Effect of low-level laser therapy on fracture healing in rabbits

Fahimeh Kamali Sarvestani 1,2, Nasrin Salehi Dehno 1, Seifollah Dehghani Nazhvani 3, Mohammad Hadi Bagheri 4, Soheila Abbasi 1, Yasaman Khademolhosseini 1, Elham Gorji 1

1: School of rehabilitation, Shiraz University of Medical Sciences, Shiraz, Iran
2: Rehabilitation Science Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3: School of Veterinary Medicine, Shiraz University, Shiraz, Iran
4: Medical Imaging Research Center, Department of Radiology, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

Background and aims: The purpose of this study was to investigate the effect of low-level laser therapy (LLLT) on radial bone fracture gap healing in a rabbit model.

Materials (Subjects) and Methods: Thirty male white New Zealand rabbits under general anesthesia had a 3mm slice of radial bone surgically removed. Fifteen rabbits were treated by 830 nm laser at 4 J/cm² and 15 were used as non-treated controls. Callus development was assessed by X-ray and radiographs every 7 days for 3 weeks.

Results: Significant radiologic changes were observed in both groups against time (P < 0.001) or from week to week (P < 0.05). However, there was no statistical difference in radiologic scores after week 2 (P = 0.087) or week 3 (P = 0.077) between control and laser treated bone.

Conclusions: Findings suggest that in this study, laser treatment did not enhance callus formation nor reduce repair time of complete fracture of the radius in rabbits.

Key words: bone • fracture healing • low level laser therapy

Introduction

Millions of fractures are reported annually around the world. Although the treatment techniques have been advanced in the last few decades, 5%-10% of fractures yet face with delayed union or nonunion. 1) Fracture repair involves complex physiological and biochemical reactions and is different from soft tissue healing. 2) In recent decades cellular activity in bone tissue has been stimulated using energy emission such as ultrasound, electrical stimulation and laser. 3-6) Recently the use of low level laser therapy (LLLT) has received attention due to its great potential on a variety of pathological condition including wound healing, 7) improving vascular density, 8) reducing pain and modulating inflammation. 9)

Laser therapy can be applied in the presence of metal devices that have been used to stabilize fractures without any thermal damage to the tissue. 10, 11) LLLT is a non-invasive treatment technique and has a relatively low cost. 12) Various experimental studies have demonstrated beneficial effects of LLLT on bone metabolism and fracture consolidation. 13-16) Sella et al. reported that the beneficial effects of LLLT in fracture healing involved not only reducing inflammation but also enhancing the reparative phase of bone repair. 17) Furthermore, Pretel et al. suggested that LLLT optimizes the tissue repair process by stimulating the healing capacity of the connective tissue as well as vessel formation from pre-existing vessels. 18)
without a gap. Contact between the bone fragments might provide a more appropriate environment for union and enhance bone repair, therefore, partial osteotomy may be not sufficiently reliable to demonstrate the efficacy of lasers as bio modulatory therapies for bone healing. Whether LLLT can affect healing in complete bone fracture is yet to be determined. This study was conducted to investigate the effect of LLLT on callus formation after complete radial bone fracture in rabbits.

Materials and Methods

This study was approved by the animal rights committee of the Shiraz University of Medical Science for dealing with laboratory animals. Thirty male white New Zealand rabbits weighing 2.0 ± 0.2 kg were housed individually in standard cages. Room temperature was maintained at about 22°C with a 12-hour light-dark cycle. Rabbits were provided free access to food and water. Each animal was anesthetized by intramuscular injection of ketamine Hcl (50 mg/kg) and Xylazin Hcl (5 mg/kg). The right foreleg was prepared for aseptic operation. An incision was made through the skin and subcutaneous tissue over the craniomedial aspect of the radius. The radial bone was exposed, a slice of 3 mm in thickness was removed by an orthopedic saw 3 cm distal to the elbow joint. The area was washed by saline and the fascia and skin was sutured. The rabbits received analgesic and antibiotics following operation for three days. The animals were allowed free activity in individual standard cages. Radiographs were obtained immediately after surgery to measure the size of the fracture gap between the bone fragments. The animals were randomly assigned to the experimental LLLT group or the untreated control group. Rabbits in the experimental group was treated with low-intensity GaAlAs laser (Azor-2k, Azor Medical Equipments, Moscow, Russia) at 830 nm wave length, power 50 mW, energy density 4 J/cm², pulse width 180 nm and frequency 1500 Hz, for 290 s, 3 times per week in 9 sessions during 21 days. The first dose was given 24 h after surgery. The laser was applied directly to the skin at three points around the fracture site: one point on the osteotomy site and two points 1 cm away on either side of the lesion site. The duration of laser emission was 2 min at the lesion site and 1 min 25 s at each of the two lateral points. During laser application both the forelegs and hind legs were immobilized in a straight, fully extended position. Callus development was assessed by X-ray. Radiographs were obtained from the operative limb in 2 views (lateral and antero posterior) at the end of each week for 3 weeks. All X-ray images were assessed by a radiologist who was blind to groups. Healing was assessed by radiological sings according to the table 1.

