DOES EXTRACORPOREAL SHOCK WAVE THERAPY LEADS TO RESTITUTION OF POSTOPERATIVE BONE DEFECTS ON MANDIBLE? AN EXPERIMENTAL STUDY IN RABBIT MODEL

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Summary

In recent years, there has been a range of studies which have proven the effectiveness of extracorporeal shockwave therapy in optimization of restitution of bone defects not only in general orthopedics scope, but also in dentistry. The aim of the study was to study changes in the quality of bone regenerate and its tissue composition in the process of restitution of postoperative BD. The experiment involved 18 pubescent outbred rabbits (males), 6 months old and above at the start of the experiment, with average weight of 2.5 ± 0.2 kg. The histological picture on day 90 in group A corresponded to restitution of BD due to the variously mineralized cancellous bone, whereas in animals of group B the newly formed bone tissue was not structurally different from the intact one. In experimental conditions, applying the methods proposed by the authors, we can achieve certain modifications in bone tissue which are radiographically and histologically confirmed.

Keywords: bone defects; maxillofacial area; extracorporeal shock wave therapy; cone-beam computed tomography; bone density.

INTRODUCTION

In recent years, there has been a range of studies which have proven the effectiveness of extracorporeal shockwave therapy (ESWT) in optimization of restitution of bone defects (BD) not only in general orthopedics scope [1-3] but also in dentistry [4-6]. However, the morphological changes of the bone regenerate in the area of healing of postoperative cavities of BD characterized by a critical size have not been studied well enough yet. Also debatable is the choice of the optimal ESWT protocol when it is necessary to influence the repair processes in bone [3,6]. Therefore, this work has its purpose to study changes in the quality of bone regenerate and its tissue composition in the process of restitution of postoperative BD.

MATHERIAL AND METHOD

The experiment involved 18 pubescent outbred rabbits (males), 6 months old and above at the start of the experiment, with average weight of 2.5 ± 0.2 kg. The animals were kept in the vivarium of Danylo Halytsky Lviv national medical university in full compliance with the guidelines for the care and use of laboratory animals. The study was conducted following the principles of bioethics in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (Strasbourg, 1986), Council of Europe Directive 2010/63 / EU, Law of Ukraine No. 3447-IV “On animal protection”, which was approved by Protocol No. 5 of the Ethics Committee meeting at the Danylo Halytsky National Medical University of 23 February 2017. The experimental animals were stratified as follows: group A - BD healing by ESWT applications with constant maximum wavefront pressure; group B - defect healing by ESWT applications with increasing maximum pressure; group C - control: BD healing only under a blood clot.

Prior to the surgery and cone-beam computed tomography (CBCT) the animals were sedated with intramuscular injection of lytic solution (0.2 ml 1% Sol. Dimedroli, 0.1 ml 0.1% Sol. Atropinii Sulfatis and 0.1 ml of 1% Sol. Nalbufini). Local mandibular and infiltration anesthesia of 0.5-0.7 ml of 4% Ubistesini forte was performed before defect modeling.

Intraoral access on the lower jaw in the area of the diastema was achieved by 1 cm incision through the mucous membrane and by developing an osteomucosal flap. Using the Implantmed W&H physiodispenser (Austria) and a straight handpiece at speed 40,000 rpm with a 0.9% NaCl irrigation, the hard-alloy trepan was applied to model a BD with the size of 4 mm in diameter and 3 mm deep (Fig. 1). After filling the BD with a blood clot, the wounds were sutured with Serafast 5-0 (Serag-Wiessner, Germany). In the postoperative period animals received 0.2 ml of 25% Sol. Analgini intramuscular twice daily for 3 days.

