photomicrographs of defects. a, b, c: Photomicrographs of defects in groups LS4, LS20 and control in the 3rd week, respectively. Newly formed bone (arrows) in trabecular pattern with bone marrow spaces (arrow heads). d, e, f: Photomicrographs of defects in groups LS4, LS20 and control in the 6th week, respectively. More bone tissues (arrows) were deposited between trabeculae reducing the area and number of bone marrow spaces (arrow heads). Photomicrographs were taken at 1.6 x 10 magnification. The scale bar is 500 µm. H & E staining.

In the 6th week after surgery, substantial compact bone was observed in the defects of all the 3 groups. Bone marrow spaces became smaller as the result of woven bone deposition among trabeculae (Fig 2, d-f). In the un-bridged defects the gaps were filled with fatty tissues and hematopoietic cells. In the control group, bone healing increased significantly in the 6th week compared with that in the 3rd week after surgery (p = 0.046), which did not occur in the 2 laser groups (p > 0.05). The statistical comparison among the 3 groups showed insignificant difference (Table 4).

Quantitative histological measurement demonstrated that the bone area fractions in the 2 laser groups were statistically significantly higher than that in the control group in the 3rd week after surgery (p = 0.011) (Table 5). However, in the 6th week after surgery, the significant positive effects of LILT could not be statistically proven despite a positive trend supporting the 2 laser groups (p = 0.082). No difference in bone area fraction within groups between 2 time points was detected.

The density of newly formed bone in the 2 laser groups was similar to that in the control group in the 3rd week after surgery. In the 6th week after surgery, the density of new bone in all the 3 groups significantly increased (p < 0.01), and that in the 2 laser groups was also statistically higher than that in the control group (p = 0.049) (Table 5). Quantitative histological analysis showed that the dosage of 4 J/cm² produced similar effects as the dosage of 20 J/cm² on bone healing in both the 3rd and 6th weeks after surgery.

Table 3 Degrees of bone healing assessed by cone beam computed tomography. Results are demonstrated as mean ± standard deviation.

Groups	Time after surgery	
	3rd week	6th week
LS4	5.68 ± 0.38	6.13 ± 0.44
LS20	5.58 ± 0.82	5.85 ± 0.68
Control	5.42 ± 0.65	5.84 ± 0.26

Comparison among groups at the same time point was made by using Linear Mixed Models, and within group between 2 time points by Mann Whitney U test.

Table 4 Findings of qualitative histological evaluation of bone healing. Results are demonstrated as mean ± standard deviation.

Group	Time after surgery	
	3rd week	6th week
LS4	5.05 ± 1.20	5.42 ± 0.92
LS20	5.00 ± 1.05	5.50 ± 1.22
Control	4.25 ± 1.37[a]	5.75 ± 0.27[b]

Comparison among groups at the same time point was made by using Linear Mixed Models, and within group between 2 time points by Mann Whitney U test. Different superscripts (a, b) in the same row mean significantly different within the same group between 2 time points, p < 0.05.

Table 5 Findings of quantitative histological evaluation of bone healing. Results are demonstrated as mean ± stand deviation.

Group	Bone area fraction		Bone density	
	3rd week	6th week	3rd week	6th week
LS4	73.45 ± 9.30*	77.35 ± 10.07	53.07 ± 8.34[a]	78.50 ± 5.05*[b]
LS20	70.60 ± 5.54*	71.90 ± 13.17	54.83 ± 8.75[a]	78.00 ± 7.69*[b]
Control	54.45 ± 13.30	62.60 ± 8.80	53.33 ± 7.89[a]	68.67 ± 7.61[c]

Comparison among groups at the same time point was made by using Linear Mixed Models, and within group between 2 time points by independent-t test. Comparison of groups in pairs when there was significant difference among 3 groups at the same time point was made by using LSD. Different superscripts (*) in the same column mean significantly different among groups at the same time point (p < 0.05). Different superscripts in the same rows mean significantly different within the same group at 2 different time points (a, b; $p \leq 0.001$), (a, c; p < 0.01).

Discussion

In this study, the assessment of bone healing with cone beam computed tomography did not show any significant difference between the treatment and control groups. The failure of cone beam computed tomography assessment in demonstration of positive influences of the LILT on bone healing in this study may be attributable to either the insensitive method or inappropriate time of evaluation. Some authors failed to see positive action of LILT, which they detected earlier, in the 3rd week after surgery (Matsumoto et al., 2009; Barbosa et al., 2013).

That the LILT promoted faster bone formation at the early stage of bone healing was indirectly implied by the qualitative histological findings since significantly increased bone formation was found in the control group, rather than in the 2 laser groups, in the 6th week compared with that in the 3rd week after surgery. It is suggested that the bone healing degree in the 2 laser groups in the 3rd week was already considerable, and comparable to that in the 6th week after surgery. Histological findings suggested that histological analysis, especially quantitative histology, should be employed in the evaluation of bone healing.

