ADVANCES IN LASER THERAPY FOR BONE REPAIR

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During the last decade, it was discovered that low-power laser irradiation has stimulatory effects on bone cell proliferation and gene expression. The purposes of this review are to analyze the effects of low- power laser irradiation on bone cells and bone fracture repair, to examine what has been done so far, and to explore the additional works needed in this area. The studies reviewed show how laser therapy can be used to enhance bone repair at cell and tissue levels. As noted by researchers, laser properties, the combinations of wavelength and energy dose need to be carefully chosen so as to yield bone stimulation. With better study designs, the results will be more credible, allowing for greater recognition of advances in bone repair using laser therapy. Many studies on the effects of laser therapy on bone healing and fracture repair have used biochemical and histological methods. However, in order to establish the effects of laser treatment on bone, additional studies need to be performed using biomechanical tests, the ultimate evidence of bone repair. Finally, future studies are needed to demonstrate that the same bone stimulation effects occurring in animals may also be seen in humans.

Keywords: Laser therapy, fracture healing.

Introduction

During the last decade, it was discovered that low-power laser irradiation has stimulatory effects on bone tissue, in the microscopic (cell proliferation [1-5] and gene expression [6] and macroscopic [1,2,4,12,13-20] biological systems. In order to understand the effects of laser therapy, its mechanism of action in the cell needs to be established. Many explanations have been proposed {7-11]. Studies have shown that poryphrins and cytochroms, natural photoacceptors located in the cell, are the main contributors to laser-tissue interaction [7-11]. Porphyrins and cytochroms absorb the light into the cell, resulting in the production of singlet $^1O_2$. The singlet oxygen then stimulates the redox activity in the mitochondria, enhances chemiosmosis, DNA production and calcium-ion influx into the cytoplasm, therby causing mitosis and cell proliferation.

The purpose of this review is to analyze the effects of low-power laser irradiation on bone cells and bone fracture repair, examine what has been done so far, and propose areas warranting further exploration.

Results and Comments

1. *Bone Cell Response to Laser Therapy*

Yamada [3] studied the biological effects of laser irradiation on cloned osteoblastic cells. The cells, prepared from newborn mouse calvaria, were irradiated with an 8.5 mW HeNE laser, 632.8 nm and .01-1.0 J/cm^2 densities. Cultured cells in the growing phase were irradiated on the second day. Cell growth and DNA synthesis were increased in the growing phase. Proliferation of the irradiated cells was significant after 2-3 days of irradiation, compared to the controls. DNA synthesis on the basis of 3H-thymidine also increased with a laser dose of 1.0 J/cm^2, resulting in a 32% increase over control values. After four irradiation sessions with a laser dose of 1.0 J/cm^2, Ca^{2+} concentration increased by 46%, compared to the control group. No significant increase in alkaline phosphatase activity was observed. The authors concluded that laser therapy photoactivates osteoblastic cells, accelerates proliferation of osteoprogenitor cells, enhances osteoblastic calcification, and may promote bone regeneration.

In another study, Barushka et al.4, with biochemical and histomorphometric methods, studied the effects of HeNe laser irradiation on bone repair in the rat tibia. The control rats were subjected to red light (660 nm, 0.4 J/cm) and the irradiated rats were subjected to 6.0mW HeNe laser (632 nm). During bone healing, the peak of alkaline phosphatase (ALP) is a good marker for osteoblastic and pre-osteoblastic activity, and the peak of tartrate-resistant acid phosphatase (TRAP) is a good marker for osteoclastic activity. The study showed that low-power laser irradiation can change the activity and/or the number of osteoclasts and osteoblasts as evidenced by changes in ALP and TRAP activity. As a unit, it can cause bone repair to occur approximately twice as fast in an in vivo model.

