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Healing of the Defect of a Long Bone after the Implantation into its Cavity of Osteoplastic Material Based on Β-Tricalcium Phosphate

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Abstract: This study was designed to evaluate the healing of the defect of compact bone tissue after implantation of osteoplastic material «Calc-i-oss®» with the definition of the dynamics of its resorption and morphological characteristics of the components of the regenerate. In the middle third of the femoral shaft of Wistar rats we reproduced perforated defect to the medullary canal, which was filled with osteoplastic materials «Calc-i-oss®». After surgical intervention fragments of bones were examined on the 15th and 30th day by light microscopy with morphometry and scanning electron microscopy. The study has established the absence of an inflammatory reaction in the area of the defect, symptoms of necrobiosis and necrosis of osteocytes in adjacent to the site of implantation maternal bone, as well as the location on the surface and inside of the particles «Calc-i-oss®» of osteogenic cells and the formation of connective and bone tissue of regenerate. Osteoplastic material throughout the observation period was subjected to resorption and substitution by bone and connective tissue of the regenerate, the ratio of which on the 15th day of the experiment was 33.57±1.53 % to 47.32±2.29 % and 19.11±0.95 % and on the 30th day 28.13±1.27 to 57.62±1.64 and 14.24±1.08. It can be concluded that osteoplastic material «Calc-i-oss®» in the area of the defect of diaphysis of the femur shows high biocompatibility, osteoconductive properties, the ability to resorption and good integration with tissue-specific structures of the regenerate.

Key words: Rats · Bone · Β-Tricalcium Phosphate Β Reparative Osteogenesis

INTRODUCTION

One of the most important problems that orthopedic physicians face in their practice is bone defects regeneration. The high frequency of occurrence of bone defects dictate the need to find tools that would ensure their full recovery. Transplantation of bone tissue, which is used to combat this pathology has a long history and has achieved a considerable success. However, despite this, the used auto-and xenografts still have serious drawbacks [1-3]. As a result, in recent years there has been a growing interest in calcium phosphate osteoplastic materials to replace bone tissue in the clinical practice [4]. The similarity of their chemical structure with that of bone tissue and inertness to biological tissues makes possible to widely use them to replace the lost bone tissue. In addition, synthetic nature of calcium phosphate material guarantees safety in practice preventing the risk of infection and clinical trials consistently demonstrate exceptional biocompatibility the materials [5].

The drug «Calc-i-oss®» and other drugs based on β-tricalcium phosphate by the time of its existence demonstrated high biocompatibility and excellent performance in the treatment of bone defects [6-8]. However, studies, on the basis of which the above facts were received, were held at the cancellous bone and on skull bone [9-11] and information on the impact of osteoplastic material «Calc-i-oss®» on the dynamics of the healing of the defect of compact bone tissue in the scientific literature has not been found. In addition, the majority of works devoted to the study «Calc-i-oss®», are concerned with morphological studies, which lack morphometric characteristics of histological preparations and electron-microscopic characteristics of the tissue-specific structures of regenerate [12-14]. Therefore

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the aim of our study was to investigate the healing process of the compact bone tissue defect after implantation of osteoplastic material «Calc-i-oss®» using histological, morphometric and electron microscopic techniques.

MATERIAL AND METHODS

The experiment was performed on 24 white Wistar rats eight months of age with the weight of 250±10 g. All procedures were agreed with the Commission on Biomedical Ethics of Sumy State University (Minutes ¹ 4/14 of 06.11.2015). The study protocol was done according to the provisions "European Community Directive of 24 November 1986 on the maintenance and use of laboratory animals for research purposes". Before surgery, animals were initially injected with 2.5 mg/kg of acepromazine intramuscular and in 5 minutes 75 mg/kg of ketamine intramuscular (Calypsol, Gedeon Richter, Budapest-Hungary). After the introduction of the animals in anesthesia under aseptic conditions in the middle third of the femoral shaft using a portable drill with a spherical cutter at low speed with cooling we reproduced the defect to the medullary canal with the diameter of 2.5 mm, without rigid fixation we filled it with the osteoplastic material «Calc-i-oss®» («Degradable Solutions Dental», Switzerland). The latter is a synthetic granular material, which is made of pure β-tricalcium phosphate (β-phase purity of> 99%, Ca / P - 1.5) with a total porosity of 50% and the size of micro pores is from 1 to 6 mm (Fig. 1).

