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FEATURES OF MINERAL METABOLISM IN BONE TISSUE DURING THE REGENERATION OF EXPERIMENTAL DEFECTS OF RATS' LOWER JAWS IN DYNAMICS

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Summary : We studied the dynamics of mineral metabolism (calcium and phosphorus) on the created bone defect model and compared the findings with results that we had found after defect's bone grafting with materials based on hydroxyapatite and polylactide with different percentage of components (1 and 2 research groups). It was established that Ca and P indexes are increasing in control group, but there are no further intensive processes of primary callus remodulation as the level of main elements concentration stay practically unchanged for 90th and 180th day.In experimental group 1, the main part of which is synthetic hydroxyapatite, the level of demineralization processes is moderate with lower increase of Ca and P concentration in blood, and the apogee of reverse processes is day 30th. In farewell terms 990th and 180th day) increased index of Ca and P level is keeping, which testifies about intensive remodulation processes.In experimental group 2, where the part of hydroxyapatite is 50%, intensive processes of mineralization are setting in faster – on day 21st. Remodulation of primary callus is also faster (90th days).

Key words: bone defect, bone tissue regeneration, hydroxyapatite, polylactide, calcium, phosphorus

Bone tissue is constantly renewing system of organism that consists of cellular elements and bone matrix [4, 5, 12], according to that the processes of "dying" and "replacing" of old cells with new, that occur in bone, are generally called physiological regeneration. However, it is distinguished such thing as "reparative regeneration", which is a process that caused by destruction of bone structures, which significantly exceeds the limits of physiological regeneration and aims to restore anatomical integrity of bones [7]. It should be noted that the mechanisms of physiological and reparative regeneration are similar, and always include the processes of disintegration of damaged cells, proliferation of viable cells, their differentiation and restructuring of regenerate. As noted by some scholars reparative regeneration is enhanced physiological regeneration [1, 6].

Researching of reparative regeneration processes of bone tissue are still in the middle of nowadays researchers' attention due to the emergence of various kinds of osteoreplacing materials for filling of bone defects. [2, 3, 8, 9, 10]

As the ideal material for bone grafting does not exist yet, it is still needed to deepen the necessary knowledge to understand the processes of regeneration based on a combination of all the above-mentioned mechanisms. It is known, that the flow of reparative ostogenesis is accompanied by activation of mineral metabolism and the main mineral components of bone matrix are calcium (Ca) and phosphorus (P) [11].

THE PURPOSE of our paper was to determine the features of the dynamics of the

above-mentioned elements (Calcium and Phosphorous) in the blood of experimental animals both in control and experimental group where the bone tissue defect was renewed by means of bone-plastic material based on hydroxyapatite and polilactide with different percentage of components.

RESEARCH METHODS. The experiment was conducted on white mature male rats with 180-200 g weight, which were kept on a standard vivarium diet. The animals were divided into groups: control, 1st and 2nd experimental. Each group was divided into subgroups, depending on the timing of withdrawal from experiment (6 animals at the time). All manipulations with experimental animals was carried out according to international requirements under "the European Convention of the protection of vertebrate animals used for experimental and other scientific purposes" Strasbourg, 1986 and "General ethical principles of animal experiments" adopted by First National Congress of Bioethics Kyiv, 2001.The surgeries were performed under general anesthesia. For this purpose was used 0.04 ml of 5% solution of sodium thiopental, which was administered intraperitoneally into the left lower abdominal quadrant. The surgery was performed by wideknown method [Chechyn A.D., 1989.], partly modified. After removal of wool cover (projection of lower jaw and submaxillary area from the left) and processing of operating margins with 3% iodine solution, we conducted skin incision parallel to and below the lower edge of mandible of 1-1.5 cm length. Then we separated soft tissues to the bone. With physiodispenser SURGEC XT (NSK, Japan) at a speed of 800 rev/min. with constant cooling of 0.9% sodium chloride we created throughout hole in the area of mandibular angle on the left. Dental boron diameter is 2 mm. At the control group of experiments the wound was sewed after antiseptic treatment. Skin stitches were moistened with 1% solution of brilliant green. In 1 experimental series was used implantation material hydroxyapatite 80% : polilaktyde 20%, for experimental group 2 was used implantation material hydroxyapatite 50% : polilaktyde 50% . To create this composition was used polilaktyde (Poly (L-Lactide) Purasorb PL 32 (Holland) and hydroxyapatite (HA) Sa10-x (PO 4) 6 (OH) 2 with a particle size of 0.1 mm (sintering temperature = 10,500 C) synthesized at the Department of Chemical Technology of Silicates NU "Lviv Polytechnic".The block (co) polymerization of the compositions was performed at the strove in air at a temperature of 348 K during 4.5 h. After the synthesis polymer samples were cooled to room temperature during 1.5-2 hours. The animals were exasperated of experiment on the 7th, 14th, 21th, 30th, 90th, 180th day with overdose of sodium thiopental solution, which was administered intraperitoneally.