Luger et al. [2] studied cultured clonal bone cells exposed to various wavelengths and several energy densities of different lasers. The light sources were a CW 632.8 nm 35 mW HeNe laser, and diode lasers: CW 635 nm (3 mW output power); CW 650 nm (3 mW); CW 670 nm (5 mW); CW 780 nm (40 mW), and CW 830 nm (20 mW). Laser irradiation was administered 24 hours after incubation of cells. Radiation times included 15s, 30s, 1min and 7min for each laser. Increased DNA synthesis, i.e. increased cell growth, was significant in the 632.8 nm, 635 nm and 830 nm irradiated samples, as compared with the non-irradiated group. The highest increase was obtained using the 632.8 nm wavelength and 52 J/cm^2 total density. When longer wavelengths (650, 670, 780, 830 nm) were used, the [3H] thymidine uptake changes never exceeded 20%, in spite of some results being statistically significant compared to their respective controls. In addition, at 780 nm with 52 J/cm^2, a decrease in DNA synthesis was observed. These results confirm the findings of Yamada [3] and Barushka et al. [4] that low-power laser irradiation applied to cultured osteoblasts can induce bone cell proliferation.

Recently, Ueda et al. [5] used a GaAlAs low-power laser with two different modes (continuous irradiation and pulsed irradiation) to evaluate cell proliferation. Samples were derived from fetal rat calvarial cells and irradiated after 1 day with a dose of 0.48-3.84 J/cm2. Cell proliferation was significantly greater in the irradiated group, compared to the control. ALP increased late in the culture, with peak expression seen at 18 days for controls, 15 days for continuous irradiation, and 12 days for the pulse-irradiated group. Both irradiated groups significantly stimulated cellular proliferation, bone nodule formation, ALP activity and ALP gene expression, compared to their control counterparts. Furthermore, pulsed irradiation stimulated these factors even more than continuous irradiation. Therefore, it is most probable that pulse frequency is an important factor affecting biological responses to bone formation [5].

Noguerol et al. [15] studied mouse bone tissue using electron microscopy. Thirty-six mice were divided into three groups: irradiated with a laser dose of 10.5 J/cm^2, irradiated with a laser dose of 31.52 J/cm^2, and a control non-irradiated group. A 7mW 632.8nm HeNe laser was used. The bone tissue was examined immediately after laser irradiation and again 10 days after irradiation. Electron-microscopy showed two differentiated osteocytic populations, the first consisting of osteocytes compatible with those in the normal state. These cells were seen in the control non-irradiated group and in the group, which received the lower irradiated dose. A higher dose of HeNe laser irradiation caused degeneration of osteoblasts. These cell alterations included a star-like image, chromatin condensation and, in some cases, complete nuclear destruction. Cellular degeneration was discovered mostly in the group irradited with the high doses and seldom in the group irradited with low doses.

2. *Bone Injury and Fracture Response to Laser Therapy*

In 1987, Trelles and Mayayo [1] performed histological measurements to determine bone repair with low-power laser irradiation. Sixty mice were divided into two equal groups - irradiated and control. An experimental tibial fracture of the hind leg, produced by digital pressure, was performed on all mice. Focusing on the fracture site, a 4mW HeNe 632 nm laser was used with a treatment schedule of 10 min per session (2.4 Joules) every 2nd day, for a total of 12 sessions. The control group was given simulated treatment with no irradiation. Electron-microscopy of the histological samples was done at the end of the experiment. After 4 irradiation sessions, a well-formed fracture, active trabecula and condocyte formation was seen in the irradiated animals. A greater amount of callus was observed in the irradiated sample, compared to the non-irradiated one. The control animal osteocytes showed a dense and more abundant chromatin. Statistically significant differences were found in the thickness of the periosteum and neoformed trabecula of the irradiated group, compared to the controls.