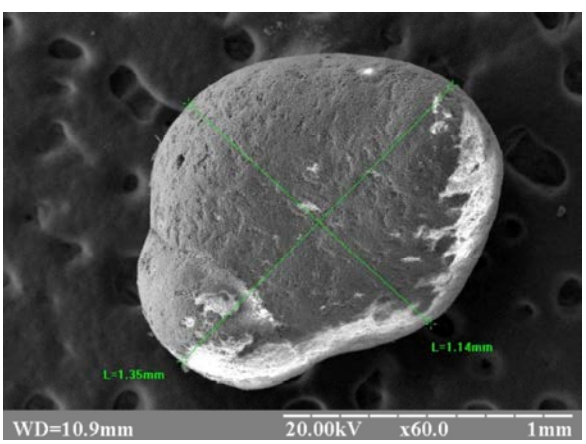

Fig. 1: The granule of osteoplastic material «Calc-i-oss®» in size of 1.14 and 1.35 microns. Electronic scanning image, X 60.

After entering into the bone defect of osteoplastic material the wound was tightly stitched with silk thread through all layers of soft cover, the seam was treated with 3% alcohol solution of iodine. Then, during the next 3 days after operation for prevention of septic complications the after-operation seam was treated with an alcohol solution of iodine and for analgesia ketorolac was injected intramuscularly at a dose of 0.6 mg 2 times a day.

Next on the 15th and 30th day after surgery animals were taken out of the experiment by decapitation under deep ether anesthesia, followed by a study of injured bones using light microscopy with morphometry and scanning electron microscopy.

For light microscopy, we extracted the fragments of femoral bones from the site of implantation of osteoplastic material and fixed them in 10 % solution of neutral formalin. After washing with water, the bone samples were subjected to decalcification in 5 % aqueous solution of Trilon B (Edetic acid), dehydrated in alcohols of increasing concentration and poured into paraffin. Histological sections were made at Sannomiya microtome "Reichert", stained with hematoxylin-eosin [15], analyzed at the light microscope «OLIMPUS» and photographed by digital camera.

Morphometric analysis consisted in identifying in the site of the defect of the area of bone and connective tissues and remnants of osteoplastic material which was performed using the program for image processing "Video-Test" and "Video-Size".

For scanning electron microscopy we extracted the fragments of the femur from implanted osteoplastic material and placed samples in glutaraldehyde holder. In one day, the samples were washed in phosphate buffer, fixed in 1% OsO_4 solution and dehydrated in ethanol of increasing concentrations. Further the bone fragments were glued on metal tables with electricity conductive adhesive, sprayed with carbon dust in standard vacuum installation of VUP-5 type and examined with an electron microscope "SEM 106-I".

Using light and scanning electron microscopy we established morphological characteristics of newly formed tissue-specific structures of the regenerate, the nature of their interaction with osteoplastic material «Calc-i-oss®». In addition, by using these methods, we investigated the state of the structure of adjacent to the site of implantation maternal bone in order to establish or refute postoperative complications due to the presence or absence of signs of necrobiosis and necrosis of osteocytes [16]. The resulting digital values were treated statistically by calculating the arithmetic mean (M) and its standard error (m). The significance of differences between the indicators of the 15th and the 30th days was evaluated using Student t-test with the use of statistical computer program MS Excel XP. The differences were considered significant at $p < 0.05$.

RESULTS

On the 15th day of the experiment in the area of the defect there were detected large granules of «Calc-i-oss®» and their small fragments that were integrated into the bone and connective tissue of the regenerate. On the outer surface and inside the granules of «Calc-i-oss®» there was a significant amount of osteogenic cells which formed foci of fibro- and osteogenesis. There were places where between the «Calc-i-oss®» and bone tissue there was located a thin layer of connective tissue as well as places of direct contact of bone tissue with the osteoplastic material (Fig. 2). Bone tissue of the regenerate bone was represented by bone gullies containing in its structure a significant number of osteoblasts, osteocytes and integrated fragments of osteoplastic material. Bone gullies formed small-and large-loop mesh structures with a total area of 47.32±2.29%. In the inter-gully spaces of the bone

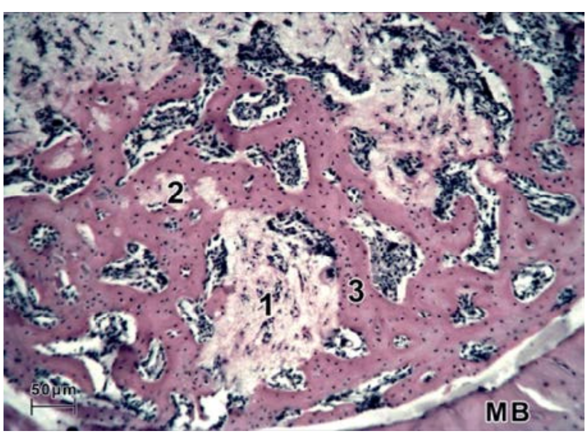

Fig. 2: Shows the area of the defect of femur of a rat on the 15th day after the implantation of «Calc-i-oss®». Osteoplastic material with osteogenic cells and foci of fibro-, osteogenesis in the intertrabecular spaces (1) and inside the trabeculae (2) of the bone tissue of regenerate (3). Adjacent to the site of implantation maternal bone (MB). Haematoxylin and Eosin staining. X 100.

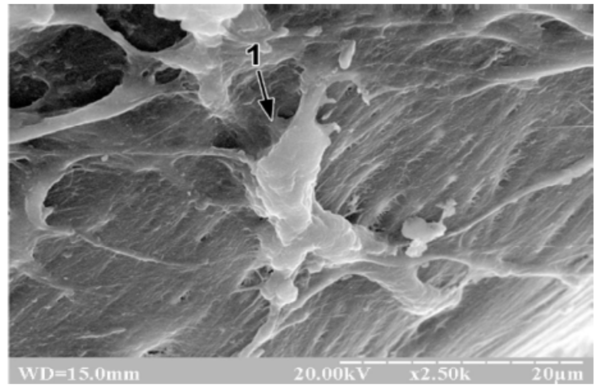

Fig. 3: The area of the defect of femur of a rat on the 15th day after the implantation of «Calc-i-oss®». Fibroblasts (1) in the composition of connective tissue of regenerate which is surrounded by osteoplastic material. Electronic scanning image. X 2500.

tissue there were found the remnants of osteoplastic material and the connective tissue of the regenerate, which occupied 33.57±1.53 % and 19.11±0.95% of the total area of the defect. Connective tissue was constructed from fibroblasts, collagen fibers and blood vessels and contained in its structure integrated small fragments of osteoplastic material (Fig. 3). In the area of the defect the signs of inflammation did not show up and adjacent to the site of the defect maternal bone was characterized by the presence in its structure of bone lacunae with typical osteocytes.

On the 30th day of the experiment in the area of the defect there were also found large and small fragments of «Calc-i-oss®», though, their quantity, if compared with the previous period of observation, reduced by 16.2 % ($p < 0.05$) and was 28.13±1.27 % of the whole area of the defect. Inside the remnants of «Calc-i-oss®», as on the 15th day of the experiment, there were distinguished osteogenic cells with the foci of fibro- and osteogenesis. In addition to osteoplastic material, the area of the defect was filled with connective and bone tissue of the regenerate, which had not only rough-fibrous but also lamellar structure. Comparing to the 15th day of the experiment the quantity of the bone tissue increased by 21.76 % ($p<0.05$) and on the contrary the quantity of the connective tissue decreased by 25.48 % ($p<0.05$) and was 57.62±1.64 % in the first and 14.24±1.08 % – in the second case respectively. In the area of the defect there were places where bone tissue with a high content of osteoblasts and osteocytes was formed directly on the surface of «Calc-i-oss®» and integrated its small fragments

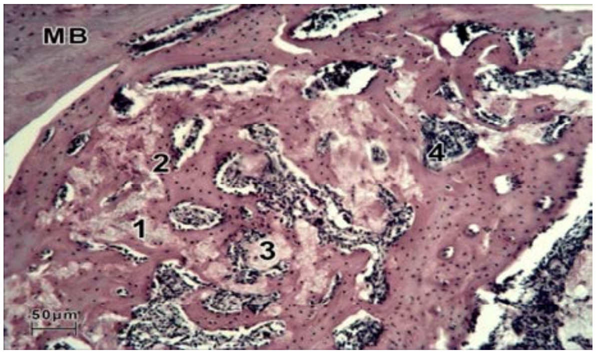

Fig. 4: Shows the area of the defect of femur of a rat on the 30th day after the implantation of «Calc-i-oss®». Fragments of the granules of osteoplastic material (1) inside the trabeculae of the bone tissue of regenerate (2) and its intertrabecular spaces (3) together with the connective tissue (4). Adjacent to the site of implantation maternal bone (MB). Haematoxylin and Eosin staining. X 100.