The serum of experimental animals was used for research. Most studies were conducted at the biochemical analyzer «Humalaizer 2000". To assess the indicators of mineral metabolism we used photometric method of the standard set of «Human» company.

The comparison of averages in different groups was performed using nonparametric statistical methods (Wilcoxon test). The differences have been taken as significant when $p < 0.05$.

RESULTS AND DISCUSSION. The resulting experimental data on the concentration of calcium (mmol/ l) in blood of experimental animals is shown in Table 1 and on Picture 1.

In the control group of animals in the first period of the study (day 7^{th}) was found the

greatest increase in the concentration of Ca, which reached 134.58% (p <0.05) of intact animals. Further, up to 30th day, was noticed the gradual decline of Ca content to 115.41% (p <0.05) at day 14th, and to 103.33% (p> 0.05) at day 21st to the significantly lower physiological level for day 30 (81.25% of p <0.05). In longer terms of experiment (day 90 and 180) the concentration of Ca was contrasted within intact animals and was 97.91% (p <0.05) and 99.61% (p <0.05) respectively. In quantitative and dynamic ratio the picture in the experimental groups was somewhat different. The initial increase in the concentration of Ca in the blood of animals of group 1 reached only 120.83% (p <0.05) out of intact animals. Next till day 30th there was a gradual reduction of this rate of 105% (p> 0.05) on the day 14th followed by a significant decrease below the physiological level to day 21st at 90.83% (p > 0.05) to a minimum of day 30th - 77.08% (p <0.05). In longer terms was found repeated and significant increase of Ca concentration to 118.75% (p <0.05) at day 90th and 111.25% (p > 0.05) at day 180th of study.

The initial increase in the concentration of Ca in the blood of animals of experimental group 2 reached 122.91% (p <0.05) out of intact animals. Similarly to previous cases, there was further decrease in the concentration of Ca to 101.25% (p> 0.05) for day 14th, and a minimum for day 21st - 80.41% (p <0.05). But at day 30th, Ca concentration increased and amounted to 83.33% (p> 0.05). The marks of Ca concentrations were higher of the level of intact animals for day 90th - 109.16% (p> 0.05). On the end of surveillance - day 180th - Ca concentration meet physiological parameters and was 100.83% (p> 0.05) from the study revealed during the research of intact animals.

Pic.1. The percentages dynamics of calcium content in the blood of experimental animals to that of intact (100%)

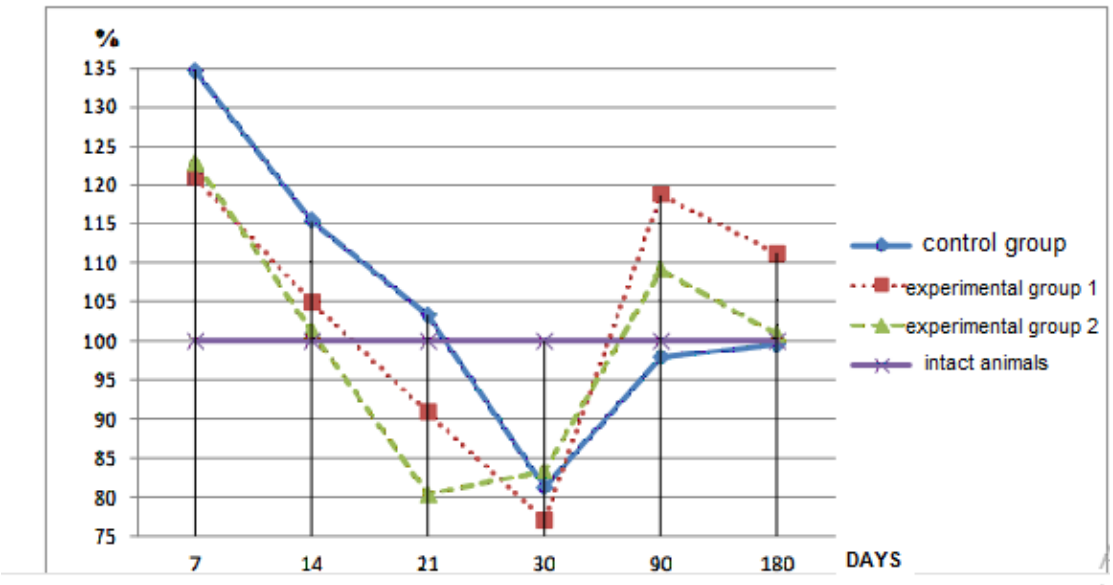


Table 1.

