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Biochemical indicators of blood serum of rats of different ages after filling the defect in the metaphysis of the femur with allogeneic bone implants

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Bone defects that do not heal on their own are a significant problem in orthopaedic and trauma surgery. One of the approaches to its solution is the use of bone alloimplants (AloI). Objective. On the basis of the analysis of biochemical indicators of the metabolism of connective tissue in the blood serum of laboratory rats, the course of metabolic processes after the filling of the defect in the metaphysis of the AloI femur was evaluated. Methods. A model of creating a transcortical defect of critical size (diameter 3 mm, depth 3 mm) in the metaphysis of the femur of 3- and 6-month-old rats was used. In animals of groups I (n = 15, age 3 months) and III (n = 15, 12 months) the defects were left unfilled, II (n = 15, 3 months) and IV (n = 15, 12 months) — filled with structural AloI. After 14, 28 and 90 days, the content of glycoproteins, total chondroitin sulphates (CS), protein and calcium, activity of alkaline and acid phosphatases in blood serum was investigated. Results. The introduction of AloI leads to an increase in the content of glycoproteins for all periods of observation in rats of both age groups. 14 days after implantation in 12-month-old rats, compared to 3-month-old rats, a 1.30 times higher level of CS in blood serum was determined (p = 0.008), which is due to their higher content in the area of connective tissue implantation; the activity of alkaline phosphatase decreased by 1.80 times (p = 0.016) and acid phosphatase by 1.50 times (p = 0.018), which indicates a delay in the formation and reorganization of bone tissue. However, the level of CS under the conditions of the establishment of AloI on the 90th day was lower compared to the corresponding groups without plasticity of the defect: in 3-month-old rats by 1.44 times (p = 0.008), in 12-month-old rats by 1.52 times (p = 0.008). Conclusions. According to the indicators of biochemical markers of connective tissue metabolism, the use of AloI for plasticity of defects of a critical size in the metaphysis of the femur of rats leads to the activation of bone regeneration with a greater manifestation in younger recipients compared to groups with an unfilled defect.

В ортопедичній і травматологічній хірургії дефекти кісток, які не загоюються самостійно, становлять значну проблему. Одним із підходів до її розв'язання є використання кісткових алоімплантатів (АлоІ). Мета. На підставі аналізу біохімічних показників метаболізму сполучної тканини в сироватці крові лабораторних щурів оцінити перебіг метаболічних процесів після заповнення дефекту в метафізі стегнової кістки АлоІ. Методи. Використано модель створення транскортикального дефекту критичного розміру (діаметр 3 мм, глибина 3 мм) в метафізі стегнової кістки щурів віком 3 та 6 міс. У тварин груп I (n = 15, вік 3 міс.) і III (n = 15, 12 міс.) дефекти залишали незаповненими, II (n = 15, 3 міс.) і IV (n = 15, 12 міс.) — заповнювали структурними АлоІ. Через 14, 28 і 90 діб досліджено вміст у сироватці крові глікопротеїнів, загальних хондроїтинсульфатів (ХС), білка та кальцію, активність лужної та кислої фосфатаз. Результати. Введення АлоІ призводить до підвищення вмісту глікопротеїнів на всі терміни спостереження у щурів обох вікових груп. Через 14 діб після імплантації у 12-місячних щурів порівняно з 3-місячними визначено підвищений рівень у сироватці крові ХС в 1,30 разу (p = 0,008), що обумовлено більшим вмістом у них в ділянці імплантації сполучної тканини; знижену в 1,80 разу (p = 0,016) активність лужної фосфатази та в 1,50 разу (p = 0,018) — кислої фосфатази, що свідчить про затримку утворення й реорганізацію кісткової тканини. Проте, рівень ХС за умов встановлення АлоІ на 90-ту добу був нижчим порівняно з відповідними групами без пластики дефекту: в 3-місячних щурів у 1,44 разу (p = 0,008), у 12-місячних — у 1,52 разу (p = 0,008). Висновки. За показниками біохімічних маркерів метаболізму сполучної тканини використання АлоІ для пластики дефектів критичного розміру в метафізі стегнової кістки щурів призводить до активації регенерації кістки з більшим проявом у молодших реципієнтів порівняно з групами з незаповненим дефектом. Ключові слова. Алоімплантат, дефект кістки, експериментальне моделювання, регенерація, біохімія, сполучна тканина

Key words. Allograft, bone defect, experimental modelling, regeneration, biochemistry, connective tissue

Introduction

Annually, millions of patients in the world require orthopedic interventions using bone grafts due to the consequences of injuries, degenerative oncological diseases, infectious complications, etc. [1].

