Histopathological study of the effect of the laser on the osteoblast cells during mandible defect in Rabbits

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Abstract

The present study clarified the efficiency of low laser power on the osteoblast cells during healing process of the mandible defect in rabbits. In 18 rabbits bone defect carried out by drilling 5 mm, then the animals were divided to two equal group 9 rabbits in each, in control group, rabbits still without any treatment, wears the second group underwent irradiated laser power 805 nm during 5 minute after the surgical operation at interval of 72 hours along two weeks. The histological examination shows that the laser power affect on the osteoblast cells during healing processing on days (7, 21, and 28) post operation the irradiated group than in control group. The conclusion, the low laser power was affected positively on healing process on the osteoblst cells. During increase in number, proliferation, and migration, and activation in bone formation.

تأثير استخدام الليزر على الخلايا البانية للعظم في إصابة الفك السفلي في الأرانب، دراسة نسجية مرضية

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الخلاصة

تم في هذا التجربة توضيح تأثير الليزر واطئ الطاقة على الخلايا البانية للعظم خلال مرحلة شفاء العظم في إصابة الفك السفلي في الأرانب. ثمانية عشر أرنبا، أجريت لها جميعا ثقب غير مخترق بقطر 5 ملم بالمثقاب الكهربائي وقد قسمت الحيوانات إلى مجموعتين متساوية من حيث العدد، وكل مجموعه مؤلفة من تسعة أرانب مجموعة السيطرة تركت بدون إي تدخلات، أما مجموعة العلاج فتم تعريضها إلى أشعة طاقة الليزر بجرعة 805 نم بفترات 5 دقائق كل 72 ساعة لمدة أسبوعين بعد العملية الجراحية. أظهرت الفحوصات النسجية تأثير طاقة الليزر على الخلايا البانية للعظم للفترات نهاية (7، 21، 28) يوما بعد العملية مقارنة مع مجموعة السيطرة. ونستنتج من هذه الدراسة بتأثير الإيجابي لأشعة الليزر واطئ الطاقة على الخلايا البانية للعظم في مراحل الالتئام من خلال زيادة الأعداد والهجرة والنشاط في بناء مادة العظم.

Introduction

The action of laser in healing is widely therapeutic by inducing local and systemic regenerative, anti-inflammatory, and analgesic effect. These effects have been demonstrated in vitro and in vivo. Particularly in studies that focus on the increase of local microcirculation activation of the lymphatic system, proliferation of epithelial cells and fibroblasts, and increased collagen synthesis by fibroblasts(1). More recently research refer that the Low Level Laser Treatment (LLLT) stimulates osteogenic cell proliferation(2). It is uncertain wither biomodulation of bone formation is an overall effect on mesenchymal cells or a direct stimulation of osteoblasts. It is possible that the observed result in irradiated specimen are due to an increased release of growth hormone factors, mainly fibroblast growth factor, which is found in bone tissue and acts on differentiated cells increasing both cell proliferation and secretion of components of the matrix (3, 4). Al-Talabani (2005) who was demonstrated the bone can absorb 805

nm more than 904 nm and this can lead to more activation of fibroblasts which are responsible for collagen fibers synthesis; and osteoblasts which are responsible for osteoid synthesis and deposition and these are important in bone formation and regeneration(5). While Diego *et. al*., (2008) found a significant difference in the degree of new bone formation between lased groups and control group in study done on Wistar rats(6). One of the Methods for bone repair stimulation is lector magnetic bone stimulation in which the stimulation is a non-invasive technology that may improve bone repair. Many studies have suggested that electromagnetic stimulation impacts many cellular pathways, including growth factor synthesis, proteoglycan, collagen regulation and cytokine production which are then increase bone repair(7). Laser irradiation alters cellular processes in a non thermal, wavelength dependent manner by affecting protein synthesis, cell growth and differentiation, cell motility, membrane potential and binding affinities, neurotransmitter release, adenosine triphosphatase (ATPase) synthesis and prostaglandin synthesis. In other word as some authors refer that the Laser irradiation may increase, inhibit or have no effect on the function of cells(8). When applied properly the gallium aluminum arsenide (Ga-Al-As) diode laser is emerging as one of the most efficient laser in low power laser therapy for a variety of applications including pain reliever, increase bone and wound healing, treatment of soft tissue trauma and normalization of abnormal metabolic state(4, 9). LLLT is an effective tool used to prompt bone repair and modeling post surgery , this has referred to the biostimulation effect of LLLT, it is directly dependent on the dose applied(10, 11). Lasers can induce phenomena in injured tissues which promote acceleration of recovery after acute trauma(12). Low level laser therapy precipitates a complex set of physiological interactions at the cellular level that reduces acute inflammation, reduces pain and accelerates tissue healing by a non-thermal and catalyzing effects which accelerate cell metabolism. Laser irradiation also increase the activity of fibroblasts and macrophages, and increase prostaglandin synthesis which has inflammatory action, efficiently clears tissue from edema and causes vasodilator agents(9, 13). Laser therapy increases cellular proliferation particularly in fibroblasts (8, 9, 11, 13), motility of phagocytes, increases synthesis of collagen, protein, prostaglandin and intracellular matrix, neurotransmitter release, transmembrane potential, oxyheamoglobin dissociation and cell granule release, myofibroblast proliferation, increase in mitochondrial size, number and function by increase ATP synthesis within the cell, monocyte stimulation and increase fracture healing which are the result of osteoblast proliferation and differentiation and intracellular changes in these cells (11,13). Stabilization of cellular membrane; Ca^{++}, Na^{+} and K^{+} concentration as well as the proton gradient over the mitochondrial membrane are increased. Laser therapy increase Ca^{++} up take in the mitochondria(9), the transport of nutrients and oxygen to the damaged cells and removal of non-viable cellular and tissue components(8, 9). The aim of this study is to evaluate the effect of laser power on the osteoblast cells.