Our quantitative histological measure showed that both dosages of LILT used could

significantly improve bone healing by increasing bone area fraction in the 3rd week and the density of newly formed bone in the 6th week after surgery. The improved bone area fraction in the 2 laser groups in the 3rd week was in agreement with those of Favaro-Pipi et al. (2010). Those authors reported that the LILT could increase the area of newly formed bone in rat tibial defects on the 14th and 25th days after surgery. Similar findings were also demonstrated by Rosa et al. (2012), who observed an increased bone area fraction in lased rat calvaria on the 15th day post-surgery. de Almeida et al. (2013) found that the bone area fraction in the laser group was approximately 4.7 times higher than that in the control group on the 30th day of healing process. LILT has been reported to stimulate the differentiation of mesenchymal stem cells into osteoblasts in the second week of culture (Abramovitch-Gottlib et al., 2005), to upregulate the proliferation of osteoblast-like cells on the 3rd day after irradiation (Khadra et al., 2005), and to positively modulate bone nodule formation on the 21st day following treatment (Shimizu et al., 2007). Furthermore, LILT upregulates vascularization and angiogenesis of rat tibial defects on the 14th day after surgery (Bossini et al., 2012). Those stimulating effects of LILT may result in advanced tissue response (Pretel et al., 2007), and earlier and faster bone formation (Barushka et al., 1995; Son et al., 2012), which may explain the increased bone volume fraction in lased groups in our study in the 3rd week after surgery.

In the 6th week after surgery, remarkable effects of the laser on bone area fraction were not seen in this study. Similar results were reported by da Silva and Camilli (2006), who stated that laser promoted bone formation during the first 2 weeks, but the same effects were not seen during the 4th and 24th weeks. Such observation was also indicated by Matsumoto et al. (2009), who found positive effects of laser on bone formation on the 14th day rather than the 21st day post-operation. Obradovic et al. (2007) succeeded in the 2nd and 6th weeks, however, failed in the 8th week in their attempts to see the increment of bone area fraction in the laser group compared to that in the control group. In the same fashion, LILT upregulated bone formation on the 15th and 45th days but failed to have stimulating effects on the 60th day (Pretel et al., 2007). Ozawa et al. (1998) suggested that stimulating effects of LILT were significant at the stages of proliferation and early differentiation of immature progenitor cells rather than at the later stages. The LILT treatment in this study was applied during 15 d after surgery, which was referred to as the stage of cellular proliferation. Furthermore, according to Javadieh et al. (2009), 3 weeks was probably a sufficient time for biological bone repair, which was independent of the LILT. In our study, at the later stage of bone healing, the fact that most of the defects were substantially filled with the bone tissues might have resulted in insignificant difference in bone area fraction among the groups.

Although LILT did not significantly increase bone area fraction in the 6th week after surgery, it was responsible for the increased density of newly formed bone tissues at this stage. Kazem-Shakouri et al. (2010) claimed that LILT treatment could improve the bone density of rabbit osteotomized tibiae during an 8-week study. Furthermore, prevention of trabecular bone loss in osteoporotic rats by using LILT has recently been suggested (Ko et al., 2013). Consistently, LILT can increase trabecular thickness and decrease trabecular separation resulting in increased bone density (Ninomiya et al., 2007). In the present study, the increased bone area fraction at the early stage of bone healing might produce larger number of functional osteoblasts at the later stage in the 2 laser groups. Increase in number of osteoblasts might augment the osteoid secretion resulting in more new woven bone depositing between trabeculae (Aspenberg and Sandberg, 2013) and advanced incorporation of different trabeculae in a single structure (Cacchioli et al., 2006). In consequence, the density of newly formed bone tissues in both laser groups increased compared with that in the control group.

Observing the positive effects of LILT on bone density at later rather than earlier stage of bone healing process in this study is of interest. Barbosa et al. (2013) used optical densitometry to analyze the action of LILT on bone mineralization and concluded that in the 2nd week instead of the 3rd week bone density of lased tissues was higher than that in the control group. It is important to notice that in the present study the density of newly formed bone was measured based on the amount of the bony tissues including mineralized, un-mineralized osteoid, and bone cells buried within osteoid rather than on the mineralization of tissues. Therefore, different trends of LILT action on the density of new bone among studies might be attributable to the disparity in measurement methods.