In orthopedic and trauma surgery, bone defects are a significant clinical problem in everyday practice [2]. They occur as a result of infections and bone tumors, which are mostly removed surgically with subsequent bone reconstruction [3]. In addition, fractures after high-energy injuries (in particular, blast and gunshot injuries) and osteoporotic disorders are often accompanied by bone defects that do not heal on their own, which necessitates bone tissue augmentation procedures [4]. Today, half a million patients are treated annually for bone defects in the United States and Europe at an estimated cost of more than $3 billion [5].

Reconstruction of bone defects is performed with the help of auto- and allo-implants, substitutes for bone tissue — biomaterials, in particular, bioactive ceramics, bioglass, synthetic or natural polymers [6].

Allo-implants have advantages over autobone, namely: a sufficient amount of material that can be used in the form of blocks, chips or granules, which allows filling defects of various configurations. There is also no need to compromise the patient's skeletal structures to obtain the graft tissue and, accordingly, complications at the donor site can be avoided, which can occur with the use of autografts [7]. In addition, the use of allo-implants can be just as successful as autologous material [8].

However, it is necessary to develop new approaches to improve the functional capacity, implantation of grafts and the quality of life of patients in a cost-effective way, and allogeneic bone plastic will definitely be a part of them [9].

Purpose: based on the analysis of biochemical indicators of the metabolism of connective tissue in the blood serum of laboratory rats, to evaluate the course of metabolic processes after filling the defect in the metaphysis of the femur with allogeneic bone implants.

Material and methods

Experimental studies were conducted in compliance with the requirements of humane treatment of experimental animals [10, 11] after the approval of the plan by the Bioethics Committee at the State Institution Professor M. I. Sytenko Institute of Spine and Joint Pathology of the National Academy of Medical Sciences of Ukraine (Protocol No. 191 dated 22.04.2019).

Experiment design

The study compared the metabolic features of bone regeneration after the creation of transcortical defects of critical size (depth 3 mm, diameter 3 mm) in the distal metaphysis of the femur of laboratory rats and their filling or absence of filling with allogeneic bone implants. 48 white rats were used: 24 aged 3 months at the beginning of the experiment, 24 — 12 months, who were randomly divided into four groups:

– I (n = 9, age 3 months) — the defect was left unfilled;

– II (n = 15, age 3 months) — a structural bone allo-implant of the appropriate size and shape was placed in the defect;

– III (n = 9, age 12 months) — the defect was left unfilled;

– IV (n = 15, age 12 months) — filling of the defect with an allo-implant.

The technique of surgical intervention and production of allo-implants is similar to that described in [12].

14, 28 and 90 days after the operation, 5 animals each from groups II and IV, 3 each from groups I and III were removed from the experiment by decapitation due to the need to obtain blood for biochemical study.

Biochemical methods

After natural coagulation, the collected blood was freed from formed elements by centrifugation for 15 min, 3000 rpm. The supernatant liquid was separated and the investigated parameters were measured in it.

The selection of biochemical analysis parameters was carried out in such a way as to investigate indicators of inflammation and metabolism of bone tissue, as well as the general somatic condition of experimental animals. The content of glycoproteins was estimated according to the modified method of O. P. Shtenberg and Y. N. Dotsenko (reaction with ammonium molybdate in a sulfuric acid medium) [13] and chondroitin sulfates according to the Nemeth–Csoka method as modified by L. I. Slutsky (reaction with rivanol) [14]; activity of alkaline and acid phosphatases by reaction with diethanolamine using kinetic methods according to the «Alkaline phosphatase — kin Sp. L» and «Acid phosphatase — kin Sp. L» instructions. Calcium content was determined by the potentiometric method using the AEK-01 electrolyte analyzer, and total protein by the biuret method [13].

Statistical data analysis was performed using the IBM SPSS Statistics 20 and Microsoft Office Excel 2007 software. The results of measurements are shown as median and quartiles (25 %, 75 %).

Mann–Whitney analysis was used to compare two groups. The difference was considered statistically significant at $p < 0.05$ [15].

Results and their discussion

Comparison in age groups

In the process of comparing the results of biochemical studies of blood serum indicators of rats after modeling an *unfilled defect* of critical size, a significant difference between the indicators of 3- and 12-month-old rats for the same duration of the experiment was found only in terms of acid phosphatase activity, which was 1.28 times higher in younger animals on the 28th day of the experiment ($p = 0.008$) (Table).

In contrast to this, under the conditions of using *allogeneic bone implants* for defect plasticity, significant differences were determined in the content of glycoproteins, chondroitin sulfates, alkaline and acid phosphatase activity in the blood serum of animals of different age groups. In particular, in 12-month-old rats, the level of glycoproteins in blood serum was lower than in 3-month-old rats by 1.10 times ($p = 0.008$) 14 days after implantation, higher by 1.40 times ($p = 0.008$) and 1. 20 times ($p = 0.032$) — after 28 and 90 days, respectively. This reflects an increase compared to 3-month-old rats at later times of the experiment in the formation of carbohydrate-protein complexes, formed at the stage of inflammation, the initial restructuring of connective tissue. The specified feature may also be a consequence of a greater content of connective tissue in the area of implantation. Also, in older animals compared to younger ones, 14 days after implantation, the level of chondroitin sulfates in blood serum increased by 1.30 times ($p = 0.008$), the activity of alkaline phosphatase decreased by 1.80 times ($p = 0.016$), the acidic one decreased by 1.50 times ($p = 0.018$), which indicates a delay in the formation and reorganization of bone tissue. The index of total protein and calcium content did not differ in age groups for any of the observation periods (Table).

Comparison in groups with allo-implant and unfilled defect

3-month-old rats with an unfilled defect on the 28th day were shown to have a 1.35-fold decrease in the level of glycoproteins ($p = 0.008$), and on the 90th day, the specified indicator increased again almost to the initial values, by 1.25 times compared to with values on the 28th day.

On the 28th day, a temporary decrease in the content of chondroitin sulfates in the blood serum of 3-month-old animals with an unfilled defect was also recorded, followed by a return to the initial level. In particular, on the 28th day, the indicator was reduced by 1.40 times, and then increased by 1.44 times ($p = 0.008$) (Table).

In 3-month-old rats with installed bone allo-implants, the level of glycoproteins in the blood serum was higher for all periods of the study compared to the group with an unfilled defect: on the 14th day by 1.20 times ($p = 0.016$), on the 28th day by 1.50 times ($p = 0.036$), on the 90th day by 1.20 times ($p = 0.032$), which can be explained by the body's response to the introduction of foreign material. Apart from this, over time, the studied index in the allo-implant group decreased significantly: by 1.40 times ($p = 0.008$) after 28 days, by 1.20 times ($p = 0.008$) after 90 compared to that on the 14th day.

The activity of alkaline phosphatase in the blood serum of animals with allo-implants compared to the group with an unfilled defect did not differ significantly at all observation periods, but decreased over time: after 90 days by 1.21 times ($p = 0.008$) compared to the value on the 14th a day

Acid phosphatase activity in animals with allo-implants was 1.59 times higher ($p = 0.008$) 28 days after surgery compared to the group with an unfilled defect and did not differ at other follow-up times. However, it decreased over time: after 28 days by 1.32 times ($p = 0.008$), after 90 days by 1.34 times ($p = 0.009$) compared to the indicator on the 14th day. This can be explained by the increased rate of bone tissue formation up to the 28th day of the experiment and, accordingly, significant functional activity of osteoclasts and osteoblasts and a decrease in the activity of the process in the future, when reorganization of the regenerate occurs.

The levels of calcium and total protein were not significantly different in the groups of 3-month-old rats with allo-implants and unfilled defect, while the level of chondroitin sulfates on the 90th day was lower in conditions of installation of allo-implants by 1.44 times ($p = 0.008$).

In animals with allo-implants, the level of chondroitin sulfates changed over time: compared to the 14th day, it was lower by 1.19 times ($p = 0.008$) after 28 days, by 1.18 times ($p = 0.046$) after 90 days, did not differ between terms of 28 and 90 days (Table).

In 12-month-old rats with an unfilled defect, a temporary decrease in the content of chondroitin sulfates in blood serum was observed on the 28th day, followed by a significant increase on the 90th day. Namely: on the 28th day of the experiment, the indicator in the analyzed group was 1.43 times lower than on the 14th ($p = 0.008$), and on the 90th day 1.78 times higher than

Table

Serum biochemical parameters of different age animals after simulation of a critical size defect in the femoral metaphysis with or without the use of allogeneic bone implants (Me, (25 %; 75 %)) (n = 5)

Period after the intervention, days	Indicator	Rat group			
		unfilled defect		allo-implant	
		3-month-old	12-month-old	3-month-old	12-month-old
1	2	3	4	5	6
14	Glycoproteins, g/l	0.76 (0.68; 0.78)	0.68 (0.62; 0.70) $p_1 = 0.200$	0.92 (0.88; 0.97) $p_2 = 0.016$	0.81 (0.78; 0.84) $p_1 = 0.008$ $p_2 = 0.016$
	Total protein, g/l	63.8 (59.4; 79.3)	62.1 (59.6; 65.6) $p_1 = 0.686$	56.7 (54.9; 63.4) $p_2 = 0.190$	65.7 (60.4; 67.3) $p_1 = 0.151$ $p_2 = 0.730$
	Ca, mmol/l	2.26 (2.12; 2.46)	2.23 (2.13; 2.31) $p_1 = 0.886$	2.02 (2.01; 2.23) $p_2 = 0.190$	2.30 (2.15; 2.35) $p_1 = 0.151$ $p_2 = 0.556$
	Chondroitin sulfates, g/l	0.367 (0.204; 0.410)	0.331 (0.301; 0.387) $p_1 = 0.886$	0.300 (0.286; 0.350) $p_2 = 0.730$	0.384 (0.374; 0.399) $p_1 = 0.008$ $p_2 = 1.000$
	Alkaline phosphatase activity, units/l	400.0 (347.3; 468.5)	318.5 (271.5; 360.0) $p_1 = 0.486$	343.0 (296.0; 408.0) $p_2 = 0.116$	295.0 (276.0;416.0) $p_1 = 0.016$ $p_2 = 0.730$
	Activity of acid phosphatase, units/l	38.4 (29.2; 47.6)	31.9 (29.8 38.3) $p_1 = 0.769$	35.5 (32.1; 37.2) $p_2 = 0.256$	28.5 (23.9; 33.0) $p_1 = 0.116$ $p_2 = 0.632$
28	Glycoproteins, g/l	0.56 (0.44; 0.68) $p_3 = 0.013$	0.65 (0.56; 0.70) $p_1 = 0.200$ $p_3 = 0.413$	0.67 (0.64; 0.71) $p_2 = 0.036$ $p_3 = 0.008$	0.95 (0.87; 1.07) $p_1 = 0.008$ $p_2 = 0.036$ $p_3 = 0.008$
	Total protein, g/l	67.3 (62.3; 72.4) $p_3 = 0.434$	74.8 (74.4; 89.3) $p_1 = 0.100$ $p_3 = 0.664$	64.1 (61.0; 70.1) $p_2 = 0.250$ $p_3 = 0.095$	68.4 (60.8; 70.4) $p_1 = 0.841$ $p_2 = 0.036$ $p_3 = 0.421$
	Ca, mmol/l	2.35 (2.35; 2.38) $p_3 = 0.738$	2.40 (2.38; 2.60) $p_1 = 0.100$ $p_3 = 0.562$	2.27 (2.18; 2.36) $p_2 = 0.250$ $p_3 = 0.016$	2.37 (2.16; 2.38) $p_1 = 0.841$ $p_2 = 0.036$ $p_3 = 0.019$
	Chondroitin sulfates, g/l	0.262 (0.177; 0.314) $p_3 = 0.017$	0.232 (0.207; 0.254) $p_1 = 0.200$ $p_3 = 0.008$	0.253 (0.218; 0.304) $p_2 = 0.856$ $p_3 = 0.008$	0.274 (0.241;0.305) $p_1 = 1.000$ $p_2 = 0.856$ $p_3 = 0.008$
	Alkaline phosphatase activity, units/l	439.0 (421.0;486.0) $p_3 = 0.127$	414.0 (383.0;469.0) $p_1 = 0.116$ $p_3 = 0.010$	348.0 (339.5; 426.0) $p_2 = 0.143$ $p_3 = 0.956$	302.0 (269.0;344.5) $p_1 = 0.046$ $p_2 = 0.008$ $p_3 = 0.256$
	Activity of acid phosphatase, units/l	42.7 (36.1; 50.1) $p_3 = 0.282$	33.4 (31.9; 36.9) $p_1 = 0.008$ $p_3 = 0.256$	26.8 (23.9; 30.7) $p_2 = 0.008$ $p_3 = 0.018$	22.8 (19.1; 25.4) $p_1 = 0.056$ $p_2 = 0.008$ $p_3 = 0.116$
90	Glycoproteins, g/l	0.70 (0.66; 0.70) $p_3 = 0.047$ $p_4 = 0.008$	0.73 (0.70; 0.90) $p_1 = 0.200$ $p_3 = 0.085$ $p_4 = 0.016$	0.80 (0.77; 0.85) $p_2 = 0.030$ $p_3 = 0.008$ $p_4 = 0.008$	0.93 (0.86; 1.14) $p_1 = 0.032$ $p_2 = 0.143$ $p_3 = 0.016$ $p_4 = 1.000$
	Total protein, g/l	71.9 (67.10; 86.40) $p_3 = 0.314$ $p_4 = 0.256$	74.6 (63.50; 77.50) $p_1 = 1.000$ $p_3 = 0.055$ $p_4 = 1.000$	76.7 (69.9; 93.0) $p_2 = 0.571$ $p_3 = 0.008$ $p_4 = 0.032$	75.6 (68.8; 96.4) $p_1 = 1.000$ $p_2 = 0.571$ $p_3 = 0.032$ $p_4 = 0.056$

