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THE IMPACT OF OSTEOPLASTIC COMPOSITIONS ON REMODELING OF BONE TISSUE IN IMMUNODEFICIENT ANIMALS: EXPERIMENTAL STUDY

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ABSTRACT — Periodontitis often occurs on the background of pathology of various organs and systems, which doubtlessly affect the course and prognosis for inflammatory and destructive processes of the periodontium. The prevalence of periodontal diseases in diabetic patients has been up to 95%. This comorbid pathology is based on universal pathogenetic mechanisms (immunological disorders, metabolic changes, free radical oxidation). The structure of periodontal pathology in patients with diabetes mellitus is dominated by generalized periodontitis of moderate and severe degree, which features the development of deep periodontal pockets, progressing loss of bone tissue and a surgical treatment that would require implantation. Implantation materials based on bioceramics with hydroxyapatite are most commonly employed. Our experimental study assessed remodeling of bone tissue in immunodeficient animals while implantating various osteoplastic compositions.

KEYWORDS — periodontitis, diabetes mellitus, immunodeficiency, osteoplastic materials.

INTRODUCTION

Periodontitis is often accompanied by pathologies of organs and systems, and this, in turn, worsens the course, clinical manifestations, as well as the outcome and prognosis for destructive changes in periodontal tissues [1, 2]. Diabetes mellitus has already assumed a leading role as a comorbidity of periodontal disease, which can be explained by its enormous prevalence (422 million people all over the world suffer from diabetes nowadays), as well as it is known as a cause of disability and a high mortality (number 3 after cardiovascular disease and malignant tumors) [3–9]. Therefore it is not a merely medical issue but also a social one.

Major disturbances of periodontitis associated with diabetes suppress the immune response of the body and affect T cells of the immune system. Diabetes mellitus might be referred as an immune-related disease that combines an autoimmune nature and immune deficiency [10]. The literature suggests that there is an influence of the immune system on bone regeneration [11, 12]. Under inhibition of the immune system, the transition to certain morphological stages of bone regeneration is delayed with a reduction in the number of osteoblasts. The interaction between the immunity and osteogenesis supposes a principal possibility for its regulation with the help of immune stimulators [13].

Aim of the study

to study the processes of bone remodeling in animals with experimental immunodeficiency during implantation of various osteoplastic compositions.

MATERIALS AND METHODS

This study involved 60 nonlinear Wistar rats weighing 200–250 g. Osteoplastic agents containing hydroxyapatite were used as implantation materials (Table 1).

Experimental model reproduction method

General anesthesia with Zoletil-50 (Virbac, S. A., France; dosage — 0.5 mg/kg of body weight) was done prior to a skin incision (1.5 cm) on the rat's inner surface of the thigh. After the joint was exposed, trepanation was performed with hard-alloy spherical burr #9 in the medial part of the femur distal epiphysis to the depth of the burr head. In the experimental series, the trepanation hole was filled with the studied composition of hydroxyapatite-based osteoplastic material, while the wound was sutured layer by layer with PGA. The following method of local tactivin application was used: 0.3 ml of tactivin solution was applied on a strip of osteoplastic material. A strip fragment (about 2×2 mm in size) containing approximately 5 mcg of tactivin was inserted into the bone cavity of the femur distal epiphysis. The immunodeficiency in the experimental animals was modeled through subcutaneous injection of cyclophosphane diluted in saline (dosage

Table 1. *Types of experimental invasion*

Group	Invasion	Number of rats
1	The experimental model reproduction in intact animals	12
2	Reproduction of an experimental model in immunodeficient animals, with no osteoplastic materials used	12
3	Reproduction of an experimental model in immunodeficient animals and the use of hydroxyapatite-based osteoplastic materials	12
4	Reproduction of an experimental model in immunodeficient animals and using a combined osteoplastic material containing hydroxyapatite and the tactivin immunostimulator (bovine thymus extract)	12
5	Reproduction of an experimental model in immunodeficient animals and using a combined osteoplastic material containing hydroxyapatite and the immunostimulator tactivin with additional subcutaneous administration of tactivin at a dose of 100 mcg per 1 / kg of animal weight	12

— 50 mcg per 1 injection), first one injection for two days in an arrow, and then, to maintain the immunodeficiency status, at an interval of 10 days.

