Effect of Low Level Laser Therapy along with the Application of DFDBA on Bone Repair in Rabbit Tibial Defects

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Objective: Low level laser therapy is increasingly used in medicine and dentistry. The purpose of this study was to evaluate the effect of GaAlAs (Gallium- Aluminum- Arsenide) low level laser along with the application of DFDBA on bone healing in rabbit tibial defect.

Methods: This experimental study was done on 12 male Albino rabbits with an approximate weight of 1.5kg and about 6 months of age. In order to perform the surgery, animals were anesthetized with intravenous injection of 10% Ketamine and 2% Xylazine. An artificial defect was created with an implant drill (4 mm height and 2.8 mm diameter) in tibial bone of rabbits and filled with DFDBA. Then, the lesions were irradiated with continuous mode GaAlAr laser (808 nm, 6 J/cm²) according to the protocol (10 days of irradiation, 14 days of rest, 10 days of irradiation, 14 days of rest). No irradiation was done in the control group. After 48 days, the animals were sacrificed, sections were made from the tibia and the specimens underwent histological analysis. Wilcoxon Signed Rank test was used for statistical analysis of the obtained data.

Results: Histological assessment revealed significantly greater osteogenesis and angiogenesis in the laser group (P<0.05).

Conclusion: The present study findings showed that GaAlAs laser irradiation along with the application of DFDBA accelerated healing and resulted in greater angiogenesis and osteogenesis in rabbit tibial defects.

Key words: Low level laser, Bone defect, DFDBA

Please cite this article as follows:

Received: 28.02.2012 Final Revision: _________ Accepted: 01.03.2012

Introduction:

Repair of bone defects caused by surgical procedures, congenital diseases, trauma, tooth extraction and etc. is among the concerns of patients and physicians. Several factors affect the rate of bone remodeling and orthodontic tooth movement velocity in the alveolar bone including the metabolic factors i.e. parathormone and estrogen, applied loads, injection of drugs, electrical stimulation and ultrasound application. Parathormone (PTH) increases and estrogen decreases bone formation. Also, strains smaller than 1000 με accelerate bone formation while strains greater than 2000 με decelerate it. Long-term application of above mentioned factors is associated with patients’ pain and discomfort (1-5).

Low level laser therapy (LLLT) is a new suggested technique for bone remodeling with several advantages. It reduces pain and inflammation and stimulates collagen formation and cell proliferation (6). LLLT can also affect
the function and activity of bone cells and result in proliferation of surface osteoblasts. Increased thickness of the osteoid has been reported in the irradiated area as well (3). Furthermore, laser radiation does not have the adverse side effects of the systemic (either orally or injection) application of chemicals (7).

Different lasers like helium-neon (HeNe, 632.8 nm wavelength), Gallium- Aluminum- Arsenide (GaAlAs, 805±25 nm wavelength) and Gallium- Arsenide (GaAr, 904 nm wavelength) have been used in various dosages and treatment regimens for therapeutic purposes (8, 9). Use of GaAlAs laser has gained considerable popularity in the past 10 years. This laser has greater depth of penetration compared to other lasers and therefore seems to be an appropriate means for repair of bone defects (10).

Allografts are a group of graft materials with various applications in reconstruction of bone defects. Demineralized freeze dried bone allograft (DFDBA) and freeze dried bone allograft (FDBA) are the two major forms of allografts. FDBA has been studied in animal models and humans and its biologic compatibility and positive clinical effects have been well confirmed (11, 12). DFDBA has also been evaluated in some studies for induction of osteogenesis and treatment of periodontal lesions. Conflicting results have been reported regarding the characteristics of the above mentioned graft materials and some studies have shown the superiority of bone induction following DFDBA application (12). A few others believe in the superiority of FDBA in this respect (13) and a third group found no significant difference in bone induction between the two materials (14).

The present study sought to assess the effect of low level GaAlAr laser therapy along with the application of DFDBA on repair of rabbit tibial defects.