Statistical analysis

The Non-parametric Mann-Whitney Test was used to compare score of radiologic changes during fracture healing between groups. Intra-group time-dependent score changes were analyzed by Friedman's test, and Wilcoxon. Signal Rank test was used to evaluate the changes within groups at each time point using SPSS 19.

Results

Radiologic changes were evaluated at 1, 2, and 3 post-operative weeks. Figure 1 shows bone repair in each group over time. Friedman's test showed significant radiologic changes in both groups against time (P=0.001), and from weak to weak (p<0.005) (table 2).

While there were no or minimal change after week 1 in both groups, after week 2 and week 3, high-
er score of radiologic changes were observed in the experimental group than in the control group; but, the difference was not statistically significant (Table 3).

Discussion

Prosser et al classified the radiologic changes in fractures into 6 categories. 22) In the present study the short-term follow-up period and the small size of the radial bone made it difficult to distinguish between category 2 (subperiosteal new bone formation) and category 3 (loss of fracture line definition). We combine Prosser’s categories 1 to 3 into 1 category 1 (no or minimal change). Bone healing evaluated by X-ray was thus scored from 1 to 4 according to the presence of callus formation (Table 1). Results showed that laser application was not able to significantly enhance callus formation. Although according to radiologic change higher grade of callus formation in the laser group than in the control group, indicating that LLLT accelerated bone healing, the difference was not statistically significant. Effects of laser therapy on bone healing are known to be dose dependent 23) and variations in laser setups and types of lasers may account for conflicting results. 18, 24-25)

In this study we used a wavelength of 830 nm to treat the fractures. This wavelength allows light to penetrate deep enough to reach the bone and thus speed healing by increasing mitochondrial metabolism in bone cells. 14, 26-27) The use of an energy density of 4 J/cm² was based on previous evidence of positive effects on bone and soft tissue in the range of 1-5 J/cm². 25) The evaluation periods (3 times per week for 3 weeks) were likewise similar to those in other studies. 28, 29) This period corresponds to the time needed for callus formation. 30) The use of LLLT, 3 times per week during 21 days has a potent effect on hematoma formation. 29) The cell proliferation phase during initial stages of the healing process led to greater laser light absorption, suggesting that LLLT has a more favorable effect at this stage than later, as previously described. 3, 10, 14, 28) The positive effects of LLLT on bone formation have been widely described, and many researchers who investigated the effects of LLLT on bone healing have concluded that laser treatment might enhance vascularization, promoting faster osseous tissue formation and thereby increasing bone cell activity within the fracture area. 9, 13, 20) However, previous studies used injury models with contact between the bone fragments. 10, 13, 20, 21) Bone defects without a gap are apparently not sufficiently reliable to demonstrate the contribution of laser in promoting bone repair.

Our finding confirm those of The results of Liu et al., who applied LLLT on complete fractures of the tibia and found no statistically significant difference in callus thickness between the treated and control groups. 3) Another recent study investigated the effect of LLLT on complete bone fractures by removing a 2-mm fragment from the middle third of the femoral

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<tr>
<th>Table 2: Mean weekly differences in score of radiologic changes in each group</th>
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<td>Postoperative week</td>
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<td>Weeks 1 and 3</td>
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<td>P Friedman’s test</td>
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Values are mean rank

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<th>Table 3: Comparison of mean score of radiologic changes between two groups</th>
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<tr>
<td>Postoperative period</td>
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<td>Week 2</td>
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Values are mean rank
shaft in rats, and concluded that LLLT played an important role in augmenting bone tissue formation, which was relevant to fracture healing. They also suggested a possible connection between the emergence of osteonectin and the formation of periosteum, both of which are involved in callus formation. Although the results of our study are not consistent with the findings by Sella et al., it was noticed that they did not evaluate the reductions in bone gaps radiologically.

Regarding the variations of methodological design and the absence of a standardized protocol for the application of LLLT, it would be difficult to compare the results of present study with other studies of LLLT on bone fracture healing. Therefore the effectiveness of laser therapy in bone formation and repair remains controversial. Further studies with different LLLT protocols, different types of fractures and longer evaluation period will be required to explain the effect of LLLT on bone healing.

In conclusion there was no significant radiographic changes between laser-treated and control (untreated) bone fractures in rabbits, although the previous studies suggest bio stimulatory effect of low-level laser therapy on the bone healing process.

Conclusions

The study finding revealed no significant radiographic difference between laser-treated and control (untreated) bone fractures in rabbits, although the previous studies suggest bio stimulatory effect of low-level laser therapy on the bone healing process.

References


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Conflict of interest
The authors declare that they have no conflict of interest.