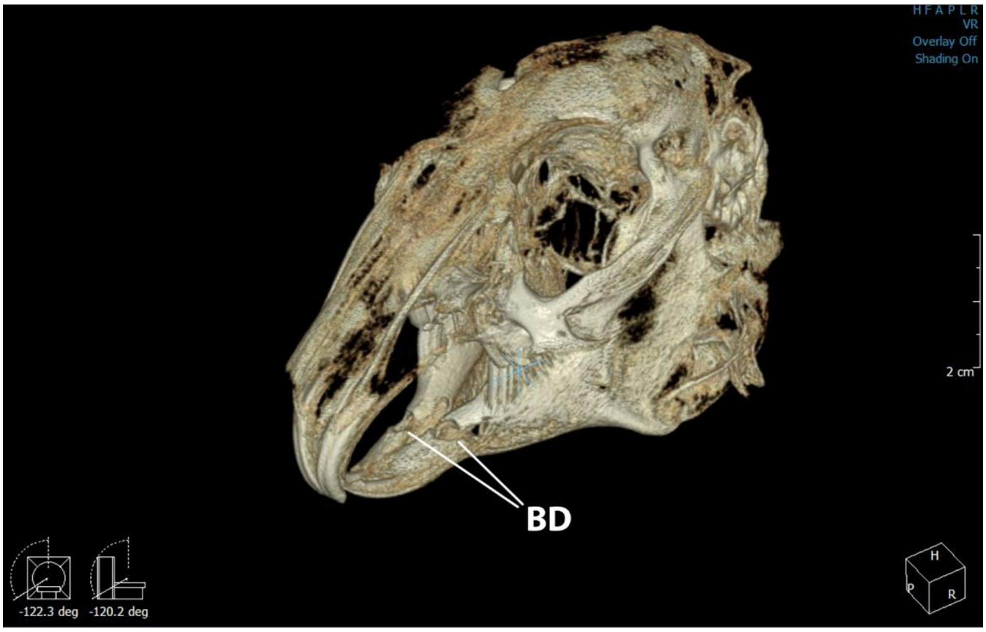

Fig.1. 3D reconstruction of postoperative BD on the lower jaw based on the CBCT image (head of the rabbit; front, left and top).

Storz Medical Master Plus MP100 (Germany) was used for ESWT applications. Animals received 3 weekly procedures of 500 impulses each (frequency 5 Hz, maximum wavefront pressure 1.2 Bar (group A), 1.2-1.6 Bar (group B).

CBCT was carried out on the 2nd, 15th, 45th and 90th days after BD formation using a PointNix Combi500 (South Korea) with the introduction of a bone-equivalent phantom into the scanning field. Unified settings were applied: a scan volume of 9x12 cm, a voxel value of 0.18 mm, the same X-ray tube settings were used, namely 7 mA and 62 kV, which was necessary to offset possible optical errors. Determination of bone density (RBD) in the BD region was performed on PointNix RealScan 2.0 platform by estimating grayscale gradients and optical density with calibration of values according to deviations of the X-ray density of the phantom. For the measurements, BD sections at 1 mm, 2 mm, and 3 mm depth were selected, which corresponded to the location of the cortical, trabecular bones, and the bottom of the defect. The percentages of growth ($^{d}\Delta_{x}$) relative to the normal X-ray density of intact bone tissue were compared at the corresponding level, in the way that the RBD difference between the areas of intact bone and bone defect at the second postoperative day was considered to be 100%:

$$^{d}\Delta_x = 100\% \times (^{d}RBD_x - ^{0}RBD_x) / (^{0}RBD_N - ^{0}RBD_x),$$

where *x* is the level of measurement of density (1-3 mm), *N* is the corresponding area of intact bone, *d* - the 15th, 45th or 90th day after the surgery, *0* - 2nd postoperative day.

Animals (2 from each group) were withdrawn from the experiment at 14th, 45th and 90th days by 10-fold overdose of 2% Sol. Nalbufini, followed by bilateral extraction of fragments of the mandibular bone 0.5x1x1.5 cm in size for histological examination. Photomicrographs of histopreparations were performed at magnification of 40x, 100x, 200x, and 400x using a light microscope MEIJI TECHNO MT4300L (Japan) with Canon EOS 550D photo camera (Japan) and Darktable software, version 2.0.3.

The obtained results were processed by the method of variational statistics using the StatSoft Statistica 10 and the Mann-Whitney U test with a confidence interval of representativeness $p < 0.05$. Average increase rates were represented in percents, standard deviation (SD) was indicated.

RESULTS AND DISCUSSION

According to the CBCT assessment, the RBD in the BD area varied depending on the measurement site and the treatment method selected (Tab. I).

	$^{d}\Delta_x$ (%, SD)								
deep	1mm			2mm			3mm		
group	A	B	C	A	B	C	A	B	C
15 day	9,6±1,1	11,0±2,8	3,4±4,1	16,9±3,9	15,8±6,3	4,0±2,7	13,2±3,8	14,2+6,9	6,5±2,0
45 day	27,9±6,9	53,4±3,3	3,4±1,3	29,3±13,2	36,9±6,8	4,0±3,5	40,2±15,6	77,3±17,2	6,8±3,5
90 day	20,2±7,3	restituted	2,1±8,0	restituted	restituted	2,8±2,5	restituted	restituted	0,4±2,6

Table I. Increase in bone density in the area of the defect.

On the 90th day of observation in groups A and B, accurate localization of BD was not possible any more due to the absence of any CT signs of a structural break in mandibular bone integrity.

