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Assessment of the effect of low-energy diode laser irradiation on gamma irradiated rats' mandibles

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ABSTRACT

Objective: The purpose of the present study was to evaluate the biostimulative and regenerative effects of low intensity laser irradiation (LILT) (applied before or after initiation of radiotherapy) on gamma irradiated rats' jaw bones.

Methods: Forty eight male Albino rats were equally divided into two groups: group 1, in which the left side of the mandible was subjected to three successive sessions of laser (LILT) prior to whole body gamma radiation (2 Gy/3 fractions/week) and group 2, received whole body gamma radiation (2 Gy/3 fractions/week) prior to three successive sessions of laser applied to left side. The right side of both groups was used as gamma irradiated non-lased control group. Each group was then subdivided into four equal subgroups (a, b, c, d) according to the time of scarification (3, 7, 14, 21 days respectively). Specimens were subjected to histological, histomorphometric and scanning electron microscopic examinations.

Results: Thin irregular bone trabeculae and widened marrow spaces were identified in the control group. The lased sides of groups 1 and 2 demonstrated regular, thick and continuous bone trabeculae. Ultrastructurally, collagen fibres of the control group appeared irregularly arranged and more spaced compared to groups 1 and 2. Normal-sized osteocytic lacunae were seen in the lased groups, as compared to the wide lacunar spaces noted in the control group. Histomorphometric analysis showed a significant increase in the area of bone trabeculae, as well as the width of compact bone, for the lased groups.

Conclusions: LILT seemed to attenuate the radiation-related damage in alveolar bones.

1. Introduction

Therapeutic lasers are identified and differentiated from surgical lasers by different names include soft, cold and low intensity laser therapy or irradiation (LILT or LILI). These types are used for the treatment of injuries of soft and hard tissue and are classified as class III medical devices.[1,2] Laser systems used for biostimulation include argon, HeNe, galium–aluminum, Nd:YAG, and the galium–aluminum–arsenide (GaAlAs) diode lasers.[3]

Previous studies suggested LILT to have bio-stimulative, regenerative, analgesic, anti-inflammatory and immune effects,[4] dependent of radiation parameters such as wavelength, dose, and intensity of laser light.[5] LILT exerts photobiostimulation or photobioinhibition effects on cells, increasing or decreasing the cellular functions. The incident light energy is absorbed by cellular photoreceptors, such as

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cytochromophores and endogenous porphyrins, and converted by cellular mitochondria into ATP increasing cell activity. In addition, some of the incident energy is converted into heat increasing the local micro-circulation through vasodilation.[6]

Clinical effects of LILT are not well documented. Asanami et al. [7] reported increased bone formation around hydroxyapatite implants in rabbit's jaw following LILT. Faster wound healing and bone formation after tooth extraction was noted using He–Ne and Ga–Al–As diode lasers compared with unlased cases.[8] Nissan et al.[9] observed that LILT application increased the rate of new bone calcification in surgically created bone cavities in rat mandibles. They proposed that the stimulatory mechanism of laser irradiation on bone formation could be mediated by growth factors, cytokines or prostaglandins, which induced osteoblast growth and differentiation by autocrine or paracrine stimulation. This was recently confirmed by an in vitro study in which low-intensity laser irradiation stimulated mineralization via increased expression of bone morphogenic proteins (BMPs) and transcription factors associated with osteoblast differentiation.[10]

A number of complications are related to radiation therapy, either acute (mucositis, infectious stomatitis, alteration of taste or smell acuity, dermatitis, pain, inflammation, and difficulty swallowing) or late (xerostomia, caries, abnormal development, fibrosis, trismus, osteoradionecrosis, and chronic pain). Osteoradionecrosis of the jaws incidence varies greatly (1–37.5%).[11] A recent histomorphometric study revealed that radiotherapy induces a dose–response depletion in osteocytes and an increase in empty lacunae. [12]

The results of previous studies permit to assume the potential of LILT to prevent osteoradionecrosis. Therefore, the aim of the present study was to evaluate the biostimulative and regenerative effect of LILT applied before or after of radiotherapy, on rat's mandible.

2. Materials and methods

Forty-eight male Swiss Albino rats (150–200 g) purchased from the Animal House of the Faculty of Medicine, Cairo University were included in the study. The experimental protocol used was approved by the Department of Animal care, Cairo University. This protocol is adherent to the European Communities Council guiding principles for the care and use of Laboratory Animals. The animals were housed in a controlled environment at 25 °C with 12 h light/dark cycle and were acclimated for 3–5 days before the start of the experiment. All animals were allowed free access to food and tap water.

The animals were divided into two groups, 24 specimens each. Group 1 received three successive applications of low intensity laser therapy (LILT) to the left side of the mandible within 1 week, then on day 7, started receiving a dose of 6 Gy of gamma radiation, fractionated within 3 sessions during 3 consecutive days. Group 2 (24 rats) received the gamma radiation first, then on day 4, started the LILT (both gamma radiation and LITT were used on the same conditions as group 1). Since the effect of laser is localised to the irradiated site,[13] the right side of each rat in both groups was used as gamma irradiated control. Each group was subdivided into subgroups according to the time of termination of the experiment – 3, 7, 14, 21 days after receiving the total dose of gamma radiation.

2.1. Laser application

The left side of the mandible received LILT using Ga–As system (model ora-laser 1030), which is a semi-conductor diode laser with 904 nm wave length (infra-red), operated continuous mode with 30 mW output power for 3 min irradiation. The average output energy is 5.4 J per session. Laser probe was applied directly on the left side of the mandible with a rotary scanning movement, using slight pressure and was repeated in three successive sessions (three times a week). The spot area of laser application was 1 cm^2.

2.2. Radiation exposure

Irradiation of rats was performed at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt using 137 Cesium Gamma Cell 40 giving a dose rate of 0.795 Gy/min at the time of experiment.

2.3. Termination of the experiment and specimens' fixation

The experiment was terminated by cervical dislocation of 6 animals from each group – 3, 7, 14, 21 days after receiving the total dose of gamma radiation. Mandibles were dissected, then 3 right halves (unlased) and 3 left halves (lased) from each group were fixed in 10% formalin for light microscopic examination, while the remaining specimens [3 right halves (unlased) and 3 left halves (lased)] from each group were fixed in glutaraldehyde for scanning electron microscopy.

2.4. Microscopic assessment

For light microscopic examination, the fixed specimens were decalcified in 20% formic acid in distilled water for 2 weeks; then processed by routine histological procedure to obtain tissue paraffin blocks. 5 μm thick sections were obtained from each block and used for hematoxylin and eosin (HE) staining.

For scanning electron microscopy (SEM), specimens were dehydrated in graded series of ethanol (50, 70, 85, 90, 100%) for 10 min at each concentration. Specimens were then mounted on SEM stubs with silver paint, sputter coated and examined at 30.0 kV on a JOEL scanning electron microscope.

2.5. Histomorphometric analysis

All HE stained sections were examined by image analyser computer system using the software SIS (Germany), which comprises a light microscope together with a microcomputer, capable of performing high speed digital image processing for the purpose of cell measurements. The image analyser is calibrated automatically to convert the measurement units (pixels) produced by the image analyser program into actual micrometer units.

For histomorphometrical analysis of HE stained sections, the surface area of spongy bone and the width of compact bone