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Medical Science

Experimental Study of Bone Tissue with Plastic Material Based on Bioactive Glass in Laboratory Animals with Walker Carcinosarcoma

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The article describes the results of an experimental study of the bone tissue of laboratory animals after the implantation of a plastic material based on bioactive glass into the defect of the tibia, and at the same time Walker carcinosarcoma was transplanted into the soft tissues of the back of the laboratory animals. As a result of the investigation, it was found that a tumor (metastasis) was discovered in the studied samples of the tibia of the rats, which at the morphological level was characterized by invasive growth into the bone and simultaneously the phenomena of osteogenesis were observed in the bone. Microscopic examination in some samples showed the development of Walker carcinosarcoma in the cortical bone, in other samples, the development of a tumor in the medullary bone was observed, and in some samples, both types of invasive growth were revealed. The highest density of mineralized foci of bone tissue in tumors was found in samples, where the material based on bioactive glass was present, and the lowest density was found in samples where material based on bioactive glass was absent. Tumor cells filled the lacunae between the newly formed trabeculae of bone tissue. There was no significant difference in the development of bone tissue in the tumor between the samples of plastic material; the difference consisted only in the density and mineralization of the newly formed trabeculae. Thus, it can be concluded that it is impossible to use a material based on bioactive glass in case of malignant tumors, since when using it, the proceeding osteogenesis is accompanied by angiogenesis, which leads to dissemination of malignant tumors into the area of implantation of the material. *© 2022 Bull. Georg. Natl. Acad. Sci.*

experimental study, microscopic examination, plastic material based on bioactive glass, osteoplastic surgery, Walker carcinosarcoma

Replacement of bone defects after removal of tumors and the search of optimal plastic materials for carrying this out is an urgent problem in orthopedics, traumatology, oncology, maxillofacial surgery and neurosurgery [1]. Currently, various plastic materials are actively used to replace bone

Table 1. Chemical composition of "Biocomposite- Syntekist"

	Na_2O	K_2O	SiO_2	CaO	P_2O_5	Ag_2O
Composition	15.54	0.173	27.58	35.45	21.27	-
The range of of permissible compositions	15-17	0.1- 0.3	25-28	34-37	20-23	0.01-0.08

defects in traumatology and orthopedics [2-4]. Data from many studies [5-7] indicate the effective use of samples of ceramic implants based on hydroxyapatite, tricalcium phosphate and bioactive glass in bone pathology [8-11]. Materials based on hydroxyapatite (HA) – $Ca_{10}(PO_4)_6(OH)_2$ are the typical representatives of bioactive materials, but when it is used, a fibrous layer is formed between the bone and the implant material, which prevents its further reconstruction [12,13]. Materials based on bioactive glass and their modifications are widespread among the implant materials, the most commonly used composition is 24.5% Na_2O, 24.5% CaO, 45.0% SiO_2, 6% P_2O_5, modifying the composition, you can change their bioactivity and resorption [14]. Materials based on bioactive glass are completely replaced by bone tissue without the formation of a fibrous layer, they actively stimulate osteogenesis, significantly enhance reparative processes in damaged tissues, which contributes to the rapid recovery of bone structure [14]. Therefore, conducting an experimental and clinical study to determine the effectiveness of osteoreparative properties of the material based on bioactive glass in the plastics of bone defects after removal of bone tumors, that remain unexplored, is a topical issue of osteoplastic surgery. It requires further study of the indications for the use of material based on bioactive glass to replace the affected areas of bone tissue, after removal of benign or malignant bone tumors [15,16]. In our study we analyze the use of material based on bioactive glass in the plasty of artificially created bone defect and the presence of Walker carcinosarcoma in the soft tissues of the animal, to determine the features of osteogenesis in the bone cavity in the presence of malignant tumors.

Materials and Methods

"Biocomposite – Syntekist" (BCS) – implant material, based on the most bioactive glass, was used during the experimental animal studies (Certificate of state registration No. 3653/2005 dated January 28, 2005), which was synthesized in the laboratory of the Institute of Materials Science NAS of Ukraine by a group under the guidance of Professor V.A. Dubok.

Implant material – BCS represents a multiphase inorganic material synthesized by means of chemical deposition and ceramic technology. Phase composition of BCS: bioactive glass – 50-65 wt.%, Hydroxyapatite – 14-17 wt.%, Whitlockite – 14-17 wt.%, Wollastonite – 7-9 wt.%. The chemical composition of BCS is given in table 1 (in terms of oxides).

The compressive strength of BCS amounts from 1 to 600 MPa for different types, the porosity is adjustable within 5-85%, the resorption period lasts from 6 months to 20 years, depending on the composition of the material. BCS is available in the form of powders and granules having various activity, dispersion and adsorption capacity, and it can also be produced in the form of highly porous blocks and implants of a complex shape. To fill the bone defects, we used BCS in the form of porous granules, having a diameter of 5-10 mm, a porosity of 40-58%, the proportion of pores with a size of 200-700 μm amounts to more than 30%.

The study was conducted in accordance with international requirements for the humane treatment of laboratory animals, in accordance with the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the Law of Ukraine On the Protection of Animals from Cruel Treatment No. 3447-IV dated February 21, 2006.

In an experiment on 16 animals (white non-inbred rats, having body weight of 300-350 grams) from the vivarium of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, porous samples of BCS (granules) were evaluated during implantation in the tibial metaphysis area.

Introduction of animals into the experiment, surgery and removal of animals from the experiment were performed under general thiopental anesthesia. The follow-up period amounted to 1-2 weeks.

The method of implantation of BCS granules into the bone cavity of laboratory animals was carried out as follows: after treatment of the operative field with 70º alcohol and chlorhexidine, the operative field was covered with sterile linen, the animal's limbs were thus fixed on a special operating table. In 16 rats (the course of the operation is shown in Figs. 1, 2) during surgery in the proximal tibia, a scalpel was used to dissect the skin on the inner surface of the tibia over the metaphysis of the tibia, soft tissues were intersected, the periosteum was treated with a raspater, then the bone was marked with an awl, and a 2 mm drill bit was used to drill bone plate up to the medullary canal, Folkman's spoon was used to excochleate the bone marrow in the area of the drilled hole, afterwards the bone cavity in the area of the trepanation hole was filled with BCS granules, soft tissues were tightly sutured over the hole, skin was sutured, the surgical suture was treated with Brilliant green or Iodine. At the same time, Walker carcinosarcoma was transplanted into the soft tissues of the rat's back.

Within the period of 1-2 weeks after the operation, laboratory animals (rats) were removed from the study in order to investigate the intensity of reparative regeneration of bone tissue at the site of BCS implantation by means of light and electron microscopy.

In order to conduct morphological studies using an optical microscope, a segment or whole tibia of rats with implanted BCS was removed and fixed in 10% neutral formalin solution, decalcified in 4% nitric acid solution, dehydrated in alcohols of increasing concentration and immersed in paraffin. Serial histological sections of 7-9 μm thick were produced using the microtome, which were stained with Weigert's Iron Hematoxylin solution and Eosin, as well as with picrofuxin according to Van Gieson.

Stained sections were analyzed on an Olympus BX-63 microscope using the CellSenceDimension 1.8.1 morphometric program. Estimation of the areas of tissues formed at the site of implanted BCS was performed between two fragments of the cortex adjacent to the implanted BCS.

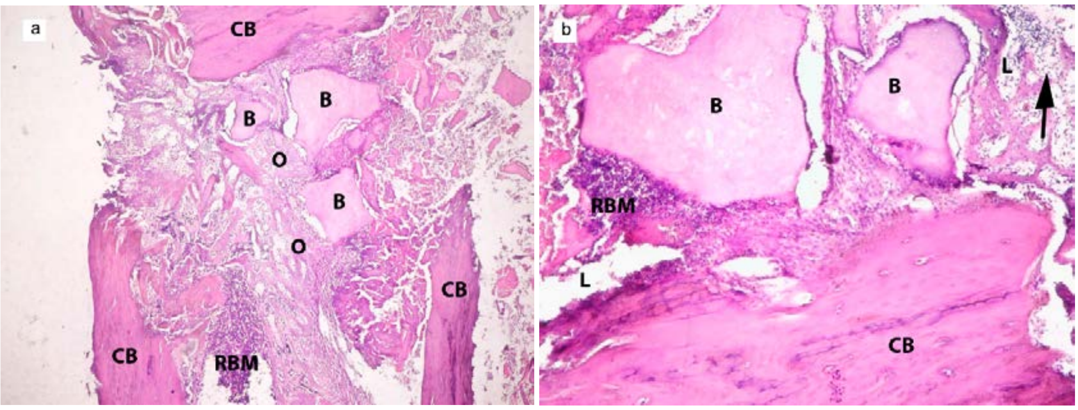

Fig. 1. Group I (4). (the right one with BCS granules). Tibial defect and biocomposite in the medullary bone. Tumor growth in the cortical and medullary bone. Note: O – osteogenesis; <– tumor cell foci; L – lacunae; CB – cortical bone; RBM – red bone marrow; B – biocomposite. Hematoxylin-Eosin, a: vol. 4, approx. 10; b: vol. 10, approx. 10.

The Results of the Study

In the studied samples of the tibia of rats, a tumor (metastasis) was found, which at the morphological level was characterized by invasive growth into the medullary bone, and above the cortical bone tissue, and was accompanied by osteogenesis (Figs. 1, 2). Some differences in tumor growth characteristics were found between the samples.

In samples I (1) (the left one with BCS granules) and I (2) (the right one without BCS granules) tumor development was detected above the cortical bone, in samples I (3) (the left one without BCS granules), II (1) (the left one with BCS granules), II (3) (the left one with BCS granules), and II (4) (the right one with BCS granules) it was detected in the medullary bone, in samples II (5) (the left one with BCS granules) both variants of invasive growth were detected. The highest density of mineralized foci of bone tissue in tumors was found in samples I (1) (the left one with BCS granules), II (3) (the left one with BCS granules), II (4) (the right one with BCS granules), and II (5) (the left one with BCS granules), and the lowest density was found in I (3) (the left one without BCS granules). In samples I (4) (the right one with BCS granules), and II (1) (the left one with BCS granules), biocomposite crystals were registered. No osteogenesis was detected around the biocomposite. Tumor cells filled the lacunae between the newly formed trabeculae of bone tissue. No significant difference in the development of bone tissue in the tumor between the samples was found, the difference was detected only in the density and mineralization of newly formed trabeculae.

Table 2 presents the relative bone density of the tibia of rats at the level of the defect (%).

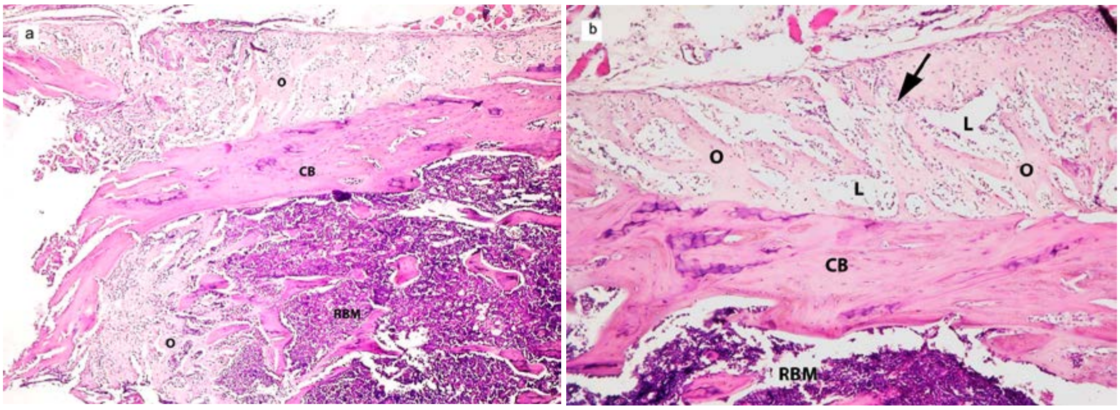

Fig. 2. Group II (5). (the left one with BCS granules). Bone defect: development of a tumor above the cortical bone (a) and in the medullary bone (b). Note: O – osteogenesis; L – lacunae; CB – cortical bone; RBM – red bone marrow. Hematoxylin-Eosin, a: vol. 4, approx. 10; b: vol. 10, approx. 10.

Table 2. The relative bone density of the tibia of rats at the level of the defect (%)

Group	The relative amount of newly formed bone tissue in the tumor	Cortical bone tissue
I (1) (the left one with BCS granules)	57.2±5.8	87.4±1.1
I(2) (the right one without BCS granules)	29.8±4.5*#	86.5±1.7
I(3) (the left one without BCS granules)	7.6±0.6*#^	84.5±9.7
I(4) (the right one with BCS granules)	37.3±4.1	87.8±1.4
II(1) (the left one with BCS granules)	16.4±1.2*#^	87.3±1.2
II(2) (the right one without BCS granules)	10.8±1.1	85.3±2.2
II(3) (the left one with BCS granules)	34.5±4.5*#	88.1±5.9
II(4) (the right one with BCS granules)	46.1±1.1#	93.8±2.6
II(5) (the left one with BCS granules)	56.7±2.5	89.1±0.8