Methods:

This experimental study was conducted on 12 Albino rabbits with an approximate weight of 1.5 kg and age of 6 months. All rabbits were generally healthy and had the same living environment (23°C temperature, 12/12 light cycle and similar nutrition). Rabbits were all matched in terms of complete general health and full growth. In order to perform the surgery, the rabbits were anesthetized with intravenous injection of 10% Ketamine and 2% Xylazine. An artificial defect was created in the tibial bone (2.8 mm diameter and 4 mm height) using an implant drill and filled with DFDBA. DFDBA powder (CenoBone, HamanandSazBaft, Iran) was mixed with an equal volume of saline solution and prepared to be implanted inside the defect. Six rabbits in the laser group underwent continuous mode GaAlAs laser irradiation (808 nm wavelength, 6 J/Cm2) of the defect site according to the set protocol (10 days of radiation, 14 days of rest, 10 days of radiation, 14 days of rest). Six rabbits in the control group did not receive any radiation.

Forty-eight days later, the animals were sacrificed after deep anesthesia with 10% Ketamine. The legs were separated and samples were placed in 10% formalin solution followed by nitric acid solution for 10 days for decalcification. After passage of samples, the specimens were immersed in 10% formalin solution for 24 hours. For dehydration, samples were placed in alcohol solutions with increasing concentrations as follows:

- 90 minutes in 70% alcohol solution
- 90 minutes in 80% alcohol solution
- 150 minutes in 96% alcohol solution
- 150 minutes in 100% alcohol solution

Samples were then stored in Xylol for 2 hours and immersed in melting paraffin for 8 to 18 hours at 56-67°C to become embedded. Paraffin embedded blocks containing tissues were cut with rotary microtome and several sections were
made of each sample. After sectioning, samples were stored in dry heat at 80-110°C and were then stained with Hematoxylin and Eosin (H & E). The slides were assessed under light microscope (E-400, Nikon, Japan) for signs of bone formation, presence of inflammation, angiogenesis and interaction with connective tissue. For the qualitative variable of bone formation, score 1 (low), 2 (moderate) or 3 (high) was allocated to the samples based on the amount of trabeculae and angiogenesis. Data were analyzed using Wilcoxon Signed Rank test.

Results:

The qualitative score of bone formation was 2 and 3 in the control and laser groups, respectively. Score of angiogenesis was 1 in the control group and 3 in laser group. Thus, laser radiation can significantly enhance bone formation in repair of bone defects (P<0.05).

Table 1. Quantitative scores of osteogenesis and angiogenesis in the two groups

<table>
<thead>
<tr>
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<th>Osteogenesis</th>
<th>Angiogenesis</th>
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<tbody>
<tr>
<td>Laser</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>1</td>
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In microscopic analysis of the samples obtained from the control group, collagen fibers, blood vessels and bone trabeculae were observed in the underlying connective tissue along with an osteoblastic rim and lacunae containing osteocytes. Bone trabeculae were normal and no considerable inflammation was detected.

In microscopic evaluation of the laser group, collagen fibers, blood vessels and bone trabeculae were seen in the underlying connective tissue along with an osteoblastic rim and lacunae containing osteocytes. Bone trabeculae were normal and no significant inflammation was observed. Rates of osteogenesis and angiogenesis were higher in the laser group compared to the control group (P<0.05).

Discussion:

The mechanism of action of low level laser has yet to be fully understood. However, it seems to have a photochemical entity. The process involves increased cell proliferation as the result of photochemical reactions following absorption of small doses of laser radiation by intercellular chromophores in the mitochondria (15-17). On the other hand, this multifactorial mechanism induces angiogenesis (18), collagen formation (19), proliferation and differentiation of osteogenic cells (20), reduction of mitochondria and ATP synthesis (21, 22). Additionally, LLLT can increase local blood circulation and result in subsequent increase in circulatory cells, nutrition, oxygenation and inorganic salt content of the bone defect (23). Yoshihide in 1999 showed that in tissues receiving LL irradiation, intraosseous blood flow and oxygen tension increased for about 80% and 15%, respectively (24). Kawasaki and Shimizu (2000) reported that low level laser therapy increased the number of osteoclasts at the pressure side during an experimental tooth movement in rats (5).

