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Morphological Assessment of Semiconductor Infrared Laser Utilization in Madibular Fracture Treatment.

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ABSTRACT

This paper deals with the assessment of semiconductor laser effect on the dynamics of morphological changes in the rabbit's mandibular bone fracture. Total 56 animals were operated, which were divided into 3 groups - 2 main (42 animals) and 1 control (14 animals). We have revealed a stimulating effect of the laser on reparative osteogenesis by activation of osteoblasts and enhancement of the proliferative activity, which reduced the healing time of the mandibular fractures in the experimental animals. The results of morphological studies revealed a stimulating effect of the high-power semiconductor infrared laser on the reparative regeneration of bone tissue, especially during the osteogenic cell elements proliferation and differentiation. We have established that the exposure to low-intensity laser radiation in the early posttraumatic period resulted in the increased proliferative activity and faster differentiation of osteoblasts into osteocytes, which indicates a positive therapeutic effect, i.e. the main groups of animals showed the consolidation of mandibular fractures on average 4-7 days earlier than control animals.

Keywords: mandibular fraction, semiconductor laser, osteogenesis, experiment.

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INTRODUCTION

Numerous experimental and clinical studies have shown the ability of optimizing the processes of bone tissue reparative regeneration by physical factors, including both red and infrared (IR) laser radiation [1,2,3]. We have found that low-intensity laser radiation is a factor that regulates growth and the structural organization of bone tissue [4,5,6]. Studies related to the osteoreparation low-level laser stimulation were performed on long bones [6,7]. However, the biological effects of the high-power semiconductor infrared laser (HPSIRL) [5,8,9] provide the possibilities that allow its use in the mandibular fractures treatment. A mandible has organospecific features that distinguish it from the long bones. We found no scientific papers in modern literature dealing with the features of mandibular fractures healing induced by the HPSIRL we have not found, which gave us occasion to develop this research field.

Objective of Research

To carry out morphological study of the dynamics of the regenerate formation in the mandibular fracture induced by the high-power semiconductor infrared laser.

MATERIALS AND RESEARCH METHODS

We conducted our experiments on 56 outbred mature rabbits of 2.5-3 kg. All animals were kept in standard vivarium conditions and on their usual diet. The experiments were conducted under intramuscular thiopental anesthesia and aseptically-administered local anesthesia, with simulation of a typical fracture of the mandible in its corner. Bone fragments were reduced and fixed with wire sutures. All animals were divided into 3 groups according to their treatment method. The first control group included 14 rabbits. Experimental groups 2 and 3 included 21 animals each. 2 days after operation, both of experimental groups of rabbits underwent laser therapy with Intradont IR laser with an output power of 20 W, at a wavelength of 0.9±0.01 mm and a pulse width of 100±50 ns (Group 2), and with ntradont IR laser with an output power of 40 W, at a wavelength of 1.9±0.02 mm and a pulse width of 250±50 ns. (Group 3). The second and third groups' exposition was 20 minutes 3 times a day every 8 hours daily, until the removal of rabbits from the experiment. Both control and experimental animals were removed from the experiment by introducing air into the auricular vein in 3, 7, 10, 15, 21, 28, and 35 days.

All experimental studies on animals were conducted with the permission of the ethics committee of Stavropol State Medical University No.288 of 17.02.2013.

An already operated half of the mandible was relieved from soft tissue, and the blocks were sawn with their further fixation in 10% buffered formalin solution. Decalcification was performed in Trilon-B solution. Blocks were embedded in paraffin, and serial sections were prepared and stained with hematoxylin and eosin, by Mallory's, and Van-Gieson's methods. In addition to studying the qualitative characteristics of the regenerate, its quantitative indicators were determined morphometrically. Quantitative analysis was conducted with the use of point calculation method. the number of osteoblasts, osteoclasts, and lymphocytes were determined in the biopsy cross-section with the use of eyepiece stereometric reticule at 400x magnification. The results of 40 random superimposings of eyepiece reticular with 100 test points were used. We considered only those cells that was in contact with the test points of the reticule. The obtained experimental material was processed by methods of variation statistics with the use of Student t-test and Microsoft Excel software package of medical statistics.

RESULTS

According to the analysis of experimental studies, no significant differences were detected upon qualitative assessment of the graft in both control and experimental groups of animals in the early posttraumatic period. For example, destructive-necrotic processes prevailed 3 days after the injury. Bone fragments, and scraps of soft tissue with the visible red blood cells, and clusters of lymphocytes and neutrophils between them, were determined in the area of mandibular fracture. Small blood vessels were dilated, with stasis observed. Bone fragments had occasional osteoclasts on their edges. The periosteum and endosteum elements were in the proliferation state. Both periosteal and inter-fragment zones had poorly differentiated connective tissue, represented by poorly differentiated cells and immature collagen fibers.

However, we observed an increase in the content of osteoblasts during quantitative study of the cellular composition in the animals exposed to the HPSIRL.

The rabbits of group 2 had this indicator 2 times higher than in control group, and the animals of group 3 - more than 5 times (the difference is statistically significant, $p < 0.05$). The number of lymphocytes and osteoclasts in animals of all groups was more or less similar.

7 days after, there was observed an activation of plastic responses, resulting in bone wound cleansing and filling of the mandibular bone defect with the developing graft. Most proliferative activity in this period was observed in animals exposed to the HPSIRL.

On day 10 of observation, there were changes in both qualitative characteristics and quantitative indicators of the graft in the control and experimental rabbits. The animals of group 1 and 2 had proliferative effects dominating in this period. The number of osteoblasts increased significantly as compared to the initial number. While the animals of group 3 showed a decreasing tendency in the number of osteoblasts. For this reason, we may suggest that the stimulation by the HPSIRL leads to faster differentiation of osteoblasts into osteocytes. Formation of callus in animals of group 3 was faster than in animals of group 1 and 2.

