

Effect of irradiation of 810nm laser on bone for 10 sec: A rabbit histological study

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Abstract: Introduction and Objective

In last decade, low level laser therapy has been evaluated for stimulation and acceleration of bone formation. In spite of promising results, biphasic 'dose' response remains. Moreover, the use of single session of low level laser on healing of bone is not explored thoroughly. The aim of this study was to determine the optimal 'dosage' for formation of bone using diode laser of 810nm under single irradiation.

Materials and Methods

Six New Zealand male rabbits were used weighing 1.5-2 Kgs and 8-10 months old for the study. Femur was chosen as site of surgery. The centre of the femur was drilled using implant osteotomy drills to the size of 2.8mm in width and 6mm in depth. 810nm Diode laser (GaAlAs, AMD Picasso®) was used in this study. Laser parameters were, wavelength of 810nm, power of 90mW, time of 10 seconds in continuous mode using the disposable fibre of 300µmm diameter in light noncontact. Contra lateral femur was used as a control and the laser was sham treated. At the end of 2 weeks samples were collected from the surgical area and slides were prepared and analysed histologically.

Results

At 14th day, the lased group showed slight areas of haemorrhage in marrow cavity with no evidence of bone formation in lased site.

Conclusion

The results of the present study using 810nm, 90mW, for 10 sec for single session did not stimulate formation of new bone in two weeks.

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I. Introduction

The term "laser" is an acronym for (L)ight (A)mplification by (S)timulated (E)mission of (R)adiation. The "light" is generally accepted to be electromagnetic radiation. The visible spectrum ranges from approximately 400 to 700 nm. The wavelength range from 700 nm to 10 µm is considered the near infrared (NIR), and anything beyond that is the far infrared (FIR). Lasers are divided into High level laser therapy (HLLLT) and low level laser therapy (LLLT). HLLLT consists of power ranging from 0.5W to 20W. During HLLLT series of photothermal events takes place such as carbonization or burn off (> 200°C), vaporization (> 100°C), vacuolization (97.5°C), coagulation (> 68°C), protein degradation (> 75°C), protein denaturation (> 40°C), thermal photoactivation (< 40°C) from the center to periphery of laser beam. All are photosurgical events. There is also nonthermal photoactivation zone present (< 36.5°C) and that is duly referred as low level laser zone.¹ Use of LLLT in animals and patients almost exclusively involves red and near-infrared light (600-1100-nm).^{2,3} There have been a large number of both animal model and clinical studies that demonstrated highly beneficial LLLT effects on a variety of diseases, injuries, and has been widely used in both chronic and acute conditions. The exact mechanism of action of LILT in biomodulation of bone healing is not known. However, previous studies have proposed number mechanisms. The results of an animal study in rabbits showed an

increased mechanical strength of the interface between the implants and bone by LLLT. The mechanism of action was attributed to an increased metabolic speed and consequently a more rapid healing process⁴. Another animal study in rats showed more bone matrix organization in sites irradiated with low-intensity laser. It was assumed that LLLT could stimulate the collagen fibers to arrange in a lamellar structure⁵. Another study showed that LLLT could stimulate the mineralization in the process of new bone formation in surgically created bony defects⁶. On the other hand, it was stated that the biostimulating effect of LLLT on bone remodeling in surgically induced bony defects might be due to stimulating the modulation of the initial inflammatory response⁷. A study in rabbits showed that the efficacy of LLLT in accelerating the process of distraction osteogenesis was related to its effect on bone turnover and consolidation⁸. Another study in the tibia of rats stated that the improvement of bone repair by LILT was due to its role in up-regulation of cyclooxygenase-2 expression in bone cells⁹. A biphasic response has been demonstrated many times in LLLT research^{10,11}. The expected dose response to light differs at a subcellular, cellular, tissue or clinical level. It can be interpreted that if more than required energy applied it may lead to bioinhibition and if insufficient energy is applied, then there will be no stimulation as the minimum threshold has not been met. So there exists an optimal window of energy as and when it is utilized then there will be biostimulation. Irradiation of laser did not have any effect on healing of hard tissues^{12,13}. In spite of promising results obtained from invitro and animal studies, the biphasic dose response remains and different results for use of different choice of lasers, wavelengths, energy density, power density, method of delivery and frequency of treatment. The single use photo biomodulation therapy on healing of bone is not explored thoroughly. The single session of LLLT is rarely used compared to multiple times as it has shown stimulation, proliferation and maturation of cells of bone.^{14,15,16} But the use of single session has an advantage of less time consumption and compliance by the patient.