Chen et al.[13] studied the effect of a CO_2 laser on bone repair. Experimental bilateral mandibular osteotomies were performed on 24 rabbits. A hole-like osteotomy, 3mm in diameter was made in the ramus. The rabbits were divided into 4 groups, matched for weight and sex. The irradiated side of each rabbit was chosen at random, and the opposite side was non-irradiated, representing the control group. A CO_2 laser system, 2W, with a power den-

sity of 255 mW/cm^2 (laser dose =153 J/cm^2) was used. The treatment was given daily, 10 min. per session for 7 days. After the animals were sacrificed, the calcium content in the irradiated calluses was found to be significantly higher than in the control calluses on the 14th, 21st, 28th post-operation days, but not on the 7th day. Phosphorus content was also reported higher than that of the contralateral bone. Therefore, calcium and phosphorus contents can be significantly increased by laser irradiation. Hydroxproline content of the irradiated callus was significantly higher than in the contralateral side within 28 days, indicating that laser irradiation stimulates bone collagen production. Since collagen synthesis is normally enhanced in a reparative process, this evidence suggests that laser treatment aides in increasing this synthesis.

Dickson et al.[14] used bone tissues extracted from murine femoral fractures to investigate the effect of GaAlAs (l= 820-830 nm) laser irradiation on alkaline phosphatase (ALP) activity and ATP. It is known that the ALP enzyme is important for calcification of bone and cartilage in normal growth and repair. ALP levels were lower in sham-irradiated femurs than in irradiated ones at all energy densities (5,10,15 J/cm^2). Compared to the control group, the 10 J/cm^2 and 15 J/cm^2 doses resulted in increased ATP levels; the 10 J/cm^2 dose yielded a significant increase in ALP levels, compared to the 15 J/cm^2 dose. Glinkowski et al. [16] studied laser therapy effect on tibial bone fracture in mice. The authors measured bone optical density from radiographs using a densitometer. An experimental tibial fracture was produced in 18 mice using Boroque's procedure. The mice were divided into three groups: first, non-irradiated control, second, irradiated with an 830nm continuous wave\ 30mW diode laser, and third, treated with a 904nm pulsed 30W (peak power) diode laser. Laser treatment was started one day following fracture. The laser dose for both irradiated groups was 4J/cm^2 for 21 days. The animals were sacrificed after the last laser treatment. Callus optical density in the pulse-irradiated group was higher than in the continuous laser-irradiated and control groups. The laser effect of pulsed irradiation was higher than with continuous laser irradiation. Barushka et al. [4] studied the effect of HeNe laser irradiation on the process of bone repair in 292 rat tibiae. An experimental 1.6 mm hole was drilled in the cortical bone of the tibia. A HeNe laser (632nm, 6.0 mW, 31 J/cm^2) was used to irradiate the site of injury on days 5 and 6 post-injury, once a day for 2 min. In the laser group, a significant increase in ALP activity was found in the repaired tissue collected on days 10 and 11 post-injury ($p < 0.01$, $p < 0.05$, respectively). The amount of new reparative bone in the hole injury increased between days 10 and 15 post-injury both in the control and laser-irradiated rats; however, the filling of the hole injury with new reparative compact bone was more rapid in the laser-irradiated tibiae, compared to control ($p < 0.05$).

David et al. [17], using a laser treatment protocol similar to Trelles and Mayayo [1], studied the effects of HeNe laser on fracture healing in rats. Sixty-two rats underwent bilateral osteotomies of the tibiae followed by internal fixation with intramedullary Kirschner wires. The rats were divided into 3 groups and then further subdivided into 3 subgroups. The right leg received laser irradiation (HeNe laser, cW, 10mW, 632.8nm of 0, 2, or 4 Joules, depending on the group) every other day for 2-6 weeks, while the left leg served as the control. Therefore, the exposures per session of the different subgroups were 0, 28, and 56 J/cm^2. Radiological, biomechanical, and histological effects of HeNe laser irradiation were then evaluated. Radiographically, no significant difference was observed between the irradiated and non-irradiated legs within the same group, regardless of energy dose. A gradual increase in healing was observed over time in all test groups, similar in the irradiated and non-irradiated legs. The bones were tested for bone strength using an Instron machine and 4-point bending method. The force/deflexion ratios, representing stiffness of the osteotomy site, were measured. The only significant result was observed in two subgroups, showing the non-irradiated osteotomy site to be stiffer than the irradiated one. Histological examination also did not show any significant effect of the laser on the fracture healing process. In the early stages, mesenchymal tissue was predominant, gradually changing into cartilagenous tissue, and bone was present finally after 6 weeks. Therefore, the radiological, histological, and biomechanical results failed to demonstrate any stimulatory effect of 10mW HeNe laser.

