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Original article

Reparative processes of periosteal cells irradiated with diode lasers

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Abstract

The current study aimed to highlight the behavior of the periosteal cells endured damages or excised surgically when irradiated with diode lasers.

Forty eight adult mail mice used in the experiment divided in to two equal groups with 24 one each, all the animal prepared for surgical operation which done on the lateral aspect of the left thigh, they receive general anesthesia followed with a surgical operation to cut through the muscles reaching to the periosteum which was scraped with a dimension of (0.2 X 0.7 cm) with the surgical knife.

The site of the operation in the animals of the treated group was irradiated with diode laser with wave length of 904 nm , power of 3mW after the operation directly continuing for 7 days, with 2 min./ session. Four animals from each group were sacrificed at the days 1, 3, 5 , 7 , 10 & 14 and the specimens were sent to the laboratory for histopathological examination.

The results of histopathological examination revealed the irradiation with the diode laser stimulate the reparative processes of the periosteal cells , promoting the role of the macrophages which to remove of the necrotized cells found in the area due to the scraping process and enhancing the microcirculatory bed of the area.

Conclusion can be made that the diode laser activate the periosteal cells regenerative processes , restoring the normal architecture and function.

Key words: Periosteal cells , repair , diode laser.

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Introduction

The outer surface of the bones are covered with a sleeve like bony forming connective tissue called periosteum. (1) , it has many functions**;** it forms a site of attachment for the muscles, tendons and ligaments and through it the forces found in these structures transferred to the bone through specialized bundles of collagen called perforating " Sharpey`s fibers" , it receives the blood vessel and nerves through holes" foramens" in the bone to communicate the osteocytes in the matrix with the marrow spaces internally , it also contains osteoprogenetor cells which are the source of osteocytes and matrix cells in growth stages and reparative processes, (2).

In early stages of fractures there is a periosteal response which is initial as a fundamental reaction for the injury, (3) this reaction leads to formation of the callus which is thought to be as a collar of reactive tissue surrounded the fracture`s site and act to immobilize it,(4).

Diode lasers known as they produce analgesia, accelerate healing nevertheless they are reported as to accelerate the collagen production , and improvement of damaged tissues, (5), such results got from treatment with red light of He – Ne laser and infrared light from Ga – As semiconductor lasers, (6).

Many diseases may affect the periosteum , so the current study planned to highlight whether the reparative role of diode lasers is significant in case of damages or excising during surgical operations.

Materials and Methods

Forty eight male mice were used in the current study , they were divided in to two equal groups with 24 one each (treated with laser and control) , each one of these animals underwent a surgical operation under general anesthesia using ketamine hydrochloride[1] 100 mg / kg B.W. i/ m , the site of the operation was the lateral aspect of the left thigh where the skin incised and the two major muscle; " Biceps femoris and Vastus lateralis" were separated to expose the femur which is scraped for a dimension of 0.2 X 0.7 cm , the area washed with normal saline and ten closed in layers.

The animal injected with penicillin[2] 1000 IU / Kg. B.W. i/ m directly after the operation and continue for three days after that. The stitches of the animals of the treated group were removed after three days post the operation while those of the control group were raised three days later.

The site of the operation in the animals of the treated group was irradiated with a Ga – Al – As[3] diode laser with wave length of 904 nm , power 3 mW directly after the operation and for three days after that for 2 min/ session by direct contact of the laser beam on the site of the operation , taking in mind that the irradiation start post the operation directly and continued for one week later.

Four animals from each group were sacrificed at the days ; 1,3, 5,7, 10 & 14 and specimens collected from the site of the scraping of the femur , placed in a fixative solution to be transmitted for histopathological examination using the ordinary staining method and a special staining using silve nitrate stain, (8) , which is a special stain for collagen fibers, then the specimens examined microscopically.

Results

Examination of the specimens collected from the animals of the control group one day after the operation showed acchymotic bleedings fill the site of the operation in addition to clots, osteoclasts seen in the area, while those collected from the treated group for the same period showed diffuse inflammatory reaction with extravasation of the polymorphic neutrophils, necrosis of the periosteal boundaries and overlying muscles, also clots can be seen in the area , specimens stained with silver nitrate showed presence of large number of large number of osteoclasts in the area.

Specimens collected from the animals of the control group three days after the operation showed presence of necrotizing tissues on the boundaries of the periosteum in addition to the inflammatory reaction and clots. Specimens stained with silver nitrate showed thin layer of collagen fibers and fibroblasts in the outer layer of the periosteum. The inner layer of the periosteum showed presence of osteoblasts which were originated from the mitotic division of the osteoprogenetor cells and osteocytes, each osteocyte is located in a lacunae, osteoclasts are also seen numerous in number in the inner layer of the periosteum.

1. Ketallar, 50 mg/ml, Pfizer warner lambert, U. K.
2. Pencom-12 Injection (Benzathine Penicillin) - 1,200,000 units (1 Vial), Alembic – India.
3. Russian-Polish Joint. Venture 103030, Moscow, Russia.