So, study is undertaken to evaluate the use of low level gallium aluminium arsenide (GaAlAs) diode laser on healing of bone using 810nm wavelength, at power of 90mW, using the fibre of 300µm with single irradiation for 10 sec.

II. Materials and Method

The study was carried out adhering to guidelines of the CPCSEA and institutional ethical committee. Six New Zealand male rabbits were used weighing 1.5-2 Kgs and 8 months old for the study. Before the surgery the animals were anesthetized using ketamine (15mg/Kg) and xylazine (10mg/Kg.). Antibiotic prophylaxis started prior to the surgery (ceftriaxone 500mg). Femur was chosen as site of surgery, the skin overlying the femur was shaved and disinfected with Povidone-Iodine solution. 3 cm incision was given upto the bone on the lateral aspect of the femur exposing the underlying fascia and bone. The muscles were retracted using surgical elevators. The centre of the femur was drilled using implant osteotomy drills and widened 0.8 mm under copious irrigation of normal saline. Final dimension of osteotomy site was 2.8mm in width and 6mm in depth.(Fig.1,2) The sites were cleaned with irrigation of saline. 810nm Diode laser (GaAlAs, AMD Picasso®) was used in this study. Laser parameters were wavelength of 810nm, 90mW, for 10 seconds in continuous mode using the disposable fibre of 300µm diameter in light noncontact with uninitiated tip. Several points were chosen for irradiation of laser along apex, mid-medial, and mid-lateral of osteotomy site.(Fig.2) Contralateral femur was used as a control and the laser was sham treated. After the irradiation, the surgical site was sutured in layers using catgut 2.0. Post operative antibiotics (ceftriaxone 500mg) was continued twice daily IM for 5 days. They were kept in the animal house and were fed vegetable diet. At the end of 2 weeks all animal were euthanized using high dose of Thiopental sodium. Samples were collected from the surgical area and restored in 10% buffered formalin. After that specimens were subjected to 4 % EDTA for demineralization. Longitudinal cuts were made to divide the bone in to two halves. Then slides of 10µm thickness is prepared and stained with Haematoxylin and eosin (H.E. stain, Merck). All tissue specimens were examined by light microscopy and were assessed histologically using compound microscope (Olympus) .

III. Results

Descriptive histology carried out. H/E section shows compact bone with osteocytes within lacunae, haversian system, along with the connective tissue is bone marrow with adipose tissue on the border, scanty lymphomatous distribution (Fig 3,4,). Areas of haemorrhage are seen in the marrow spaces. (Fig 5,6) There is no evidence of new bone formation is seen. (Fig 3,4,5,6)

IV. Discussion

Wound healing is complex process involving numerous cells, enzymes, inflammatory mediators, growth factors. Bone healing is slow compared to soft tissue healing and involves complex processes.¹⁷ The bone healing mechanism can be hindered by local factors such as trauma, infection, and radio-osteonecrosis or systemic factors such as Paget Disease, Fibrous Dysplasia, Diabetes Mellitus, the use of corticosteroids or hormonal disturbances.^{18,19} To date, several methods such as bone grafts, growth factors, platelet-rich fibrin, low-

level pulsed ultrasound and low-level laser therapy have been proposed to promote bone healing.^{20,21,22} Among all these methods, use of LLLT outshines other modalities due to the advantage of being non-invasive and the ease of application in clinical use.

Photobiomodulation (PBM) is the latest term used to replace the LLLT. PBM gives the better understanding of parameters of laser and its effect on tissues. It signifies the non-thermal application and tissue temperature equal or less to the body temperature. It differs from antibacterial and antifungal effects of Photodynamic therapy (PDT) and high power laser. Laser wavelength used for PBM includes visible 650nm, 660nm, near infrared 780nm, 810nm, 830nm, 940nm and infrared 1040nm, 2940nm and 10,6400nm.²³ PBM is used in wound healing, reduction of postoperative pain, regeneration of nerve, treatment of trismus and swelling. It has bio modulatory effect on tissues leading to repair of nerve injury, regulation of hormone and immune system, relief of pain, inflammation and acceleration of healing of bone, epithelial and fibroblast proliferation.²⁴⁻³²

The aim of our study was to investigate the effect of GaAlAs low level diode laser under single irradiation on healing of bone defect. Our study showed that irradiation with diode laser for 10 sec did not stimulate new bone formation. The use of laser in healing of wound is controversial and has conflicting reports. The laser exhibits biphasic dose response and it depends on type of laser and irradiation dose. If the irradiation dose or time is increased too high then there is an inhibition and they are too low then there will be no response.³³ Wavelength dependent effects are seen on various cells such as green light has an effect on fibroblasts and lymphocytes and red light was on fibroblasts and epithelial cells and phagocytic activity of leucocytes³⁴ and infrared on osteoblasts.³⁵ Studies by Rosa et al³⁶ Horva't karajz K,³⁷ Hou J,³⁸ Abramovovitch Gottlieb L,³⁹ Mvula B,⁴⁰ Eduard⁴¹, showed positive effect of laser although the fluence varied in these studies from 1-5 J/cm². Study by Rodrigo Ré Poppi⁴² et al. evaluated the effects of LLLT (660- and 808-nm wavelengths) on the process of repairing bone defects induced in the femurs of female rats submitted to ovariectomy. Bilateral ovariectomies were performed on 18 female Wistar rats, which were divided into control and irradiated groups after the digital analysis of bone density showed decreased bone mass and after standardized drilling of the femurs. The irradiated groups received 133 J/cm² of AsGaAl (660-nm) and InGaAlP (880-nm) laser radiation. The animals were euthanized on days 14 and 21 after the bone defects were established. Detailed descriptive histological evaluations were performed, followed by semi-quantitative histomorphometry. The results from days 14 and 21 showed that the irradiated groups presented increased density of osteoblasts, fibroblasts, and immature osteocytes on the tissue surface compared with the control (non-irradiated) groups (p<0.05). Additionally, inflammatory infiltrate evaluations showed that LLLT decreased the accumulation of leukocytes when compared to the control treatment (p<0.05). Authors concluded that both wavelengths (660-nm and 880-nm) inhibited the inflammatory process and induced the proliferation of cells responsible for bone remodeling and repair. Saito S, Shimizu N⁴³ investigated the effects of low-power laser irradiation on bone regeneration during expansion of a midpalatal suture in rats. Gallium-aluminum-arsenide diode laser 100 mW irradiation was applied to the midpalatal suture during expansion carried out over 7 days (3 or 10 minutes per day), 3 days (7 minutes per day for day 0-2 or 4-6), and 1 day (21 uninterrupted minutes on day 0). The bone regeneration in the midpalatal suture estimated by histomorphometric method in the 7-day irradiation group showed significant acceleration at 1.2- to 1.4-fold compared with that in the nonirradiated rats, and this increased rate was irradiation dose-dependent. Irradiation during the early period of expansion (days 0 to 2) was most effective, whereas neither the later period (days 4 to 6) nor the one-time irradiation had any effect on bone regeneration. These findings suggest that low-power laser irradiation can accelerate bone regeneration in a midpalatal suture during rapid palatal expansion and that this effect is dependent not only on the total laser irradiation dosage but also on the timing and frequency of irradiation. Authors suggested laser therapy may be of therapeutic benefit in inhibiting relapse and shortening the retention period through acceleration of bone regeneration in the midpalatal suture.

On the contrary, studies by Coombe et al,⁴⁴ Baguette⁴⁵ et al, Stein et al⁴⁶, Bergetgaz⁴⁷, showed no difference between proliferation and differentiation of cells of bone in laser and control groups. Bölükbaşı Ateş G, Ak Can A, Gülsoy M⁴⁷ conducted detailed in vitro study, the osteoblast cells were irradiated with 635 and 809 nm diode lasers at energy densities of 0.5, 1, and 2 J/cm². Cell viability, proliferation, bone formation, and osteoblast differentiation were evaluated by methylthiazole tetrazolium (MTT) assay, Alamar Blue assay, acridine orange/propidium iodide staining, alkaline phosphatase (ALP) activity, Alizarin red staining, and reverse-transcription polymerase chain reaction (RT-PCR) to test the expression of collagen type I, ALPL, and osteocalcin. The results indicate that studied energy doses have a transient effect (48 h after laser irradiation) on the osteoblast viability and proliferation. Similarly, laser irradiation did not appear to have any effect on ALP activity. These results were confirmed by RT-PCR analysis of osteoblast markers. Authors concluded that several irradiation parameters and variations in the methods should be clearly established in the laboratory before laser treatment becomes a postulated application for bone tissue regeneration in clinical level. Atasoy KT, Korkmaz YT, Odacı E, Hancı H⁴⁸ evaluated the efficacy of low-level 940 nm laser therapy with energy