The hip defect was created on the 12th day against the developed immune pathology. The experiment duration was 60 days. The animals were decapitated under anesthesia on the 5th, 15th, 30th, and 60th day after the surgery. These terms reflect the major stages of reparative osteogenesis. The femoral bones were isolated and fixed in a 10% neutral formalin aqueous solution, dehydrated in alcohols, and encased in paraffin following the generally accepted method. 7–8 micron sections were made to be further stained with Ehrlich hematoxylin solution and water-alcohol eosin. The work on the laboratory animals was performed following the Helsinki Declaration (2000) on the humane treatment of animals.

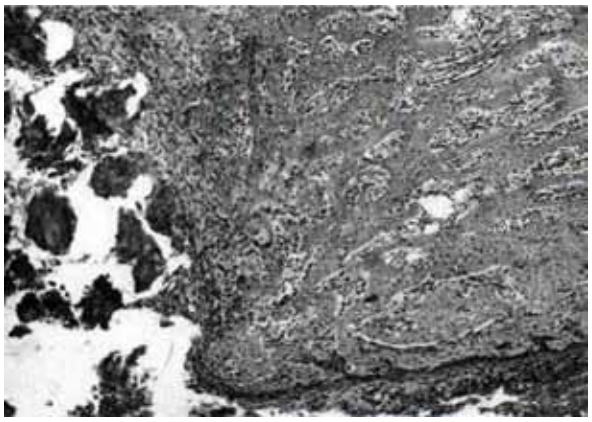

Fig.1. *Group 1. 60 days into the experiment. A powerful periosteal callus merges with the distal bone reparation, embracing the bone defect and its contents. Hematoxylin and eosin. x 40.*

RESULTS AND DISCUSSION

The morphological study outcomes suggest that the suppression of the immune system cells activity has a negative effect on the healing of experimentally reproduced bone defects.

Group 1. 5 days. In the proximal epiphysis, there is a bone defect embracing the cortical plate and spongiosa on ½ of the bone perimeter. 15 days. Intensive development of periosteal bone callus registered, which has a trabecular structure. The periosteal bone callus descends into the defect at the level of the spongiosa, joining the bone regenerate moving from the endosteum. The regenerate has a trabecular structure. 30 days. The bone defect, almost completely to the level of the cortical plate, is a trabecular bone callus. The bone trabeculae in the central areas of the callus feature a broad-mesh structure. Closer to the cortical plate, the mesh structure gets finer. The bone structures are getting dense. 60 days. The periosteal bone reparation makes a uniform spongy mass of bone callus that fills the area of the traumatic destruction (Fig. 1).

Group 2. 5 days. The bone defect penetrates through the cortical plate to occupy part of the spongiosa, and partially reach the bone-marrow canal filled with destructed red bone marrow. The defect lumen show unstructured masses, lots of osteocyte-free bone fragments. The granulation tissue development as well as of connective tissue components of the preparation is poor. 15 days. A fall in the red bone marrow cells number, total death of the bone cambial elements. Absence of these cells in the growth zone. 30 days. The red bone marrow is atrophic. The structures of the epiphysis spongiosa are rarefied, while their bone substance reveals degenerative changes with the rarefaction sites. The growth area and the plate are absent. 60 days. Partial recovery of the red bone marrow. The cortical plate defect is filled with bone material. The periosteal bone reparation features poor development (Fig. 2).

Group 3. 5 days. The bone defect is filled with osteoplastic material, where granules from hydroxyapatite crystalline mass and the oxyphilic fibrous remains