The specimens collected from the animals of the treated group for the same period showed large number of macrophages aliened on the boundaries of the periosteum, in addition to high diffuse network of fibrin and primitive proliferative capillaries but the necrotized tissues decreased to a high degree , the special staining showed slight increase in the thickness of the collagen fibers and the fibroblasts in the outer layer of the periosteum, Fig 1 , slight increase in the number of the osteoblasts, osteocytes and osteoclasts found in the Howshp`s lacunae deep in the inner layer of the periosteum, Fig 2.

Five days post the operation specimens collected from the animals of the control group had free erythrocytes between the muscles, clots which contains free fibrin

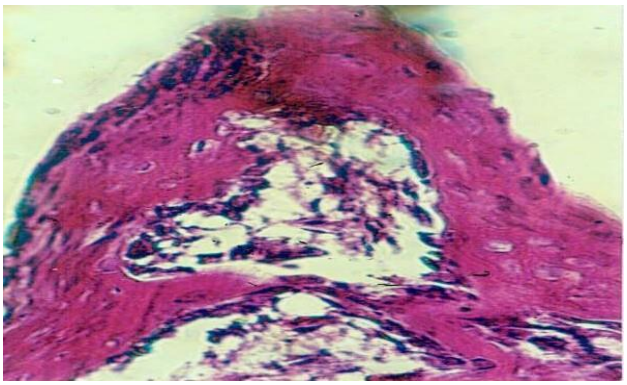

Fig.1; osteoblasts, osteocytes and osteoclasts. H&E , 40X100 .

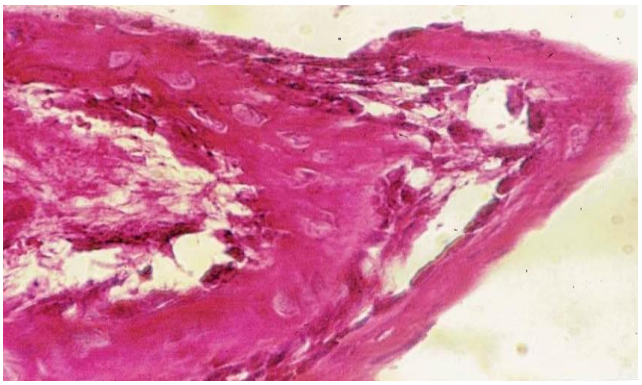

Fig.2 ; osteoclasts found in the Howshp`s lacunae . H&E , 40X100 .

networks, and the necrotizing tissues still can be seen, but the special stain revealed presence of fibroblasts and collagen fibers in the outer layer of the periosteum, the inner layer of the periosteum showed presence of osteoblasts, osteocytes and osteoclasts.

Osteoclasts found few in number at the site of the operation, while specimens collected from the animals of the treated group revealed the active migration of the osteoblasts from the boundaries of the periosteum toward the center in addition to appearance of thick outer layer
of periosteum consist of collagen fibers , increasing numbers of osteocytes and osteoblasts and decreasing in the number of the osteoclasts.

Specimens collected from the animals of the control group seven days post the operation showed a loose network of fibrin from the outer layer of the periosteum lined with newly formed capillaries. Specimens stained with special stain revealed presence of thick collagenous
layer at the outer boundaries of the periosteum while it`s inner layer showed increasing in the number of the numbers of osteocytes and osteoblasts with few number of osteoclasts. While the specimens collected from the treated group for the same time revealed well developed outer layer which revealed a dense fibrous tissue , while the inner layer was filled with flattened cells which were differentiated at the same site to form osteoblasts, special stain revealed high increase in the thickness of the outer layer of the periosteum consisting collagen, the inner layer of the periosteum showed high
increase in the number of the osteoprogenetor cells which were differentiated to osteocytes in the same region. Fig 3 .

Trabecular formation in the cancellous bone could be seen in addition to early formation of Haversian systems in the compact bone.

Ten days post the operation specimens collected from the animals of the control group showed osteoblasts at the margin of the periosteum which migrated toward the center of the site of the operation , the capillaries were more developed , increasing activity of the osteoprogenetor cells, in addition to well developing outer layer which contains dense fibrinous tissue, Fig. 4 .

Specimens collected from the treated group for the period and stained with special stain showed high numbers of osteoblasts which migrate toward the center of the site of the operation, osteocytes also present in the same region while the osteoclasts seen just few in number , the osteoblasts were cuboidal shaped , well developed blood vessels penetrate the area and the bone to reach the marrow cavity, there is also increasing in the thickness of the collagenous layer on the outer surface of the periosteum.
Examination of the specimens collected from the animals of the control group fourteen days post the operation showed well developed outer layer which consists of collagen fibers while the inner layer began to show numerous osteoblasts which differentiated at the periphery near the intact periosteum in to osteocytes.