intensities of 5, 10 and 20 J/cm² on bone healing in an animal model. A total of 48 female adult Wistar rats underwent surgery to create bone defects in the right tibias. Low-level laser therapy (LLLT) was applied immediately after surgery and on post-operative days 2, 4, 6, 8, 10 and 12 in three study groups with energy intensities of 5 J/cm², 10 J/cm² and 20 J/cm² using a 940 nm Gallium-Aluminium-Arsenide (Ga-Al-As) laser, while one control group underwent only the tibia defect surgery. All animals were sacrificed 4 or 8 weeks post-surgery. Fibroblasts, osteoblasts, osteocytes, osteoclasts and newly formed vessels were evaluated by a histological examination. No significant change was observed in the number of osteocytes, osteoblasts, osteoclasts and newly formed vessels at either time period across all laser groups. Although LLLT with the 10 J/cm² energy density increased fibroblast activity at the 4th week in comparison with the 5 and 20 J/cm² groups, no significant change was observed between the laser groups and the control group. These results indicate that low-level 940 nm laser with different energy intensities may not have marked effects on the bone healing process in both phases of bone formation. In their study Irradiation was performed in continuous wave mode for 10 seconds and there were , no significant change was observed between the laser groups and the control group. In their study, although the different energy densities were achieved by altering the power applied during a constant irradiation time of 10 seconds, LLLT showed no substantial promoting effect on the repair process of bone defects. It may be suggested that the time of irradiation applied to the tissue in the protocol was not sufficient to stimulate bone metabolism. In our study also there was formation of new bone, but results were not statistically significant. Pedro silva et.al⁴⁹ did not find any positive effect of laser on growth of cells. Red and infrared laser at 90J/cm² and 150J/cm² did not stimulate preosteoblast cell growth and ALP production invitro. Increased dosage may lead to early conflux of cells in time after that they have tendency to inhibit and die⁵⁰. Authors attributed lack of results to single irradiation, methodology used and high doses of laser.

The different doses, application protocols and experimental models complicate comparisons between the studies. Many variables may affect the LLLT biostimulatory effects such as, laser wavelength, energy, exposition time, power, and the biologic state of the cell, power density, beam profile, energy density, number and frequency of treatment and duration of treatment.

V. Conclusion

The results of the present study using 810nm, 90mW, for 30 sec for single session did not stimulate formation of new bone in two weeks. So the above parameters can be regarded as ineffective dose for formation of new bone.

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Figures:

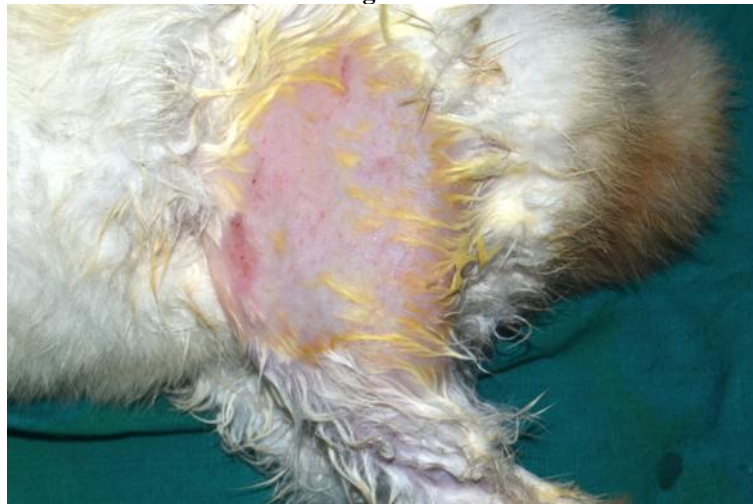


Fig1: Femur of rabbit

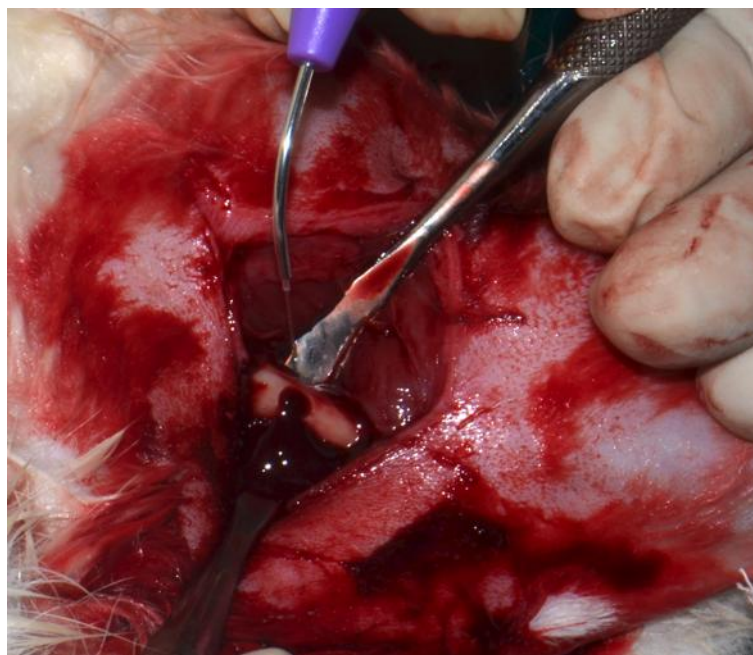


Fig2: osteotomy site and laser irradiation

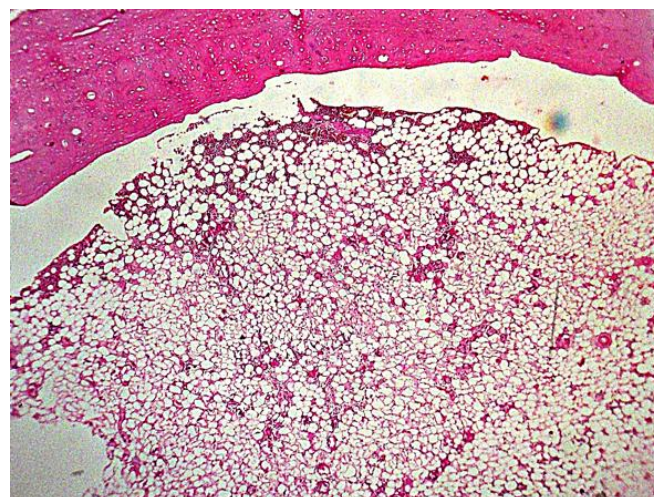


Fig3: Control (4X)

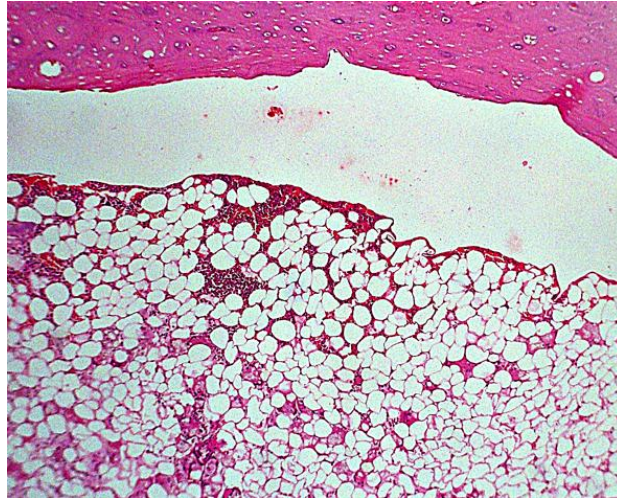


Fig 4: Control (10X)

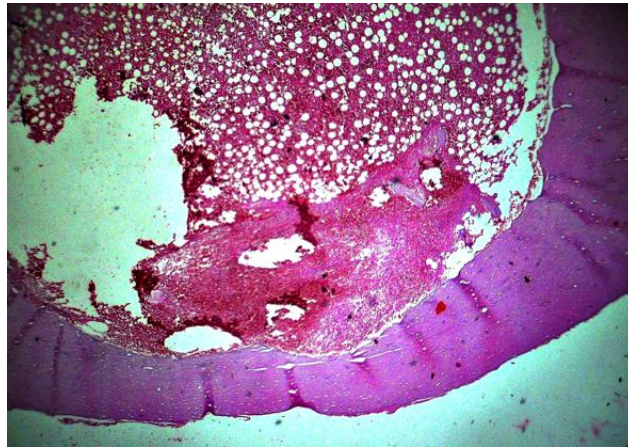


Fig 5: Laser (4X)

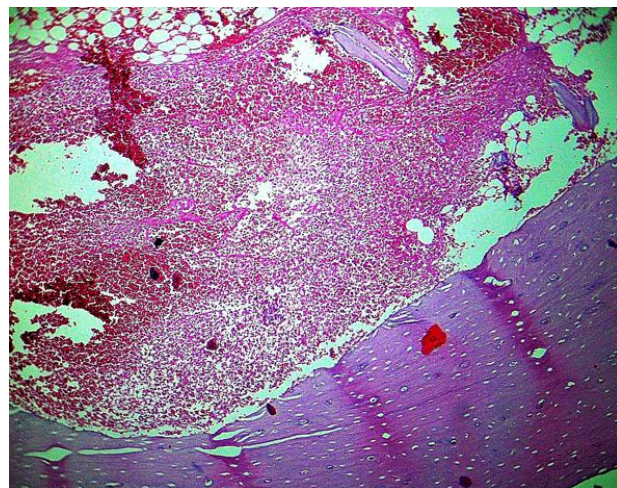


Fig 6: Laser (10X)

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