The content of calcium (mmol/l) in blood of experimental animals, (M ± m, n = 6)

Terms of observations (days)	Groups of animals			
	Intact	Control	Experimental 1	Experimental 2
7	2,40±0,10	3,23±0,09 p_1<0,05	2,90±0,09 p_1<0,05 p_2<0,05	2,95±0,06 p_1<0,05 p_2<0,05 p_3>0,05
14	2,40±0,10	2,77±0,11 p_1<0,05	2,52±0,07 p_1>0,05 p_2>0,05	2,43±0,12 p_1>0,05 p_2>0,05 p_3>0,05
21	2,40±0,10	2,48±0,07 p_1>0,05	2,18±0,10 p_1>0,05 p_2>0,05	1,93±0,03 p_1<0,05 p_2<0,05 p_3>0,05
30	2,40±0,10	1,95±0,16 p_1<0,05	1,85±0,08 p_1<0,05 p_2>0,05	2,0±0,09 p_1<0,05 p_2>0,05 p_3>0,05
90	2,40±0,10	2,35±0,11 p_1<0,05	2,85±0,07 p_1<0,05 p_2<0,05	2,62±0,08 p_1<0,05 p_2>0,05 p_3>0,05
180	2,40±0,10	2,38±0,07 p_1>0,05	2,67±0,04 p_1>0,05 p_2<0,05	2,42±0,10 p_1>0,05 p_2>0,05 p_3<0,05

Notes: p1 - the value of credibility ratio to the intact animals' group

p2 - the value of credibility ratio to the control group

p3 - the value of credibility ratio to the experimental group 1

More monotonous and plain picture was during the study of the phosphorus concentration (P), the data of which is presented in Table 2 and Picture 2.

Primary growth (day 7th) of P concentration in the blood of animals in the control group was within the 114.19% (p>0.05) out of the intact animals' level.

Similarly to previously described research, there was a gradual slow and reduce of P concentration to 108.64% (p> 0.05) at day 14, 105.55% (P> 0.05) at day 21st to the most minimal and below the physiological level's data for day 30th - 89.51% (p> 0.05). Deadlines of observation were characterized by normalization of this index and accounted of 98.76% (p> 0.05) for day 90th and 100.61% (p> 0.05) for day 180th.

In animals of experimental group 1 for day 7th, P concentration increased to 112.34% (p>

0.05) and tended to continue its reduction of 102.46% (p> 0.05) for day 14th, 94.44% (p> 0.05) for day 21st and 80.24% (p>0.05) for day 30th. In this group, for the final terms of the research were found the increasing concentration of P to 114.81% (p <0.05) for day 90th and its slight decreasing for day 180th to 108.02% (P< 0.05).

For day 7th animals in experimental group 2 the P concentration growth rate was recorded within the 111.72% (p> 0.05). For day 14th we observed the subsidence to 101.23% (p> 0.05), which further continued for day 21st, when the concentration of P was amounted to 85.80% (p <0.05) versus intact animals. For the next term of observations were found the trend to increasing, and the concentration of P was amounted to 89.50% (p> 0.05). Slightly larger than in intact animals was result on day 90th, which was 106.17% (p> 0.05). The entire normalization in P exchange was observed in this group for day 180th (100.61% of p> 0.05).

Table 2.

Phosphorus content (mmol/l) in blood of experimental animals, (M ± m, n = 6)