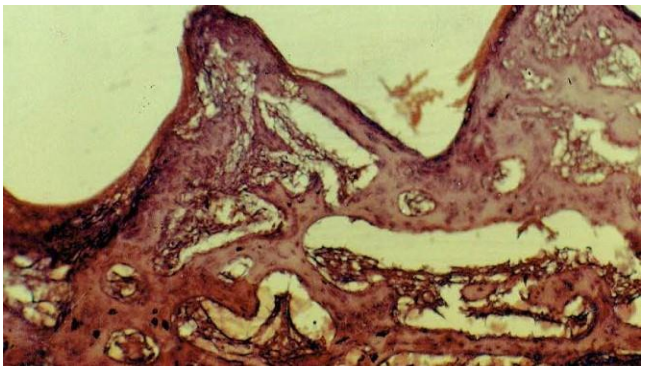

Fig 3; osteoprogenetor cells differentiated to osteocytes. Silver Nitrate stain , 40X100 .

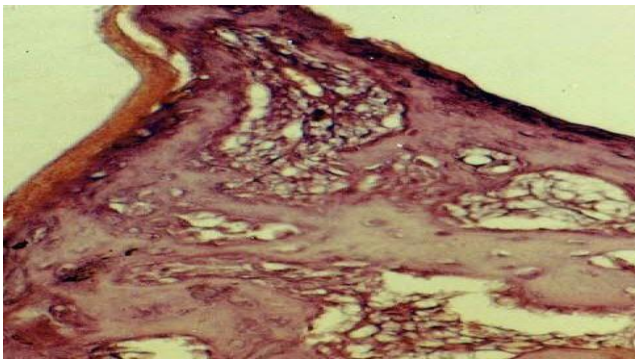

Fig 4; well developed blood vessels. Silver Nitrate stain , 40X100.

The examination of the specimens collected from the treated group for the same period showed presence of few numbers of osteoclasts situated in the "Howship`s lacunae" deeply in the inner layers of the periosteum, while there were large numbers of osteocytes and osteoblasts in the same region.

Special stain for the specimens collected from these animals at the same time showed significant increase in the thickness of the outer layer of the periosteum consisting of collagen. The inner layer showed high number of osteocytes and osteoblasts , decrease number of osteoclasts, trabecular formation developed in the cancellous bone , in addition to Haversian system and canaliculi.

Comments

Low level laser therapy means using of laser radiation to obtain photochemical non thermal process without any visible changes in the mammal`s tissue and to provoke such effect , it is necessary for the radiation to be absorbed by the tissue, the deep penetration of the wavelengths between "800 – 1064 nm" make them have great advantages when been used, (9).

Specimens collected from the animals of both groups showed great variations, so the treated group animals clots, inflammatory reactions and necrotized tissues. The direct application of the window of the laser device on the site of the operation help in decreasing the bleeding through enhancing the anastomosis between the capillaries and the blood vessels as early as the radiation start,(10 & 11).

The cell membrane play a fundamental role in the response to the laser radiation that it founded a case of membrane permeability alteration, decreased membrane Ca^{+2} binding ability leading to extravasation of the blood contents out the vessels and a diffused inflammatory response initiated as the laser used, (12) , the inflammatory reaction help in attacking of necrotizing cells which were present at the inner margin of the incised periosteum , large numbers of macrophages appeared lined the incised periosteum removing the necrotizing tissues and thus the degenerated tissues decreased to a great ration at the third day post the operative in the treated group , and in some regions they disappeared, this finding is analogues to that obtained by , (13), the laser therapy assisted in increasing the reparative processes , (14) , so the examination of the specimens collected from the animals of the

treated group at the third post operative day revealed a diffuse fibrin network and primitive proliferative capillaries while those collected at the fifth days revealed absence of necrotizing tissues contributed to the action of the macrophages and thus the inner margin of the incised periosteum seemed as if they were debrided by the surgeon and became ready for reparative process which began by migrating of the osteoblasts from the margins to the center of the area , this finding also referred by , (15).

The outer layer of the periosteum of the animals of the treated group began to appear due to the presence of the fibroblasts, protein synthesis, increase in the rate of the metabolism, which increase the mitotic activity and fibrin organization, (16).so the outer layer became more compact and began to restore it`s original structure, the outer layer of the periosteum has the ability to stop the projection of the osteoprogenetor cells toward the overlying tissues and that ruination couldn`t be classified , but it was thought that it is ionically active mechanism of the membrane initiate a case of the membrane initiate a case of ionic state between the interstitial media of the membranes overlying the periosteum and the extravascular fluids of the periosteum, this fact contribute the early reparative processes initiated in the periosteum at the early stages of fracture healing, (17).

The osteoprogenetor cells of the inner layer of the periosteum helped in binding of the ends of the fractured bone , the laser radiation enhancing the cell oxygenation , reduces the inflammatory reaction, decrease the inflammatory reaction, increase the rate of inflammatory tissue reabsorption, increase the blood supply and microcirculation, this fact contribute the early maturation of the blood vessels in the animals of the treated group when compared with the control one, the matured blood vessels penetrated the bone reaching to the medullary space. Scraping of the periosteum in the current study may cause damage in the cells of the outer surface of the femur shaft , that contribute to presence of the osteoclasts in the deep layer of the periosteum , they start the stage of the remodeling , (18).

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