In the histological preparations of the mandible, corresponding to the condition of bone and adjacent tissues on day 15 after BD modeling, in groups A and B, the area of BD was peripherally (for 2/3) restituted by a composition of well-vascularized fibrous and loose connective tissue with low expression of lymphocyte-macrophage infiltration. Beyond the BD, single osteoclasts were identified, indicating remodeling processes in operatively unmodified bone. From the side of intact walls of the BD and the multilayered periosteum, isolated single osteoid trabeculas with low mineralization and with osteoblastic rim along the perimeter were observed.

On the 45th day (Figs. 2-4) the BD areas were unevenly filled with mainly osteoid (O), *de novo* osseous tissue of different degree of mineralization (MB) with osteoblastic rim (Ob), single osteoclasts (Oc) along the periphery and loose connective tissue (LCT). At the same time, the osteoid with low mineralization prevailed in group A, while group B was characterized by a considerable amount of bone tissue with osteon formation, in particular from the periosteum (P) side. Residual inflammatory infiltrate was represented by lymphocytes, macrophages, and plasmatic cells. The histological picture on day 90 in group A corresponded to restitution of BD due to the variously mineralized cancellous bone, whereas in animals of group B the newly formed bone tissue was not structurally different from the intact one.

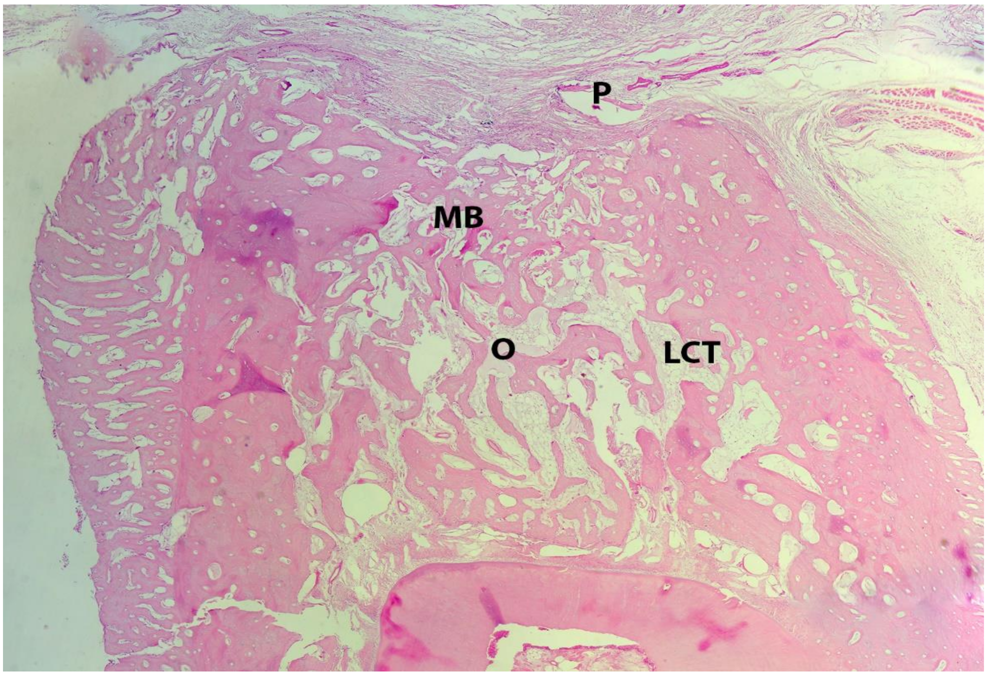

Fig.2. Healing of a hollow bone defect, day 45, group A, cross section of the mandible, 40x magnification, H&E stain (see above).