Note: * - P<0.05 regarding I(1); # - P<0.05 regarding II(5); ^ - P<0.05 regarding II(5)

Findings

According to the results of microscopic examination in case of Walker transplantable tumor, in some samples the development of a tumor over the cortical bone was observed, in other samples the development of a tumor in the medullary bone was observed, in some samples both variants of invasive growth were detected. The highest density of mineralized foci of bone tissue in tumors was found in samples where the biocomposite was available, and its lowest density was found in samples where the biocomposite was absent. No osteogenesis was detected around the biocomposite. Tumor cells filled the lacunae between the newly formed trabeculae of bone tissue. No significant difference in the development of bone tissue in the tumor between the samples was found, the only difference is in the density and mineralization of the newly formed trabeculae. Thus, we can conclude that it is impossible to use a material based on bioactive glass in malignant bone tumors, since when using this material, osteogenesis occurs, which is accompanied by angiogenesis, and which leads to dissemination of malignant tumors into the area of implantation of the material.

სამედიცინო მეცნიერება

ძვლის ქსოვილის ექსპერიმენტული კვლევა პლასტიკური მასალით ბიოაქტიურ მინაზე უოლკერის კარცინოსარკომის მქონე ლაბორატორიულ ცხოველებში

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** უკრაინის სამედიცინო მეცნიერებათა ეროვნული აკადემია, ტრავმატოლოგიისა და ორთოპედიის ინსტიტუტი, უკრაინა*
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(წარმოდგენილია აკადემიის წევრის რ. ხეცურიანის მიერ)

წინამდებარე სტატიაში აღწერილია ლაბორატორიული ცხოველების ძვლის ქსოვილის ექსპერიმენტული კვლევა ბიოაქტიური მინის საფუძველზე, წვივის დეფექტისას პლასტიკური მასალის იმპლანტაციის შემდეგ; ასევე, უოლკერის კარცინოსარკომის გადანერგვა მოხდა ლაბორატორიული ცხოველების ზურგის რბილ ქსოვილებში. კვლევის შედეგად დადგინდა, რომ ვირთხების წვივის ნიმუშებში აღმოჩენილ იქნა სიმსივნე (მეტასტაზი), რომელიც მორფოლოგიურ დონეზე ხასიათდებოდა ინვაზიური ზრდით ძვალში და, ამავდროულად, დაფიქსირდა ოსტეოგენეზის ნიშნები ძვალში. ზოგიერთ ნიმუშში მიკროსკოპულმა გამოკვლევამ აჩვენა უოლკერის კარცინოსარკომის განვითარება კორტიკალურ ძვალში, სხვა ნიმუშებში დაფიქსირდა სიმსივნის განვითარება მედულარულ ძვალში, ზოგიერთ ნიმუშში კი გამოვლინდა ორივე ტიპის ინვაზიური ზრდა. სიმსივნეებში ძვლოვანი ქსოვილის მინერალიზებული კერების ყველაზე მაღალი სიმკვრივე დაფიქსირდა ნიმუშებში, სადაც იყო ბიოაქტიურ მინაზე დაფუძნებული მასალა და ყველაზე დაბალი სიმკვრივე აღმოჩნდა იმ ნიმუშებში, სადაც ბიოაქტიურ მინაზე დაფუძნებული მასალა არ იყო. სიმსივნურმა უჯრედებმა შეავსეს ძვლოვანი ქსოვილის ახლადწარმოქმნილ ტრაბეკულებს შორის არსებული ფოსოები (ლაკუნები). პლასტიკური მასალის ნიმუშებს შორის ძვლოვანი ქსოვილის სიმსივნის განვითარებაში მნიშვნელოვანი განსხვავება არ იყო; განსხვავება იყო მხოლოდ ახლადწარმოქმნილი ტრაბეკულების სიმკვრივესა და მინერალიზაციაში. ამრიგად, შეიძლება დავასკვნათ, რომ ავთვისებიანი სიმსივნეების შემთხვევაში ბიოაქტიურ მინაზე დაფუძნებული მასალის გამოყენება შეუძლებელია, ვინაიდან მისი გამოყენებისას მიმდინარე ოსტეოგენეზს თან ახლავს ანგიოგენეზი, რაც იწვევს ავთვისებიანი სიმსივნეების გავრცელებას მასალის იმპლანტაციის არეში.

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