Continuation of Table

1	2	3	4	5	6
90	Ca, mmol/l	2.38 (2.35; 2.55) p_3 = 0.251 p_4 = 1.000	2.40 (2.25; 2.45) p_1 = 1.000 p_3 = 0.071 p_4 = 1.000	2.41 (2.38; 2.55) p_2 = 1.000 p_3 = 0.013 p_4 = 0.208	2.38 (2.33; 2.42) p_1 = 0.310 p_2 = 0.393 p_3 = 0.776 p_4 = 0.204
	Chondroitin sulfates, g/l	0.367 (0.324; 0.412) p_3 = 1.000 p_4 = 0.008	0.415 (0.380;0.465) p_1 = 0.100 p_3 = 0.0460 p_4 = 0.008	0.255 (0.241; 0.288) p_2 = 0.008 p_3 = 0.046 p_4 = 0.222	0.273 (0.267;0.296) p_1 = 0.222 p_2 = 0.008 p_3 = 0.008 p_4 = 1.000
	Alkaline phosphatase activity, units/l	311.0 (271.0;379.0) p_3 = 0.032 p_4 = 0.008	250.2 (208.0; 321.1) p_1 = 0.100 p_3 = 0.407 p_4 = 0.008	278.0 (244.5; 308.5) p_2 = 0.393 p_3 = 0.008 p_4 = 0.008	245.0 (210.5;292.5) p_1 =0.690 p_2 = 0.865 p_3 = 0.841 p_4 = 0.116
	Activity of acid phosphatase, units/l	39.9 (35.6; 44.2) p_3 = 0.956 p_4 = 0.256	34.0 (26.4; 42.6) p_1 = 0.129 p_3 = 0.400 p_4 = 0.956	26.5 (19.9; 35.6) p_2 = 0.032 p_3 = 0.007 p_4 = 0.009	28.2 (21.8; 34.3) p_1 = 0.045 p_2 = 0.748 p_3 = 0.863 p_4 = 0.051

Note. p_1 — comparison of indicators in rats of different ages with the same type of defect filling at the same time after the intervention; p_2 — comparison of the indicators of the group with allo-implants with the indicators of the group with an unfilled defect in rats of the same age at the same time after the intervention; p_3 — comparison of indicators at different times of the experiment in animals of the same age and type of defect filling with indicators of the same group on the 14th day after the intervention; p_4 — comparison of indicators in groups of rats of the same age and type of defect filling on the 90th day after the intervention with the indicators on the 28th day after the intervention.

on the 28th one (p = 0.008) and even 1.31 times higher than on the 14th day (p = 0.008), which may indicate activation in both age groups with an unfilled defect in the formation of connective tissue on the 28th day (Table). Moreover, in younger rats, this process subsided on the 90th day, and in older rats, the active formation of connective tissue continued.

In addition, on the 90th day, 12-month-old rats with an unfilled defect showed a 1.66-fold decrease in alkaline phosphatase activity compared to the 14th day (p = 0.008) and a 1.66-fold decrease compared to the 28th (p = 0.008), which indicates a slowdown in the process of bone tissue formation.

In 12-month-old rats with installed bone allo-implants, the level of glycoproteins in blood serum was 1.2 times higher (p = 0.016) after 14 days, and 1.5 times (p = 0.036) after 28 compared to the group with an unfilled defect and was not statistically significantly different after 90 days. In contrast to 3-month-old rats with allo-implants installed, with the passage of time the studied indicator in the group of 12-month-old animals increased: compared to the 14th day, it was 1.20 times greater (p = 0.008) after 28 days, and 1.14 times (p = 0.016) after 90 days.