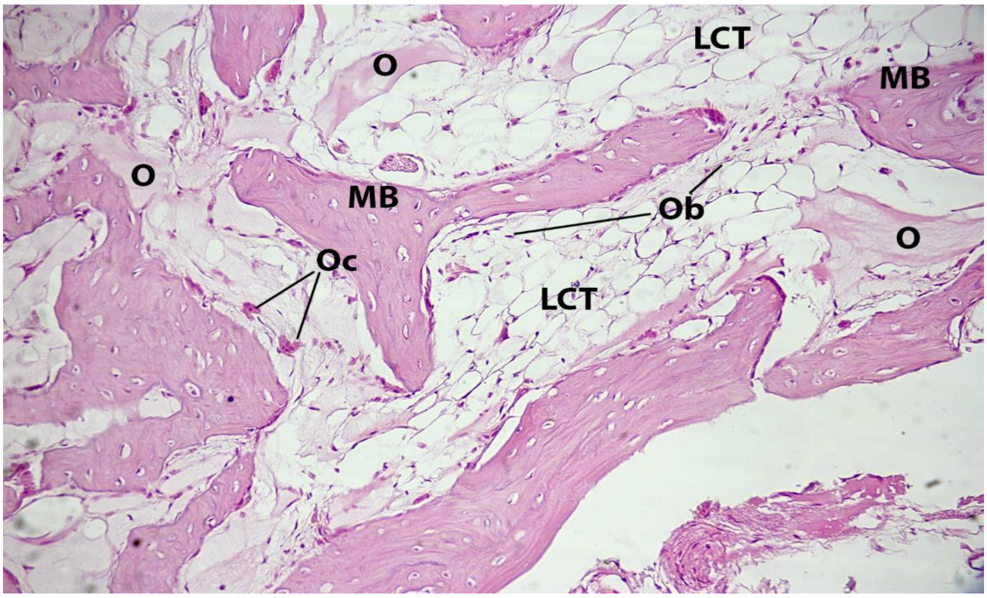

Fig.3. Restitution of the defect with bone trabeculas of different degrees of ossification, day 45, group A, cross section of the mandible, 200x magnification, H&E stain (see above).

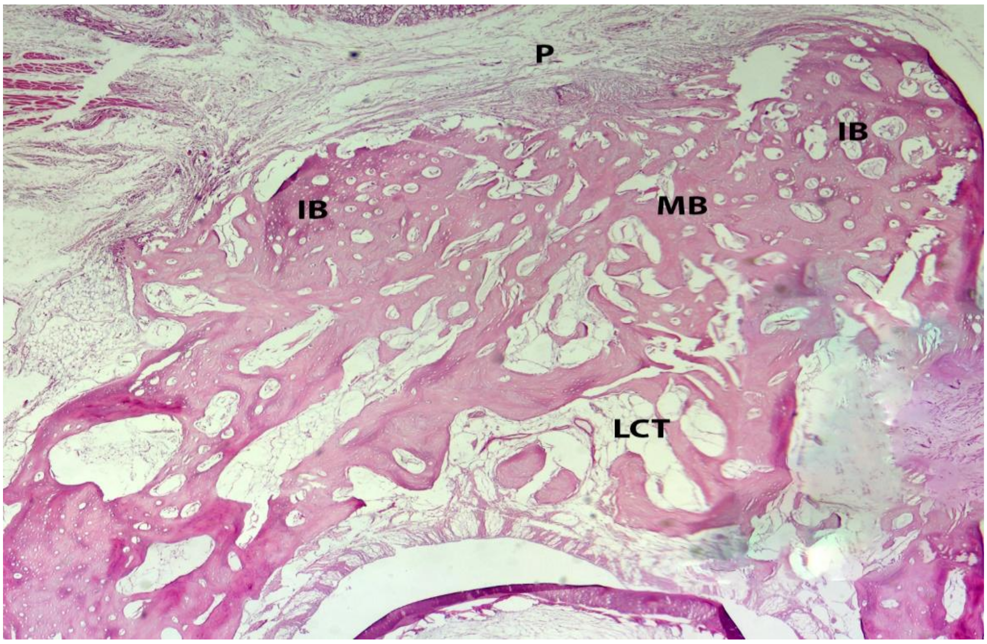

Fig. 4. Healing of a hollow bone defect, day 45, group B, cross section of the mandible, 40x magnification, H&E stain (see above).

On the 15th day, the lower jaw micropreparations in Group C had the BD area filled with loose connective tissue (LCT) infiltrated by neutrophils and macrophages (as a result of a surgery). On the 45th (Fig. 5,6) and the 90th day, BDs were filled with unmodified loose connective tissue (LCT) with diffuse round cell lymphocyte-macrophage infiltration. The periphery of the intact bone (IB) was characterized by the rim of inactive osteoblasts (Ob). Despite the appearance of single small trabeculas of the bone with low mineralization, the modeled postoperative BD cavities were not subject to spontaneous repair.