Materials and Methods

Eighteen New-Zealand white rabbits, of both sexes, weight was about 1.5-2 kg and their age was 9-12 months. In All the animals bone defect carried out in the mandible, a protocol of general anesthesia was done by using (Combelen 0.5 mg/kg) was injected intramuscularly as premedication then injected mixture of Ketamin (50 mg/kg) and Xylazine (20 mg/kg) intramuscularly. The mandible was prepared by routine manner with restrict secure aseptic condition. A circular defect of 5 mm in diameter was done by electrical driller. The control group of nine rabbits was left without receiving any treatment, while in the treatment group of the equal number they all received diode laser with wavelength 805 nm, 900 mW in power and exposure time 5 min, at intervals of 72

hours for two weeks. The laser device which is used in this study was (K4d). The K-laser a diode laser with wavelength of 805 nm which is in the infrared region of the electromagnetic spectrum. The skin and the Masseter muscle were incised to expose the body of the mandible, and then the periostium was reflected. After performing the defect cavity, it was washed using distilled water to remove bone debris. The Masseter muscle was sutured by 3/0 catgut suture with continuous mattress suture, the skin was sutured by 3/0 silk suture using vertical mattress suture technique. The rabbits were given antibiotic which was a combination of streptomycin and penicillin (0.25 mg/kg) intramuscular injection for 7 days. Laser irradiation was done immediately after operation in treated group. The histopathological evaluation was done at the end of the first week, then at the twenty one days, and at the twenty eight days after operation in the both group and three rabbits for each period, the specimen were prepared and stained by H&E and examined (17).

Results

- **Histopathological result of the bone at the end of (7 days):** Control group the granulation tissues formed in the space, are contains many inflammatory cells, collagens fibers, and there were many differentiated mesenchymal cells to osteoblast, and production of bone matrix (osteon) by the osteoblast. With signs of the old bone which surrounded by the osteoblast cells**.** Treatment group at five minute. The granulation tissues invade the hematoma, and inflammatory cells are presents in the lumen with active osteoblast lined the bone. The granulation tissues which formed is more active and more evident than in control group. The dense connective tissues which can be evident from the regular collagens fiber which formed in the space and invade the bone, signs of bone matrix formation (osteon) with active osteoblasts cells which lined at the surface of the bone, the old bone is observed with many lacunas and the osteocyte inside it. In other section there were good events for the immature bone formation during the trabecular bone forming. There were signs of immature bone formation through the trabecular bone formation.
- **Histological result of the bone (21 days):** Control group mature and dense fibrous connective tissues with osteoblast on the surface of the bone. There were still not filled space surrounding the bone. Treatment group at five minute the mature connective tissues which filled the space is attached to the newly immature bone formed with large and active osteoblast lined the surface of the newly formed bone, the trabecular bones which were formed is filled the defect with lacuna and with active osteocytes many harversian canal with precipitation of the lamellar bone around.
- **Histological result of the bone (28 days):** Control group. The space filled with granulation tissues characterized by congested blood vessels, fibroblast and collagen fibers, addition to inflammatory cells, mainly microphages (osteoclast) and lymphocyte. In other section the granulation tissues attached to the necrotic bone. There is a sign of lamellar bone formation with few osteoblasts on the bone surface, and the osteocyte in the formed bone. There are few osteoblast surrounded the trabecular bone and extended within the granulation tissues in the space. In other section there is fragment of the necrotic bone characterized by osteocyte with dilated lacuna space. Still there were space and gap not completely filled with compact bone. Treatment group at five minute. The bone trabecula ion which is formed is thicker and the osteoblast is more active than in the previous week. The osteocytes were impeded in the newly formed trabecular bone active osteoblast surrounding the bone harversian system is formed. The amount of the compact bone which is formed is already to fill the space.

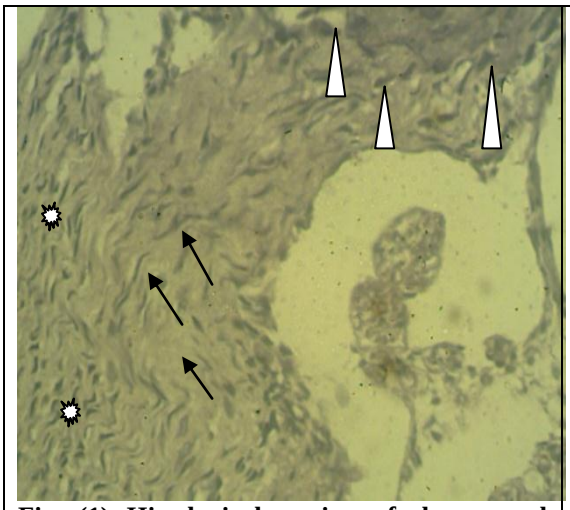

Fig. (1) Histological section of the control group on 7 days (H&E ×40)

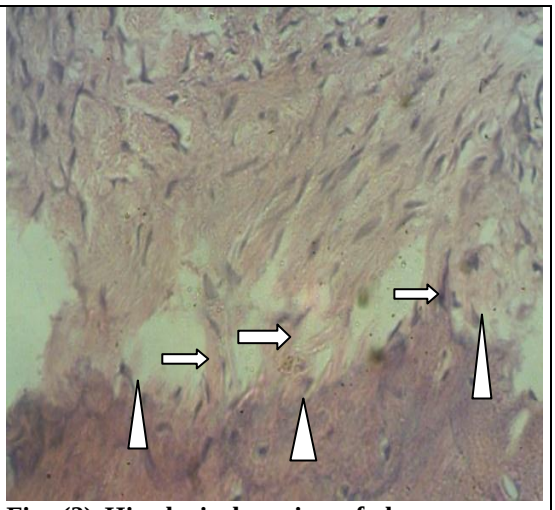

Fig. (2) Histological section of the treatment group on 7 days (H&E ×40)

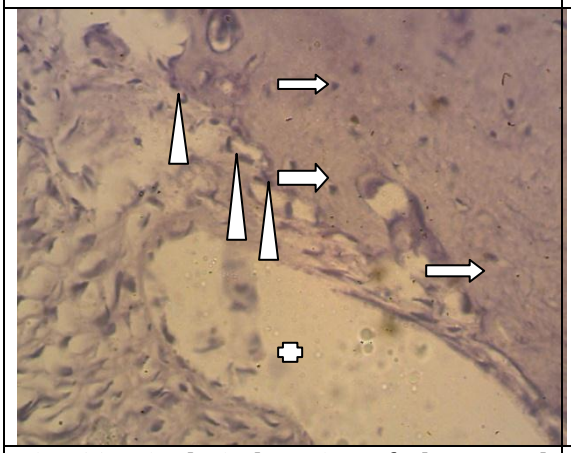

Fig. (3) Histological section of the control group on 21 days (H&E ×40)

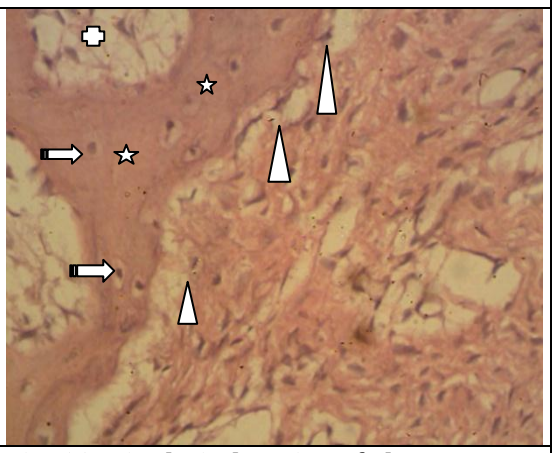

Fig. (4) Histological section of the treatment group on 21 days (H&E ×40)