Luger et al. [18] also studied the therapeutic effects of low-power laser irradiation on the mechanical properties of bone fracture healing in rats in a randomized double blind study. Fifty rats were divided into two groups: the first group was treated with laser and the second was used as a control group. An experimental fracture of the tibia was made by first inserting a Kirschner wire into the medullary cavity of the right tibia, progressing down toward the distal end of the tibia. Three holes were then drilled in the midshaft of the tibia. The tibia was broken by light manual bending while the Kirshner wire was held in place. Immediately after the operation, laser irradiation was administered to the first group. A 632.8 nm, 35mW HeNe laser with continuous wave was used for 30 minutes each day for 14 consecutive days. The overall dose of irra-

diation used on the skin in this study was chosen due to the finding that skin reduces the energy level of the HeNe laser beam to 3-6% of its original intensity (Nissan et al. [21]). Thus, the energy levels between 26 J/cm^2 to 52 J/cm^2 that were needed to induce proliferation of bone cells, as shown in our previous work (Luger et al. [2]) could be achieved. The five parameters studied included: maximum load at failure, stress high load, maximum load extension, structural stiffness of the tibia (calus stiffness) and maximum sample area of the callus (mm^2). The bones were tested at tension until failure using a Lloyd LR 50K testing device. The following parameters were found to be significantly higher in the laser-irradiated group, compared to their control counterpart: maximum load at failure ($p= 0.014$) , stress high yield ($p= 0.016$) and callus stiffness ($p= 0.0023$). The differences between the properties of the calluses of the two groups indicated that callus in the non-irradiated group tended to be larger in volume, weaker in strength, more fibrocartilaginous and less ossified, while the bone in the irradiated group had already begun to unite and, therefore, the callus was reabsorbed. Another interesting finding was that gross non-union of fracture at the time of examination (4 weeks post-surgery) was found in 4 of the 19 rats in the control group, but in none of the irradiated group. This may be another parameter which shows the positive effect of laser therapy on rat bone fracture healing.

Recently, Morrone et al. [19] studied the effect of a GaALAS laser on the osteochondral lesion repair of the knee in rabbits. Surgical procedures were done on 18 rabbits and experimental lesions were made on both sides. An arthrotomy of each knee was performed and an osteochondral lesion of 2.5 mm in diameter and 2 mm in depth was drilled. All the left lesions were irradiated immediately with a GaAlAs diode laser (1W, wC=780 nm, 300J/cm^2, 10 min). The laser-treated condyles showed earlier repair of bone (at 2 weeks post-operation), compared to the controls (55% versus 39% $p = 0.043$). The irradiated condyles showed earlier healing and better quality of the reconstituted tissue, compared to untreated condyles. After 6 and 12 weeks, the matrix revealed more hyaline-like cells mixed with fibrocartilage, than in the untreated specimens. In addition, the reconstitution of the articular surface appeared more regular and complete in the laser-treated condyles, while the untreated ones showed an abnormal bony growth beyond the articular profile with a fibrous coating present even at 12 weeks. In fact, better and faster healing of the surgical wound, as well as a lower incidence of swelling in the knee, were observed in the laser-treated lesion, compared to the control group.