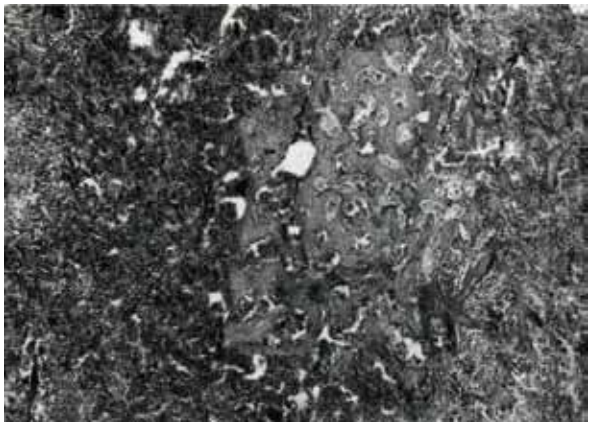

Fig. 2. *Group 2. 60 days into the experiment. The endosteal callus (center and right) is build of defective osteoid beams. Bone marrow (left) features significant destruction. Hematoxylin and eosin. × 40*

of collagen can be identified. 15 days. The bone defect consists of loose connective tissue. There are rounded bodies of hydroxyapatite granules with a distinct crystal structure to be observed. Fibroblastic elements grow into the tubules that penetrate the granules. 30 days. The growth zone cartilage plate is narrow, while its structural arrangement is disturbed. The transition zone to bone structures is undeveloped. The area of injury still features a cortical plate defect. There is no periosteal bone callus. 60 days. The red bone marrow shows active regeneration process; the area of injury reveals complete closure of the cortical plate defect by the bone callus. The osteoplastic material is sealed into the mature trabecular bone tissue. Complete integration of the mineral with the bone tissue (Fig. 3).

Fig. 3. *Group 3. 60 days into the experiment. The area of injury features a bone callus (center) with granules of hydroxyapatite (dark color) sealed in. Hematoxylin and eosin. x 40*

Group 4. 5 days. The injury area contains a cortical plate defect, which penetrates the epiphysis thickness. The bone defect edges are smooth, with no osteocytes. 15 days. The red bone marrow is reduced through a significant length. The injury area presents a cortical plate defect with smooth edges. Regeneration can be observed from the endost part only. 30 days. Active regeneration is underway in the red bone marrow. The bone defect is filled with remnants of necrotic detritus of hydroxyapatite, cellular-fibrous and fibrous connective tissue. For a considerable length, the bone defect is replaced with a bone regenerate with a mature structure, which presents powerful bone beams, sometimes developed osteonic systems. 60 days. The red bone marrow is in a state of active functioning. The bone defect is preserved only at a small section of the cortical plate and epiphysis. It is filled with fibrous connective tissue surrounded with compacted bone callus (Fig. 4).

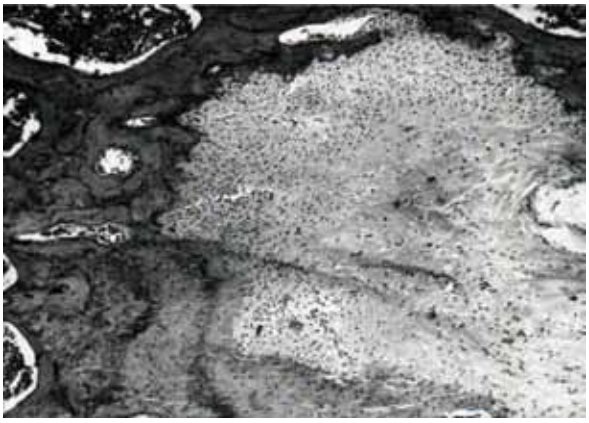

Fig. 4. *Group 4. 60 days into the experiment. The bone defect is filled with fibrous connective tissue. Hematoxylin and eosin. x 63*

Group 5. 5 days. The area of injury reveals a bone defect at the level of the cortical plate with hydroxyapatite granules. 15 days. The bone defect is filled with regenerate tissue surrounding hydroxyapatite granules. 30 days. A bone defect in the injury area features intensive progress of soft tissue regenerate around the hydroxyapatite granules, the bone regenerate connects to the endostal bone callus, which is actively built along the defect edges. 60 days. Closure of the defect with a bone callus. Hydroxyapatite particles integration with the bone preparation structures is observed in the newly developed bone as well as their gradual replacement with bone matter (Fig. 5).

CONCLUSION

1. The obtained data suggest that remodelling of a condition of immunodeficiency is accompanied by suppression of the immunocompetent cellular elements.

2. Implantation of osteoplastic material with immunocorrecting properties improves the course of reparative osteogenesis.

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