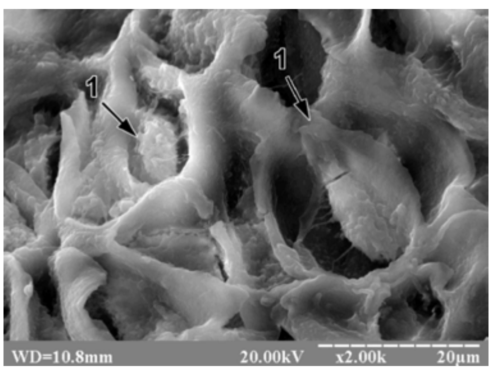

Fig. 5: The area of the defect of femur of a rat on the 30th day after the implantation of «Calc-i-oss®». Osteoblasts (1) in the composition of coarse fiber bone tissue of the regenerate, which is formed directly on the surface of osteoplastic material. Electronic scanning image. X 5000.

into its structures (Fig. 4, 5). However, inside the inter-gully spaces of rough-fibrous bone tissue the remnants of osteoplastic material were located together with the connective tissue. On the surface of the large fragments of osteoplastic material there was also found not only bone but also connective tissue of the regenerate, which consisted of collagen fibers, fibroblasts and blood vessels. It should also be noted that on the 30th day of the experiment, as in the previous period of observation, in the area of the defect of the there were no signs of inflammation and the adjacent to the site of the defect maternal bone was characterized by the presence of typical osteocytes in its structure.

DISCUSSION

The conducted microscopic examination of the diaphysis of the femur of rats after implantation into their defect of osteoplastic material «Calc-i-oss®» found that the latter has a high biocompatibility, as evidenced by the absence of an inflammatory reaction in the area of the defect and no signs of necrosis and necrobiosis of osteocytes in adjacent to the site of implantation maternal bone. With the help of the pilot study it was also determined high tropism of osteogenic cells to osteoplastic materials, as evidenced by their location on the surface and inside the particles of «Calc-i-oss®» and the formation of foci of osteo- and fibro genesis. It is known that the osteoplastic material, which provides the necessary support for the cells to attach, gives them the opportunity to divide and maintain its differential phenotype. Therefore, the use of the osteoplastic material by the osteogenic cells as a platform for attaching and generating on the surface and in its cavities of new bone tissue is the proof of its osteoconductive properties [17].

In addition, the microscopic examination found that in the area of the defect there were revealed the signs of only desmal osteogenesis, as evidenced by presence in it of the bone, connective tissues and the absence of the cartilage. At the same time «Calc-i-oss®» integrated well with components of regenerate, as indicated by the formation of connective and bone tissues on the surface of osteoplastic material and their close immurement in its structure. Gurin *et al*. [18] also observed connective and predominantly bone tissue of regenerate on the surface and inside the β-tricalcium phosphate granules on the 30th day after their implantation into the defect of the epiphysis of femoral bones of rats. However Berchenko *et al*. [19] found trabecular bone formation on the surface of β-tricalcium phosphate granules on the 30th day after its implantation into tibial defects of rats only in certain areas. Basically there was active neoplasm on the surface and inside of the implant of connective tissue. In our study we observed a direct integration of small remnants of «Calc-i-oss®» to the bone tissue of regenerate and between large fragments of osteoplastic material and the bone tissue of regenerate there remained a thin layer of connective tissue.

It is also important to note that most of the works that have been devoted to the study of «Calc-i-oss®», testify to its ability to resorption and replacement by the bone tissue [20, 21]. However, these data were obtained on spongy bones and without morphometric measurements.

In our study, in the defect of diaphysis of the femoral shaft we also observed a gradual resorption of the osteoplastic material and its replacement with the tissues of regenerate. At the same time morphometric method gave another opportunity to establish the dynamics of changes in the number of implants, bone and connective tissues, the ratio of which on the 15th day of the experiment was 33.57±1.53% to 47.32±2.29% and 19.11±0.95% and on the 30th day 28.13±1.27% to 57.62±1.64% and 14.24±1.08%.

CONCLUSIONS

Thus, osteoplastic material «Calc-i-oss®» in the area of the defect of diaphysis of the femur showed high biocompatibility, osteoconductive properties, the ability to resorption and good integration with tissue-specific structures of the regenerate.

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