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Abstract

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INFLUENCE OF ANTITUMOR CHEMOTHERAPEUTICS ON THE STRUCTURE AND PHOSPHORUS-CALCIUM METABOLISM OF INJURED LONG TUBULAR SKELETAL BONES

The high frequency of fractures in cancer patients is due to a decrease in bone strength which is associated with bone metabolism disorders such as osteoporosis, metastatic bone disease, and pathological fractures. Anticancer chemotherapy is prescribed for long-term periods and affects bone metabolism, in particular mineralization of bony tissue.

Objective. To study the structure and macronutrient composition of long tubular bones in rats under the influence of antitumor chemotherapeutics.

Materials and methods. The study involved 96 white laboratory 7 month-old male rats weighing 230 ± 10 g that were cut by a ball-shaped dental burr to obtain a 2 mm diameter perforation defect to the medullary cavity in the middle third of the femoral shaft. The animals were divided into the control (n = 24) and three experimental groups (Group I, II, and III, n = 72), which were given intraperitoneal antitumor chemotherapeutics after the cut procedure: Group I (n = 24) – doxorubicin (60 mg/m²), Group II (n = 24) – 5-fluorouracil (600 mg/m²), Group III (n = 24) – methotrexate (40 mg/m²). The therapy was repeated every 21 days throughout the experiment. On the 15th, 30th, 45th, and 60th day after the injury, the animals were sacrificed with subsequent removal of the injured long tubular bones. The samples were studied using scanning electron microscopy and X-ray energy dispersive spectroscopy. Statistical analysis of the obtained digital values was performed with the help of MX Excel XP statistical computer program using the Student's t-test. The difference was considered significant at $p < 0.05$.

Results. Antitumor chemotherapy slows down the formation of bone regenerate in the area of the defect and causes disorders of phosphorus-calcium metabolism in the injured bone. This is manifested by a decrease in the intensity of newly formed organic matrix mineralization in the area of the defect and a decrease in the level of calcium and phosphorus in the native bone and on its border with the regenerate. Doxorubicin and methotrexate provide the most negative impact on mineralization process among antitumor chemotherapeutic agents.

Conclusions. The use of antitumor chemotherapeutic agents –

doxorubicin, 5-fluorouracil and methotrexate – slows down the processes of reparative regeneration at all stages of recovery after injury and reduces the phosphorus-calcium metabolism of injured long tubular bones.

Keywords: reparative regeneration, scanning microscopy, spectral analysis, phosphorus-calcium metabolism, mineralization, anticancer chemotherapy.

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Резюме

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ВПЛИВ ПРОТИПУХЛИННИХ ХІМІОПРЕПАРАТІВ НА СТРУКТУРУ ТА ФОСФОРНО-КАЛЬЦІЄВИЙ ОБМІН ТРАВМОВАНИХ ДОВГИХ ТРУБЧАСТИХ КІСТОК СКЕЛЕТУ

Велика частота виникнення переломів у онкологічних пацієнтів зумовлена зниженням міцності кісток внаслідок розвитку порушень кісткового метаболізму у вигляді остеопорозу, метастатичного враження кісток та патологічних переломів. Необхідна для лікування раку протипухлинна хіміотерапія призначається курсами на тривалий проміжок часу та впливає на кістковий метаболізм, зокрема на мінералізацію кісткової тканини.

Мета роботи. Вивчити структуру та макроелементний склад довгих трубчастих кісток щурів в умовах впливу протипухлинних хіміопрепаратів.

Матеріали та методи. Дослідження проведено на 96 білих лабораторних щурах-самцях 7-місячного віку вагою 230 ± 10 гр, яким наносився дірчастий дефект діаметром 2 мм шароподібною фрезою до кістковомозкового каналу в середній третині діафіза стег ової кістки. Тварини були поділені на контрольну (n = 24) та три експериментальні групи (I, II, III, n = 72), яким після нанесення травми вводили внутрішньоочеревинно протипухлинні хіміопрепарати: I-й (n = 24) – доксорубіцин (60 мг/м²), II-й (n = 24) – 5-фторурацил (600 мг/м²), III-й (n = 24) – метотрексат (40 мг/м²) та повторювали їх введення кожну 21-у добу протягом усього експерименту. На 15-ту, 30-ту, 45-ту, 60-ту добу після травми тварин виводили з експерименту з наступним вилученням травмованих довгих трубчастих кісток. Досліджувані зразки вивчали за допомогою скануючої електронної мікроскопії та рентгенівської енергодисперсійної спектроскопії. Статистичний аналіз отриманих цифрових показників проводили за допомогою статистичної комп'ютерної програми MX Excel XP з використанням t-тесту Ст`юдента. Відмінності вважали значущими за $p < 0,05$.

Результати дослідження. Протипухлинні хіміопрепарати сповільнюють формування кісткового регенерату в ділянці дефекту та спричиняють порушення фосфорно-кальцієвого обміну травмованої кістки. Це проявляється зниженням інтенсивності мінералізації новоутвореного органічного матриксу в ділянці дефекту та зниженням рівня кальцію та фосфору в самій материнській кістці та на її межі з регенератом. Найбільш негативний вплив серед протипухлинних хіміопрепаратів на мінералі-

зацію мають доксорубіцин та метотрексат.

Висновки. Застосування протипухлинних хіміопрепаратів доксорубіцину, 5-фторурацилу та метотрексату призводить до сповільнення процесів репаративної регенерації на всіх етапах відновлення після травми та зниження фосфорно-кальцієвого обміну травмованих довгих трубчастих кісток скелету.