In our last study Shlomi et al. [20], the effectiveness of low-power laser irradiation on the organic and non-organic components of the bone, using infrared spectroscopy as one of the most informative and accurate methods for physical-chemical analysis of bone, were investigated. The high informative value of the infra-red spectroscopy method enables us to understand the particular features of the structure of bone tissue, and to identify the chemical composition of its main components at the molecular level. The bone injury in rats was induced by penetration of alveolar processes of the mandibular bone using a 3 mm drill. In the laser-treated group, the intensity of absorption of the inorganic component in the region of 850-1200 cm^{-1} increased 56 % compared to the control injured area, and decreased only 11.4% in the intact bone. The wavelength characteristics of the inorganic component remained unchanged, i.e. the organic component was similar to intact bone. For quantitative analysis of the regenerative bone process, the Mineralization Index was used:

$$MI = \frac{API}{APO}$$

Where MI = Mineralized index, API = Absorption peak of inorganic group and APO = Absorption peak of organic group.

Mineralization Index in the laser-treated group increased significantly to 1.86 compared to 0.63 in the control group. An increase in this Index indicates an intensive regenerative bone process.

Bone Cells and Tissue Sensitivity for Laser Irradiation

In 1991, Friedmann et al. [11] discovered that a certain amount of energy is needed to enhance cell proliferation. Higher doses causes the production of too much energy, leading to cell damage. In a few studies on bone tissue the lower or higher doses of low power laser irradiation showed no positive response. Luger et al. [2] found that when wavelengths greater than 632.8 nm were used, the labeled thymidine uptake never exceeded a 20% change, causing little effect on bone cells. At 780 nm, a decrease in DNA synthesis was observed. For bone tissue, Dickson et al. [14] found no significant effect with a 5 J/cm^2 energy dose. Noguerol et al.15 showed that mice bone tissue irradiated at 31 J/cm2 caused cell deformation and destruction. In addition, David et al. [17] found no significant effects with laser doses (0 J/cm^2, 28 J/cm^2, 56 J/cm^2) on bone.

Conclusions

Laser therapy can be used to enhance bone repair at the

cell and tissue levels. However, as suggested by researchers [15,18,19,22], laser properties need to be carefully chosen. Since bone is sensitive to laser irradiation, the combinations of wavelength and energy dose should be carefully chosen so as to yield bone stimulation. In the studies reviewed, in which gas-type lasers (GaAlAs, CO_2, HeNe) were used with continuous and pulsed irradiations, wavelengths of 632.8 and 780 nm and energy doses between 10-52 J/cm^2 showed positive results. David et al. [17] found no significant effect on bone repair using the HeNe laser. However, the doses the authors used were very low, for *in vivo* study, which may suggest no effect with laser therapy. Glinkowski and Rowinski [16] and Ueda and Shimizu [5] found pulsed irradiation gave even better results than continuous irradiation. The influence of low-power laser irradiation on bone repair has been more accurately proven with the use of biomechanical tests.

Recommendations for Future Studies

Many studies on the contribution of laser therapy to bone healing and fracture repair have used biochemical and histological methods. However, in order to establish the effects of laser on bone, additional studies should be performed using biomechanical tests, the ultimate evidence for bone strength. In addition, further randomized studies with pulsed and continuous laser irradiations should be done, since a few authors suggest no better results using pulsed laser than treatment with continuous laser irradiation [5,16,19]. Finally, future studies are needed to prove that the same bone stimulation effects occurring in animals may also be seen in human.

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Congress host and WALT President Nick Nicolopoulos (second from left) pose for a picture with dignitaries at the Third Congress of WALT, May, 2000

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Professor Chukuka S. Enwemeka greets Professor Krishna Rao and his wife (India) when they meet at the U.S.A. 2000 Congress of the Japanese Soceity for Lasers in Surgery and Medicine. Professor Rao was serving as the President-Elect of the International Soceity for Lasers in Surgery and Medicine at that time.