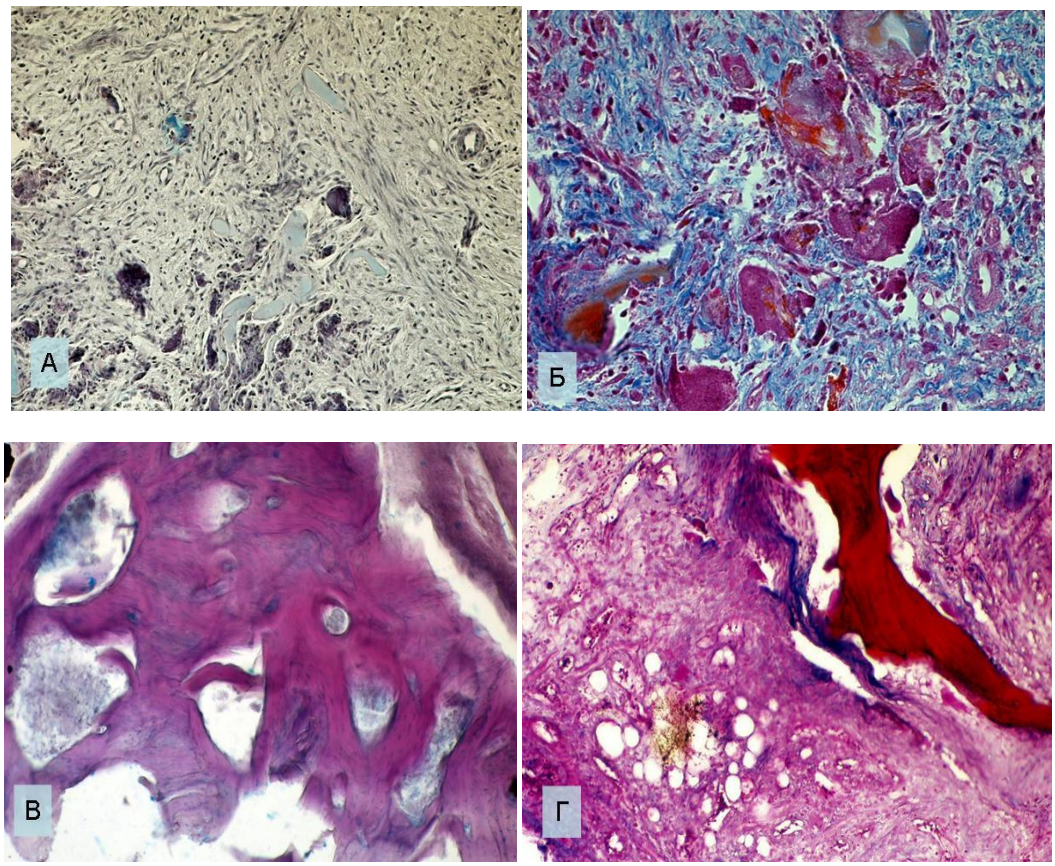

Figure 1: Microslides. Morphological pattern in the mandibular fracture 15 (A, B), 21 (C), and 28 (D) days after the start of the experiment. A – a developing bone callus with visible independent bone tabeculae. Hematoxylin and eosin stain. Ocular 10, Objective 20; B – enchondral ossification. Malory's stain. Ocular 10, Objective 20; C – interfusion of bone trabeculae; development of loop-shaped structures. Van Gieson's stain. Ocular 10, Objective 20; D – a large number of osteoblasts around the newly formed bone trabeculae. Malory's stain. Ocular 10, Objective 20

On day 15, the animals of control group demonstrated a partial consolidation of the fractures. The callus, which connects the mandibular fragments, had clearly visible, frequent trabeculae, separated from each other and surrounded by a layer of osteoblasts (Figure 1-a).

The trabeculae had equidistant line of bone growth. Intertrabeculae spaces were filled with poorly differentiated connective tissue with blood vessels of different diameter observed therein. Zones of newly formed cartilage tissue were observed somewhere in the fracture area (Figure 1-b). It should be noted that starting from day 15 and until the end of observation, the control group demonstrated a gradual decrease of blast cells content in the graft. The rabbits exposed to the HPSIRL showed alternation of periods of reduced osteoblastic activity and their increased proliferation (day 15 and 21).

After 21 days, all animals demonstrated productive bone-developing processes prevailing in the bone wound and resulting in the primary callus formation and reconstruction (Figure 1-c). The exposure to the HPSIRL intensified clearly the formation of osteoid trabeculae. Thus, after 28 days, the rabbits of group 3 showed a huge number of active blast elements in the graft represented by broad interconnected trabeculae (Figure 1-d).

SUMMARY

The control animals showed the mandibular fracture consolidation on average 4-7 days later than the animals of group 2 and 3. Summarizing the results of morphological studies, and comparing quantitative and qualitative characteristics of the grafts in the control and experimental animals, we revealed a stimulating effect of the high-power semiconductor infrared laser on the reparative regeneration of bone tissue, occurring especially during the osteogenic cell elements proliferation and differentiation.

CONCLUSION

Thus, the exposure to high-power semiconductor infrared laser (Group 3) contributed to the improvement of the reparative osteogenesis and shortened the healing period of mandibular fractures in experimental animals. The exposure to low-intensity laser radiation (Group 2) in the early posttraumatic period resulted in the increased proliferative activity and faster differentiation of osteoblasts into osteocytes. Under exposure to HPSIRL, we determined the features of bone tissue reparative regeneration such as alternation of the periods of decreased osteoblast activity and their enhanced proliferation. More pronounced stimulating effect was observed in group 3, which was exposed to the high-power semiconductor infrared laser.

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