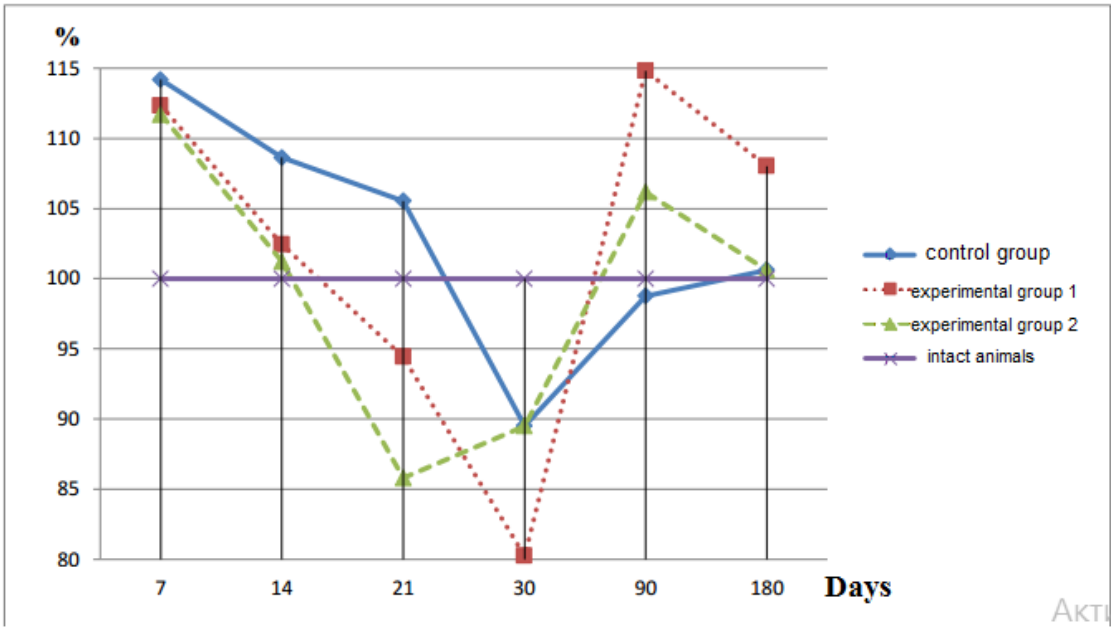
Terms of observations (days)	Groups of animals			
	Intact	Control	Experimental 1	Experimental 2
7	1,62±0,07	1,85±0,06 p_1>0,05	1,82±0,06 p_1>0,05 p_2>0,05	1,81±0,07 p_1>0,05 p_2>0,05 p_3>0,05
14	1,62±0,07	1,76±0,09 p_1>0,05	1,66±0,08 p_1>0,05 p_2<0,05	1,64±0,04 p_1>0,05 p_2>0,05 p_3>0,05
21	1,62±0,07	1,71±0,09 p_1>0,05	1,53±0,07 p_1>0,05 p_2<0,05	1,39±0,04 p_1<0,05 p_2>0,05 p_3>0,05
30	1,62±0,07	1,45±0,02 p_1>0,05	1,30±0,06 p_1<0,05 p_2>0,05	1,45±0,05 p_1>0,05 p_2>0,05 p_3>0,05
90	1,62±0,07	1,60±0,04 p_1>0,05	1,86±0,04 p_1<0,05 p_2<0,05	1,72±0,07 p_1<0,05 p_2<0,05 p_3<0,05
180	1,62±0,07	1,63±0,08 p_1>0,05	1,75±0,05 p_1<0,05 p_2>0,05	1,63±0,08 p_1>0,05 p_2>0,05 p_3<0,05

Notes: p1 - the value of credibility ratio to the intact animals' group

p2 - the value of credibility ratio to the control group

p3 - the value of credibility ratio to the experimental group 1

Pic. 2. The percentages dynamics of phosphorous content in the blood of experimental animals to that of intact (100%)



CONCLUSIONS: Based on these data we can draw the conclusion that after application of significant defect in its volume in the body of rats' lower jaw appears the concentration growth of both its mineral components, especially calcium, it is due to the intense demineralization in the area of injury. For day 30th microelements' content is below the physiological, indicating the reverse processes, which is an integral part of osteoregeneration. But intensive processes of remodulation of primary bone moseley does not happen, as the concentration of main elements that create the mineral part of bone stay virtually unchanged in blood at day 90th and 180th.

During the introduction of osteoimplantant into experimental defect, most of which is a synthetic hydroxyapatite, the level of demineralization processes is moderate with lower increase of Ca and P concentrations levels in blood, and the apogee of mineralization processes is day 30th, when the level of concentration is the lowest and significantly lower than physiological. In the longer terms (day 90th and 180th) there is preserved increased index above the physiological level of Ca and P, which suggests intensive processes of primary bone seam remodulation in this period.

The reduction of hydroxyapatite in bone substitute and increasing the share of polilaktyde to 50% (experimental group 2) also reduces the intensity of organism's demineralization, as evidenced by moderate growth of Ca and P concentrations in the blood of animals in this group. Note that intensive processes of mineralization, which were shown in the largest decrease of mineral components concentration occurs the fastest for day 21. Because of this, remodulation primary callus is also occur faster (day 90th), when it was revealed increasing concentrations of Ca and P in blood. And for the finalization of all the observations, all bone regeneration

processes are fully completed, as evidenced by normalization of investigated at this work stage indicators of osteogenesis markers.

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