Due to the varying oxidation and reduction states and subsequent change in cell pH, one cell can respond differently to the irradiation of a specific type of laser. The effect of laser becomes more complicated when complex mechanisms are involved or more than one cell type is present at the time of radiation. Furthermore, in some cases the effect of radiation is not only reinforced or weakened, but also its entity changes and becomes inhibiting or stimulating alternately (25).

The immunologic, neurologic and physiologic processes involved in osteogenesis, chondrogenesis and repair of bone defects in a
host are so extensive, complicated and full of mutual interactions that drawing a clear cause and effect pathway to show the effects of a specific type of laser irradiation is extremely difficult and almost impossible based on the current available data. On the other hand, there are numerous confounding factors responsible for the controversial results in different studies with apparently similar in-vitro conditions such as the host immunity reactions against laser irradiation, metabolic status and cell pH at the onset of radiation, actual, paradoxical and unknown effects of tissue mediators on autocrine, paracrine and endocrine reactions and last but not least complex effects of various types of lasers on these unknown reactions. However, role of intensity and power of laser, method of laser radiation, cell culture technique and histological analysis methods in this respect cannot be ignored (25).

In the present study we used an external control group since the systemic effects of LLLT have been documented in several studies (26-28). It has been revealed that LLLT can enhance the release of growth factors and cytokines into the bloodstream and affect the nonirradiated side of the body in animals or humans as well. The mentioned issue may be the reason why some studies that used an internal control group could not find significant differences between the radiated and control (other side of the human or animal body) groups. Selection of GaAlAs low level laser was because of its repeated use in other studies.

GaAlAs laser used in the present study has a deeper penetration compared to other lasers and is available for use by the clinicians as an effective penetrating tool. It has been reported that radiation of 820-840 nm GaAlAs laser has a greater tissue penetration due to the reduction in water and hemoglobin absorption (10, 29).

In a study by Nicola et al, on 48 rats, it was concluded that LLLT increases the activity of bone cells at the site of repair without changing the bone structure (30). In a study by Bossini et al, in 2012 on 30 rats suffering from osteoporosis it was demonstrated that LLLT enhanced bone repair by inducing new bone formation, fibrovascularization and angiogenesis (31). In a study by Kang et al, in 2011 similar results were reported in cortical bone of osteoporotic rats with minimally invasive laser needle system (32). In another study da Silva (2011) showed that LLLT after REM increases the proliferation and expression of osteoblastic cells in mid-palatal suture (33). For DFDBA the reported results have been controversial. Koutouzis and Lundgren in 2010 revealed that implants inserted in post-extraction sockets augmented with DFDBA had a bone loss similar to that of implants in native bone (34). Pinheiro et al, in their study in 2003 created a defect on the femur of 24 rats and divided them into three groups of control, inorganic bone and inorganic bone plus low level laser therapy. They observed a more advanced repair in the inorganic bone group compared to controls. Also, bone repair was the greatest in the group of laser irradiation plus inorganic bone (35). In another study, Lee and coworkers (2010) showed that bone regeneration effects of human bone substitutes were not significantly different for repair of bone defects (36).

Based on the available literature in this respect, low level laser therapy has had a significant effect on repair of bone defects. However, for DFDBA, the obtained results have been conflicting. The present study evaluated the simultaneous effect of LLLT and DFDBA and revealed improved bone formation.

Conclusion:

GaAlAs laser irradiation along with the use of DFDBA enhances the repair of bone defects. For better comparison of the results and evaluation
of other therapeutic effects of low level lasers on pain and inflammation, further studies are required to evaluate GaAlAs laser radiation at various power and energy levels and application of other low level lasers like helium-neon.

Acknowledgement

The present article was originally the doctoral thesis of Faezeh Atri. Thesis supervisor was Dr. Masood Seifi from School of Dentistry, Shahid Beheshti University of Medical Sciences.

References:


