The Effects of Helium-Neon Light Therapy on Healing of Partial Osteotomy of the Tibia in Streptozotocin Induced Diabetic Rats

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Abstract

Objective: The effect of light therapy (LT) on surgically created partial osteotomy in streptozotocin (STZ)-induced diabetic rats was examined. Background Data: LT has been shown to enhance bone repair in healthy human and animal models. Materials and Methods: Forty male rats were divided into groups 1 to 5. Diabetes was induced in rats of groups 1, 2, and 3 using an intraperitoneal injection of STZ. All diabetic rats were maintained for 30 days after STZ injection. Under general anesthesia and sterile conditions, a partial transversal standardized osteotomy was made in the mid-portion of the right tibia. The defects in groups 2, 3, and 5 were treated using a helium-neon (He–Ne) laser (632.8 nm, 10 mW, circular beam shape). Groups 1 and 4 were diabetic placebo and normal placebo groups, respectively. A dose of 369.4 J/cm² for groups 2 and 5 and a dose of 66.8 J/cm² for group 3 were applied three times a week. Six weeks after surgery, the right tibia was collected. The specimen was subjected to a three-point bending test. Results: LT with 369.4 J/cm² energy density resulted in significantly greater bending stiffness in group 5 (41.8/C6 5.2) than in groups 1 (18.5/C6 4.1), 2 (17.7/C6 1.6), and 3 (11.5 ± 4) (least significant difference (LSD) test, p < 0.01, p < 0.001, and p < 0.001, respectively). LT with 369.4 J/cm² energy density resulted in a significantly higher stress load in group 5 (10/C6 0.4) than in groups 1 (4.9/C6 1.5), 2 (5.7/C6 0.52), and 3 (3.9/C6 1.1) (LSD test, p < 0.01, p < 0.01, p < 0.001, respectively). Conclusion: LT with a He–Ne laser in STZ-induced diabetic rats did not enhance bone repair of a partial transversal standardized osteotomy.

Introduction

Diabetes mellitus (DM) is a heterogeneous group of disorders characterized by high blood glucose levels.1 DM, for a long time considered a disease of minor significance to world health, is now taking its place as one of the main threats to human health in the 21st century.2 It is the most common noncommunicable disease worldwide and the fourth to fifth leading cause of death in developed countries.3 The global figure of people with DMs set to rise from current estimates of 150 million to 220 million in 2010 and 300 million in 2025.4 DM has profound effect on various systems of the human body, including musculoskeletal abnormalities such as decreased bone volume affecting especially the trabecular bone,5,6 diminished bone formation,7–9 retarded bone healing,10 decreased biomechanical parameters,11,12 and osteoporosis.13 It is estimated that each year 260,000 American suffer an ankle fracture, 25% of whom undergo surgical stabilization.14 Ganesh et al. have reported significant increases in in-hospital mortality, rates of in-hospital postoperative complications, duration of hospital stay, rates of nonroutine discharge, and total charges diabetic patients with DM.15

The term light therapy (LT) in the present investigation is applied to the therapeutic effects of lasers. The use of LT as a therapeutic modality was originally prescribed in Europe more than 30 years ago. Professor Endre Mester in Hungary reported the earliest application of LT in medicine in 1968. He described how irradiation with ruby and argon lasers at low intensity accelerated the healing of chronic ulcers.16 There are reports that LT can improve fracture healing. Motomura et al.17 revealed that a helium-neon (He–Ne) laser stimulated callus formation. Trelles and Mayayo18
demonstrated that laser biostimulation significantly increased vascularization and modulated the formation of osseous tissue in fractured tibiae in mice. Luger et al.\textsuperscript{19} showed the positive effect of LT on healing fracture by measuring the biomechanical properties of the repairing bone. In Luger et al.,\textsuperscript{19} healing indices such as maximum load and energy absorption of the bones were shown to have improved significantly after LT. Recently, it has been indicated that He–Ne laser irradiation stimulated the growth of trabecular area and hastened organization of matrix collagen.\textsuperscript{20} More recently, Rochkind et al.\textsuperscript{21} demonstrated that the use of the He–Ne laser for repair of normal bone defects can significantly improve the quality of recovery and decrease recovery time. The objective of the current study was to survey the effects of LT on surgically created osteotomy defects in rats with streptozotocin (STZ)-induced DM. The results are demonstrated according to biomechanical parameters.

Materials and Methods

Animals and study design

Forty adult (4 months old) male Wistar rats weighing 275 ± 7.7 g were used in this study. Rats were divided into five groups. They were provided with food and water ad libitum and were weighed twice a week throughout the study. DM was induced in rats of groups 1, 2, and 3. A partial transversal standardized osteotomy was made with a drill in the right tibiae of all rats. The right tibiae of groups 2, 3, and 5 were treated using a He–Ne laser. Groups 1 and 4 were diabetic and nondiabetic placebo groups. Six weeks after surgery, the rats were sacrificed and their right tibiae submitted to a biomechanical test. The institutional medical ethics committee approved all procedures.

Induction of DM

DM was induced in rats of groups 1, 2, and 3 using an intraperitoneal injection of pancreatic β-cell toxin STZ (Zanosar Pharmacia & Upjohn Co, Kalamazoo, MI) dissolved in sterile distilled water at a single dose of 60 mg/kg of body weight.\textsuperscript{22} Rats of groups 4 and 5 received a control injection of distilled water. DM was defined as blood glucose concentration greater than 250 mg/dl. in an orbital sinus blood sample (Gm 300 Biomine, GmbH, Switzerland) 7 days after STZ injection. Blood glucose level was monitored once a week throughout the study. All diabetic rats were maintained for 30 days after STZ injection.\textsuperscript{23}

Surgery

Thirty days after STZ injection and establishment of detrimental side effects of DM on bone,\textsuperscript{23} rats were anesthetized using 50 mg/kg of ketamine hydrochloride (ROTEX MEDI-CA, Germany) intramuscularly injected along with 5 mg/kg of diazepam (Jaber ben Hayan, Tehran, Iran). After being cleaned with povidone iodine, the skin was cut longitudinally below the knee on the medial side. A circular partial transversal standardized osteotomy deep to the central medullary canal on the midpoint of the medial side of the right tibia was made using a drill with a low-speed, terminal, 1.5-mm-diameter drill bit (delab, dental fabrikteffurt, Germany) irrigated using saline solution to avoid burning. The muscles were sutured with 03 cat gut (SUPA, Iran), and the skin was sutured with 04 nylon reverse cutting sutures. Antibiotic therapy with ceftriaxone (Jaber ben Hayan, Tehran, Iran) at a dose of 50 mg/kg was administered immediately before surgery and 24 and 48 hours after surgery.

LT

A He–Ne laser (Iranian Atomic Energy Agency, Bonab, Iran) with 632.8-nm wavelength, 10 mW average power, circular beam shape, and 0.0314 cm$^2$ of surface area was used. LT started immediately after surgery. A dose (energy density) of 369.4 J/cm$^2$ for groups 2 and 5 and 66.8 J/cm$^2$ for group 3 was applied to each of two points around the osteotomy (0.5 cm proximal and 0.5 cm distal to the center of osteotomy, respectively) three times a week. The duration of irradiation for each point of groups 2 and 5 was 1166 s and that of group 3 was 210 s. The energy density used was calculated according to the formula:\textsuperscript{24,36}

\[
\text{Energy density} = \frac{\text{Output power (mW)} \times \text{time(s)}}{\text{Surface area of laser beam (cm}^2\text{)}}
\]

The power of output of the irradiated laser was checked using a power meter. There were no methodological differences between the study groups except for the use of LT. The rats in groups 1 and 4 received placebo LT with inactivated laser equipment.

Biomechanical examination

Six weeks after the beginning of LT, rats of the study groups were sacrificed using inhalation of chloroform in a closed space. Tibiae were collected, wrapped in gauze that had been soaked in physiologically balanced saline, and frozen at −20°C for later biomechanical testing. Before biomechanical testing, the specimens were slowly thawed at room temperature and kept moist for all handling and testing procedures. Biomechanical properties of six bones of groups 1, 2, and 5 and five of groups 4 and 5 were examined. Bones were subjected to three-point bending on a material testing device (ZWICK Z 2.5 H 15WN, Germany) until fracture occurred. All bones were oriented similarly in the testing machine. A supported device with two loading points 19 mm apart was used to mount each bone, and a press head was then activated to compress the midline of the bone shaft until fracture occurred. Compressive loading speed was 0.08 mm/s in all tests. Data were automatically recorded to the material testing device from the load-deformation curve, and bending stiffness (N/mm), energy absorption (N mm), and stress high load (N/mm$^2$) was calculated. Briefly, these biomechanical parameters may be defined as follows. Bending stiffness is the slope of the liner proportion of the load-deformation curve (the ratio of load to deformation in the elastic region of the curve). Energy absorption is the amount of energy absorbed by bone until it breaks. Stress high load was calculated by dividing the maximum force value by surface area (mm$^2$) of the bone at the site of osteotomy.\textsuperscript{19,22,25,26}

Statistical analysis

Normality of data was analyzed using the Kolmogorov-Smirnov test. Data were subjected to one-way analysis of variance. Multiple comparisons were performed using least
significant difference (LSD). \( P \leq 0.05 \) was considered statistically significant. The results are expressed as means \( \pm \) standard errors of the mean.

**Results**

Of 30 rats in groups 1, 2, and 3, 18 developed clinical evidence of DM after STZ injection; 12 rats did not show an increase in blood glucose level, so they were omitted, and other rats were added and examined.

Rats with DM showed a significant decrease in body weight by the end of study (257.2 ± 7.7 vs 208.3 ± 6.1) (paired Student \( t \) test, \( p < 0.001 \)). Blood glucose level rose to 531 mg/dL in rats with DM.

Results of biomechanical examination are classified into diabetic laser-treated and diabetic control (non-laser-treated) bones, (2) normal laser-treated and normal non-laser-treated bones, and (3) diabetic bones and nondiabetic bones (Figures 1–4).

**Diabetic–laser-treated and control bones**

Mean bending stiffness of diabetic control bones (18.5 ± 4.1, group 1) was higher than that of laser-treated diabetic bones (17.7 ± 1.6, group 2; and 11.5 ± 4, group 3), but there was no significant difference between groups 1, 2, and 3. Mean energy absorption in group 3 (34.2 ± 6) was higher than in group 1 (31.8 ± 11.2) and group 2 (25.7 ± 4). There was no significant difference between study groups. Mean stress high load in group 2 (5.7 ± 0.52) was higher than in group 1 (4.9 ± 1.5) and group 3 (3.9 ± 1.1). There was no significant difference between study groups.

Mean tibial surface area at the site of the osteotomy in group 1 (8.2 ± 1.8) was higher than in group 3 (8.2 ± 1.9) and group 2 (5.0 ± 0.3). There was no significant difference between groups 1, 2, and 3.

**Normal laser-treated and normal non-laser-treated bones**

Mean bending stiffness of normal bones (45.7 ± 4.5, group 4) was higher than that of normal laser-treated bones (41.8 ± 5.0, group 5), but there was no significant difference between group 4 and group 5. Mean energy absorption in group 4 (54.5 ± 6.8) was higher than in group 5 (41.7 ± 5.7). There was no significant difference between group 4 and group 5. Mean stress high load in group 5 (10.0 ± 0.4) was higher than in group 4 (7.7 ± 0.54). There was no significant difference between group 5 and group 4. Mean tibial surface area at the site of the osteotomy in group 4 (8.4 ± 1.2) was higher than in group 5 (5.1 ± 0.3). There was no significant difference between group 4 and group 5.

**Diabetic and nondiabetic bones**

Mean bending stiffness in normal laser-treated bones (group 5) was significantly higher than in diabetic laser-treated bones (18.5 ± 4.1, group 1; 17.7 ± 1.6, group 2; 11.5 ± 4.0, group 3) (LSD test, \( p < 0.001 \)). Mean bending stiffness in normal laser-treated bones (group 4) was significantly higher than in groups 1, 2, and 3 (LSD test, all \( p < 0.001 \)). Mean energy absorption in group 4 (54.5 ± 6.8) was significantly higher than in diabetic bones of group 1 (31.8 ± 11.2) and group 2 (25.7 ± 1.6) (LSD test,
Reddy et al.\textsuperscript{12} may be because of variation in methodologies control bone. The difference in our results and those of measurement of the current study reveal that the healing bone diabetic bone, the results from the bending stiffness mea-
reported that bending stiffness was significantly greater in group.\textsuperscript{32} 

The rats in the Reddy et al. study were diabetic for 7 weeks, and there were not any defects in their bones.\textsuperscript{18} In the present study, there were no significant differences in biomechanical parameters between nondiabetic healing bones and nondiabetic laser-treated (369.4 J/cm\textsuperscript{2}) healing bones.

It seems that 369.4 J/cm\textsuperscript{2} energy density has a detrimental effect on the bone healing process. The output of the laser used in the present investigation was 10 mW, which can include lasers capable of producing up to 500 mW of power (up to a class 4 laser\textsuperscript{44}), which do not produce a thermal effect.

In this regard, Cumming stated that the He–Ne laser is a physical agent reputed to be medically and cost-effective in facilitating the healing of dermal wounds and is considered to be a type of low-energy laser.\textsuperscript{35}

Griffin and Karselis\textsuperscript{36} reported that a laser that did not generate an appreciable thermal effect is considered a low-power laser. Luger et al.\textsuperscript{19} anesthetized two groups of rats and fractured their tibiae. The first group was treated with LT (He–Ne laser, 632.8 nm, 35 mW, 3-mm\textsuperscript{2} spot size of laser beam) with three shots for 14 consecutive days. The duration of each shot per session was 10 minutes. Luger et al. claimed that the energy density was 52 J/cm\textsuperscript{2}. Recently, coworkers of Luger in another study\textsuperscript{21} treated the injured right-side alveolar process of rats using the same laser. They irradiated laser by one shot for 20 minutes, but they did not report the energy density of the laser used. Calderhead\textsuperscript{37} emphasized that laser type, wavelength (nm), output power (Watt), and spot size or irradiated area should be given. We assumed that, in these studies,\textsuperscript{19,21} the real energy densities might be more than the authors claimed.

Energy density is calculated according to the formula:\textsuperscript{24,36}

\[
\text{Output power in watts (W)} \times \text{exposure time (s)} \times \frac{1}{\text{Irradiated area in cm}^2} = \frac{J}{\text{cm}^2}
\]

The energy density of the Luger et al.\textsuperscript{19} and Rochkind et al.\textsuperscript{21} studies according to the formula were 700 J/cm\textsuperscript{2} and 1400 J/cm\textsuperscript{2}, respectively, for each shoot in the first study. Both studies showed positive effects of LT on the bone healing process in the normal animal model of bone defect.

Recently, Lopes et al.\textsuperscript{39} assessed the bone quality of healing bone around dental implants after infrared laser photobiomodulation. Fourteen rabbits received a titanium implant on the tibia; eight of them were irradiated with \( \lambda \) 830-nm laser (7 sessions at 48-h intervals, 21.5 J/cm\textsuperscript{2} per point (4 points), 10 mW, spot size \( \sim 0.0028 \text{cm}^2 \)), and six acted as controls. Specimens were routinely prepared for Raman spectroscopy and scanning electron microscopy. The laser-treated bones had significantly higher concentrations of calcium hydroxyapatite (CHA) than the control bones. Lopes et al. assumed that the reason for the observed difference in the rate of deposition of CHA between irradiated and control subjects was probably correct choice of wavelength, with higher penetration in the tissue and thus greater changes at the cellular level.

Results of Reddy et al. studies\textsuperscript{39,40} showed that He–Ne laser photostimulation significantly accelerated the cutaneous wound-healing process of STZ-induced diabetic rats. So it seems future work should investigate the probable positive effect of LT on bone repair in diabetic animal models as well as in humans.
Although there is widespread acceptance of LT in the clinical setting, there is still a lack of scientific evidence and insufficient guidelines for the use of the most-effective parameters for laser treatment. Therefore, rigorous trials that use laser units and doses that are continuously modified are required to establish the optimal parameters in animal models and under clinical conditions. Delayed wound healing is a clinical problem seen mainly in elderly patients, people with DM, and patients who undergo radiation treatments, such as people with cancer. These, together with patients with burns and with acute and chronic wounds stand to benefit greatly from further research performed in this field. LT with a He–Ne laser in STZ-induced diabetic rats did not enhance bone repair of a partial transversal standardized osteotomy in rat. Further studies need to be conducted to determine the optimal parameters for He–Ne LT in bone repair in diabetic animal models.

Acknowledgments

We wish to thank the late Mrs. Jamileh Rezaie. We also wish to thank the Vice-Chancellor of research of the Medical Faculty of Shahied Beheshti University, M.C., for financial support (grant No. 13/17989) and Shahied Beheshti University, M.C. for cooperation in financial support (grant No. 10012), Miss Sahar Bayat for assistance in feeding rats, Mrs. Mina Koohi for typing the manuscript, and Mr. Ezadee for drawing figures.

Author Disclosure Statement

No competing financial interests exist.

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