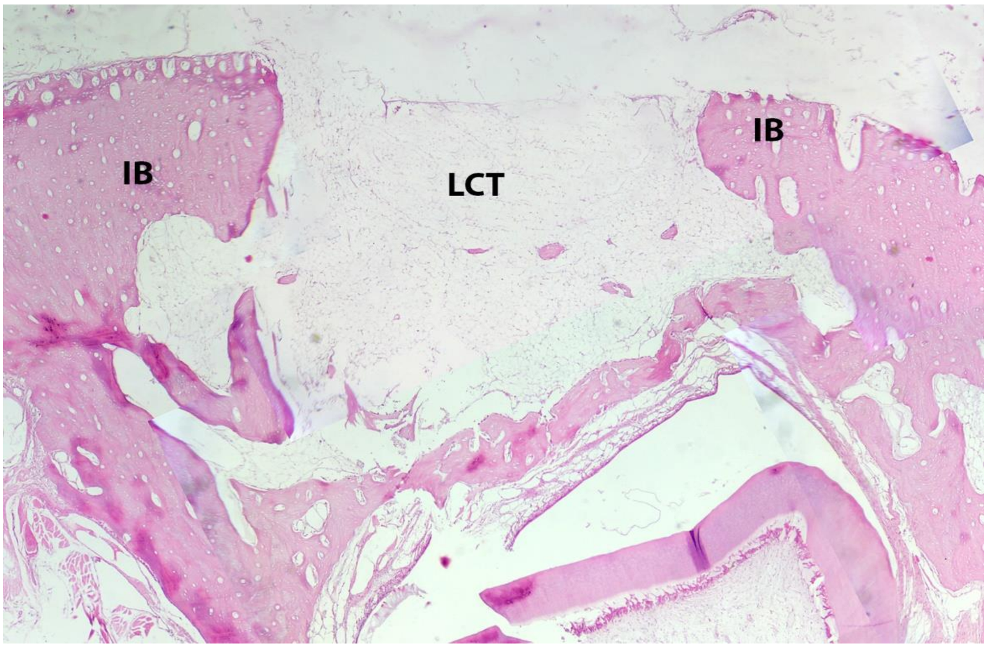

Fig. 5. Hollow bone defect, day 45, group C, cross section, 40x magnification, H&E stain (see above).

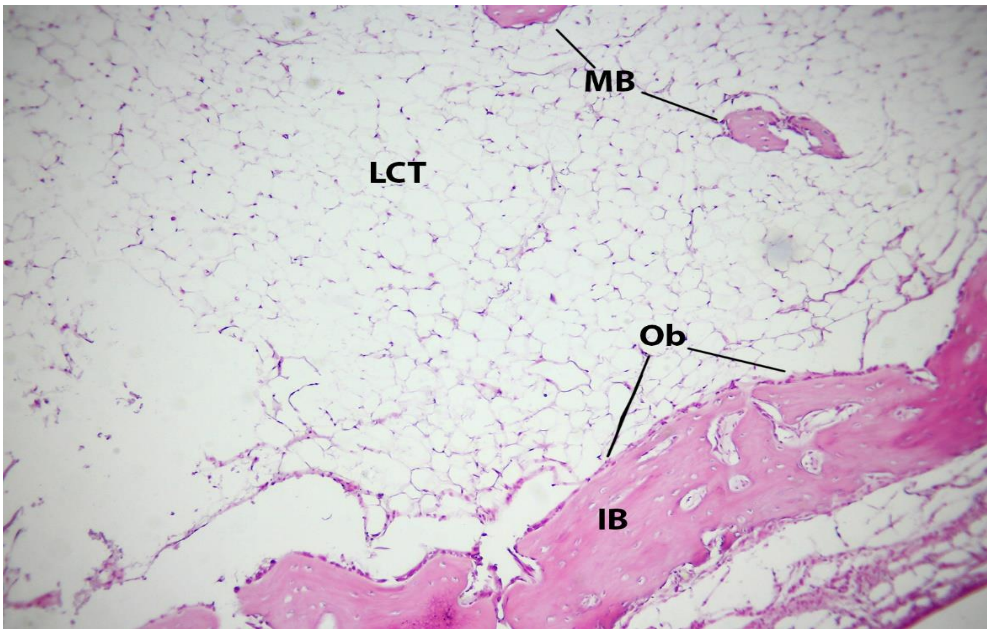

Fig.6. Bone defect wall, group C, cross section, 100x magnification, H&E stain (see above).

CONCLUSIONS

In experimental conditions, applying the methods proposed by the authors, we can achieve certain modifications in bone tissue which are radiographically and histologically confirmed and indicate the initiation of repair processes both within the intact bone surrounding the bone defect and from the side of the periosteum. Using ESWT with a constant maximum wavefront pressure, BD restitution occurs due to the diversification of minerals in the trabecular bone at all levels. Whereas with increasing rates of maximal pressure from 1.2 Bar to 1.6 Bar, the newly formed bone tissue is not structurally different from the intact one.

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