Previous studies suggested that different dosages of LILT might generate different effects on bone cells (Aihara et al., 2006) and bone tissues (da Silva and Camilli, 2006). Aihara et al. (2006) studied the impact of 4 dosages of laser on growth of bone cells and found that the dosages of 9.33, 27.99 and 55.98 J/cm^2 promoted osteoclast differentiation and activation, whereas the dosage of 93.30 J/cm^2 failed to have similar results. da Silva and Camilli (2006) used 2 dosages of laser, i.e. 5.1 and 10.2 J/cm^2, and confirmed that the higher dosage might better increase the bone regeneration of rat skull defect. Our results supported those of Bossini et al. (2012) that 2 dosages of 60 J/cm^2 and 120 J/cm^2 had similar potential to accelerate the bone repair of osteoporotic rats. Moreover, the osteoblastic cells' attachment and proliferation were equally induced by 2 LILT dosages of 1.5 J/cm^2 and 3 J/cm^2 in an *in vitro* study (Khadra et al., 2005). Similar stimulating effects of the 2 dosages of LILT in this study suggested that a minimum dosage of laser may be sufficient for promotion of bone healing, and may be preferable since it saves time and energy, and eases animal restraint.

It is worth pointing out limitations of the present study. Since the evaluation of the defect healing was performed in the 3rd and 6th weeks after surgery, other interesting information about effects of LILT on bone healing might be absent. An earlier sampling with one week interval until the completeness of healing process would help one understand thoroughly the action of LILT on repair of bony tissues.

In conclusion, our findings supported our hypothesis that LILT accelerated the healing of rabbit tibial defects by increasing bone area fraction at the earlier stage, and bone density at the later stage of the bone healing process. Similar promotive results of the 2 settings lead to a suggestion that the lower dosage, automatically followed by a shorter duration, may be more convenient for the treatment of bone fractures/defects. As the mechanism of LILT at bone cell level should be similar in all bones and species, therefore, the dosage setting in the present study could be applied to other bones and species that are under similar condition.

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บทคัดย่อ

ผลการกระตุ้นของแสงเลเซอร์ที่มีพลังงานต่ำต่อขบวนการซ่อมแซมกระดูกหน้าแข้งในกระต่าย

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การวิจัยครั้งนี้ใช้กระต่ายเพศผู้ สายพันธุ์ New Zealand White อายุ 7 เดือน น้ำหนักระหว่าง 3.5-4.5 กิโลกรัม จำนวน 18 ตัว โดยมีวัตถุประสงค์เพื่อศึกษาถึงผลของการใช้แสงเลเซอร์ที่มีพลังงานต่ำ (low intensity laser therapy, LILT) ต่อขบวนการซ่อมแซมของกระดูกหน้าแข้ง (tibia) ทำกระดูกหน้าแข้งส่วนด้านในของขาหลังทั้งสองข้างให้เป็นรูวงกลม จากนั้นสุ่มแบ่งการทดลองออกเป็น 3 กลุ่ม คือ กลุ่มที่ 1 ได้รับการรักษาด้วย LILT ที่ตั้งค่าพลังงาน 4 J/cm^2 (LS4) กลุ่มที่ 2 ได้รับการรักษาด้วย LILT ที่ตั้งค่าพลังงาน 20 J/cm^2 (LS20) และกลุ่มที่ 3 เป็นกลุ่มควบคุมซึ่งไม่ได้ใช้ LILT โดยการใช้ LILT เริ่มครั้งแรกที่ 24 ชั่วโมงหลังการผ่าตัด และทำซ้ำทุกๆ 48 ชั่วโมง รวมทั้งสิ้นจำนวน 8 ครั้ง กลุ่มกระต่ายได้รับการทำการุณยฆาต 3 และ 6 สัปดาห์ภายหลังการผ่าตัด จากนั้นบริเวณกระดูกที่ถูกทำให้เป็นรูได้รับการประเมินขบวนการซ่อมแซมโดยการใช้เอกซเรย์คอมพิวเตอร์ (cone beam computed tomography) และการศึกษาทางจุลพยาธิวิทยาเชิงคุณภาพและเชิงปริมาณ จากการศึกษาพบว่าการใช้ LILT ช่วยเร่งขบวนการซ่อมแซมของกระดูกอย่างมีนัยสำคัญ โดยพบการเพิ่มขึ้นของ bone area fraction ภายหลังการผ่าตัดที่ 3 สัปดาห์ และการเพิ่มความหนาแน่นของ newly formed bone tissues ภายหลังการผ่าตัดที่ 6 สัปดาห์ จากผลการศึกษาจึงสามารถสรุปได้ว่า LILT ช่วยกระตุ้นขบวนการซ่อมแซมของกระดูกโดยเฉพาะอย่างยิ่งในช่วงแรก อย่างไรก็ตามไม่พบความแตกต่างในขบวนการหายอย่างมีนัยสำคัญระหว่างกลุ่มที่ใช้ค่าพลังงานของ LILT ต่างกัน ดังนั้นการใช้ LILT ที่ค่าพลังงานต่ำกว่าที่ 4 J/cm^2 จึงเพียงพอและเหมาะสมในการใช้รักษา

คำสำคัญ: สัดส่วนพื้นที่กระดูก ความหนาแน่นกระดูก การซ่อมแซมกระดูก แสงเลเซอร์พลังงานต่ำ กระต่าย

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