The content of calcium and total protein in the blood serum of 12-month-old rats with allo-implants 28 days after surgery was slightly lower (1.10 times, p = 0.036) compared to the group with an unfilled defect, and did not differ significantly at other times. The level of total protein was 1.20 times higher (p = 0.032) 90 days after the operation compared to the 14th day and did not differ at other times.

The level of chondroitin sulfates in the blood serum of age-matched rats with allo-implants is 1.52 times lower (p = 0.008) 90 days after surgery compared to the group with an unfilled defect, reflecting a reduction in the formation of connective tissue in them. No statistically significant differences between groups were found for this indicator at other follow-up periods. At the same time, in animals with allo-implants, the content of chondroitin sulfates gradually decreased: compared to the 14th day, it was lower by 1.42 times (p = 0.008) after 28 days, by 1.41 times (p = 0.008) after 90.

In animals with an unfilled defect, the peak values of chondroitin sulfate content in blood serum were determined on the 90th day of the experiment — an increase of 1.25 times (p = 0.008) compared to the 14th day, despite the fact that on the 28th the indicator was lower , than on the 14th in 1.42 times (p = 0.008).

In blood serum of 12-month-old rats with allo-implants on the 28th day, alkaline phosphatase activity was 1.37 times lower, acid phosphatase activity was

1.47 times lower (p = 0.008) compared to the group of this age, where the defect was left unfilled. The indicators did not change during the experiment.

Discussion

Based on the results of a biochemical study of the blood serum of 3- and 12-month-old rats, which were modeled with a defect of critical size (depth 3 mm, diameter 3 mm) in the metaphysis of the femur, it was determined that the introduction of allo-implants into the defect area leads to an increase in the content of glycoproteins in the blood serum for all periods of observation, regardless of the recipient's age. This may be related to the reaction to the implant as a foreign body and the process of its reconstruction, which is still ongoing 90 days after the operation. Indeed, histological studies conducted within the framework of this experiment showed that about 10 % of the initial size of the allo-implant remained in the area of the defect at the end of the study (90 days) in rats of both age groups [12]. In clinical conditions, incomplete reconstruction of structural allo-implants with the formation of connective tissue was determined as a result of histological analysis of patient tissues [16, 17].

We found a higher level of chondroitin sulfates in the serum of 12-month-old allo-implanted rats 14 days after implantation compared to 3-month-old animals, which is probably due to a 2.1-fold (p = 0.005) higher content in in the area of connective tissue implantation [12]. In addition, at this time, the activity of alkaline phosphatase in blood serum was determined to be lower in older rats than in younger rats, which indicates a slowdown in the processes of bone tissue formation, namely, a decrease in the activity of osteoblasts. As a result, the relative area of bone tissue in 12-month-old rats was 2.4 times (p = 0.0001) smaller compared to 3-month-old rats [12].

Signs of activation of bone regeneration reorganization began to appear on the 14th day in animals with allo-implants, continued on the 28th day and minimized on the 90th with a greater severity in 3-month-old animals. In 12-month-old rats with installed allo-implants, the reactions were slower: signs of activation of bone tissue remodeling according to the content of metabolic markers appeared on the 28th day and continued with a partial decrease on the 90th day.

Conclusions

Biochemical study of blood serum showed that the introduction of allo-implant resulted in an increase in the content of glycoproteins for all periods of observation in rats of both age groups. An increase in the level of chondroitin sulfates was recorded in 12-month-old rats 14 days after implantation compared to 3-month-old animals, which was due to their higher content in the area of connective tissue implantation. A decrease in the activity of alkaline phosphatase was also determined for this period in older rats compared to 3-month-old animals, indicating a slowdown in bone formation processes, namely, a decrease in the activity of osteoblasts.

Treatment of experimental rats with a critical size defect in the metaphysis of the femur with allo-implants resulted in biochemical signs of activation of regenerative processes in them, but this activation was insufficient and required additional strengthening due to certain external influences.

Conflict of interest. The authors declare no conflict of interest.

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BIOCHEMICAL INDICATORS OF BLOOD SERUM OF RATS OF DIFFERENT AGES AFTER FILLING THE DEFECT IN THE METAPHYSIS OF THE FEMUR WITH ALLOGENEIC BONE IMPLANTS

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