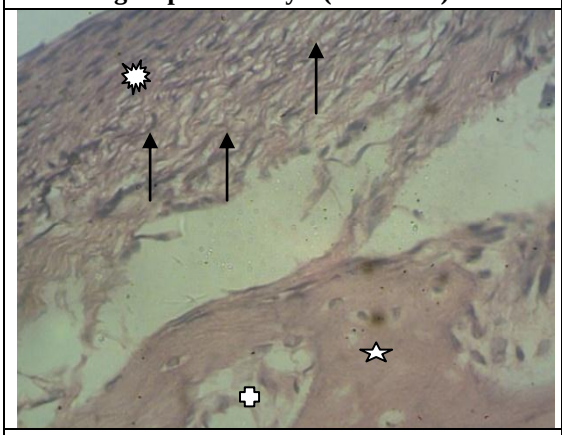

Fig. (5) Histological section of the control group on 28 days (H&E ×40)

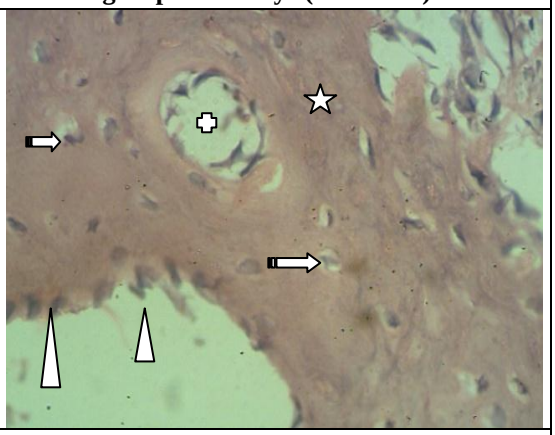

Fig. (6) Histological section of the treatment group on 28 days (H&E ×40)

CONNECTIVE TISSUE: OSTEOBLAST: BLOOD VESSELS

MESENCHYMALCELLS: OSTEOCYTE: TRABECULI BONE

Discussion

From the histopathological results of bone which confirm that the laser in treatment group accelerates bone repair, and this finding in considered with many authors and workers (6, 10, 14, 15, 16, 17). The histopathological results at the end of the seven days especially at the treatment group represents the effect of the laser dose on the osteoblasts cells compare with the control group by its activation on these cells in both number and activation for the production of the bone matrix at the surface of the bone. And this agree with others workers (6, 15, 16, 17). The trabecular bone formed at this time declare that the effect of the laser dose employed in this study, in the treatment group were more thicker than the control group, and these finding in agreement with (6) who found a significant difference in the degree of new bone formation between lased groups and control group in study done on Wistar rats. The effect of the irradiated group with laser power is very evident through the type of dense connective tissue formed in the treatment group than the control group from the size and amount and the collagen fiber formed in the space, and this finding is agreement with (14, 15). There is significant difference in trabecular bone is formed from the end of the seven days till the 21 day postoperatively in the treatment group is thicker than that formed in the control group, which represents the active osteoblast due to laser treatment in this period in the treatment group is more evident than in the control group. The trabecular bone formation is seen more thicker with the active osteoblast lined on the bone surface and within the newly woven bone. And this finding explain and fixed that the suggested dose with their time of using laser therapy suitable and efficient for process for activation and proliferation and differentiation of fibroblast and osteoblast cells for collagens and osteon synthesis and deposition is essential for bone formation and regeneration(5). On other side the low dose treatment of laser will affect on the osteogenic cells and proliferation. This finding insure that the laser dose employed in this study have an active effect on the cells were responsible for the bone repair like fibroblast, osteoblast for synthesis and deposition collagen and bone matrix respectively. And this agree with many authors whom show from their works the essential dose of laser may be enough for penetration the tissues and reach the bone and its effect on bone forming cells (5, 6, 17). And this agree with the results of the study done by (5) in which 904 nm diode laser was used. Who found that bone can absorb 805 nm more than 904 nm and this can lead to more activation of fibroblasts which are responsible for collagen fibers synthesis; and osteoblasts which are responsible for osteoid synthesis and deposition and these are important in bone formation and regeneration. At days 28 in treated group showed compact bone with Haversian canals fill the gap, compare with the control group same changes except in the amount of the compact bone formation. And this finding can be with good agreement with (5).

It concluded that the laser dose which used in this study is essential for the repair of mandible defect, and this results confirmed by study done by (15) in which defects were prepared on the mandibles of Holtsman rats and in which the lased group showed bone regeneration faster than control group. (dose and duration times of laser) with wavelength 805 nm and power density (1.79 W/cm^2) with exposure time equal to 5 min. every 72 h for 14 days after operation. Can produce active effect on proliferation and activation of the osteoblast cells in the mandible defect which induced in rabbits. And has good effect on collagen synthesis. The best result which was evident is, in the trabecular bone formation (thickness of the woven bone) which formed, after the end of the seven days to the twenty one days, in the treatment group compare with control group. The lamellar bone which is formed in treatment group more significant than that of control group in the 28 days after operation.

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