Ключові слова: репаративна регенерація, скануюча мікроскопія, спектральний аналіз, фосфорно-кальцієвий обмін, мінералізація, протипухлинні хіміопрепарати.

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Introduction/Вступ

Fractures are the most common lesions of the bone system. In connection with considerable growth of oncological diseases in present days, the percent of fractures among the patients of this group is also constantly increasing. This is due to the development of bone metabolism disorders such as osteoporosis, metastatic bone disease, and pathological fractures. Disorders of bone continuity are especially common in breast, prostate, thyroid, kidney, and lung cancers [1, 2]. Fractures lead to long-term and significant disability of patients, and particularly in cancer patients, they cause cessation or postponing of the necessary anticancer chemotherapy.

An important factor in the occurrence of fractures is represented by a decrease in bone mineral density due to changes in its mineral composition [3]. The process of bone mineralization is important for bone hardness and strength. Bone metabolism and tissue properties depend on macronutrients, which can influence the regulation of mineral metabolism, as well as proliferation or activity of osteoblasts and osteoclasts.

According to the literature, extracellular calcium (Ca^{2+}) and inorganic phosphate (P) are the two important factors that determine bone mineralization [4]. Calcium is a macronutrient that is important for the development, growth, and maintenance of the bone tissue, as well as for cellular cytoskeleton stability. The total Ca content in the body of an adult is about 1200 g, which is about 2% of body weight. About 99% of Ca in the body is contained in the bones and teeth in the form of hydroxyapatite, which is responsible for tissue mineralization [5]. Insufficient Ca intake during the growth period can adversely affect bone maturity and contribute to the further increased risk of osteoporotic fractures.

Ca deficiency is a major factor in the development of osteoporosis [6]. The main factors that maintain blood Ca concentration at a constant level are 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF_{23}). Decreased serum Ca content leads to inactivation of the calcium-sensing receptor (CaSR) in the parathyroid glands, which thus stimulates the release of PTH. The latter binds to its receptor in the kidneys, which increases Ca reabsorption and production of $1,25(OH)_2D_3$. Circulating PTH and $1,25(OH)_2D_3$ bind to respective receptors on osteoblasts, which enhances expression of the receptor activator of nuclear factor kappa-B ligand (RANKL). RANKL stimulates osteoclastic bone resorption and Ca and P release into the circulation. It restores Ca levels and triggers down-regulation mechanisms. They involve the release of calcitonin from the thyroid gland, which reduces Ca reabsorption in the kidneys and Ca absorption in the intestine, as well as inhibition of osteoclastic bone resorption, thus maintaining Ca level within optimal limits [7]. FGF23 (phosphatonin) is an endocrine hormone, which is produced by osteoblasts and osteocytes. FGF23 effect is due to the inhibition of renal phosphate reabsorption and vitamin D_3 ($1,25(OH)_2D_3$ hormone synthesis, as well as to the maintenance of normal renal sensitivity to PTH and regulation of bone mineralization [8].

Phosphorus is the major component of bone tissue, being second only to Ca. It is present in the human body in the amount of 550–770 g, of which almost 85% is stored in bones and teeth in the form of phosphoproteins and hydroxyapatite crystals. Phosphorus homeostasis is regulated by three main hormones: PTH, $1,25(OH)_2D_3$ and FGF_{23}. An appropriate level of inorganic P is crucial for osteoblasts and osteocytes activity in the process of matrix mineralization. Deficiency of P in most cases is due to impaired reabsorption of P in the kidneys rather than to its low content in food and leads to impaired mineral deposition and non-mineralized osteoid formation [9].

Bone tissue serves as a depot of calcium and phosphorus in the body and has an extremely high (up to 65%) content of calcium phosphate as compared to other body tissues. Calcium salts in the form of hydroxyapatite crystals are linked to collagen fibers (type I collagen). The effect of calcification (adhesion of calcium salts to the organic bone matrix) is ensured by specific proteins of bone tissue: osteonectin, osteocalcin, and bone sialoprotein. Osteoblasts, which play a major role in the formation of organic intercellular bone matrix, provide continuous growth of hydroxyapatite crystals and act as mediators in the binding of mineral crystals to the protein matrix [10].

It is known from the literature that decreased mineral content leads to a slowdown in the formation of mineralized bone matrix and significantly increases the risk of osteoporotic fractures [11].

The problem of reparative bone regeneration in cancer patients is underexplored today. Antitumor chemotherapy being one of the main methods of cancer treatment is prescribed for long-term courses and affects the mineral metabolism of the injured bone.

Objective: to study morphological features of reparative osteogenesis and macronutrient composition of long tubular bones in rats under the influence of antitumor chemotherapeutics.

Materials and methods. The study involved 96 white laboratory 7 month-old male rats weighing 230 ± 10 g. Throughout the experiment, the rats were kept in the vivarium of the Medical Institute of Sumy State University on a standard diet and water intake regime with free access to food and water. The rats were kept under standard light conditions (12 hours day, 12 hours night). The study was performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986); the General Ethical Principles for Animal Experiments approved by the First National Congress on Bioethics (Kyiv, 2001); the Declaration of Helsinki of the General Assembly of the World Medical Association (2000).

Under ketamine anesthesia (50 mg/kg), all the rats were cut in a sterile operating room by a ball-shaped dental burr to obtain a 2 mm diameter perforated defect to the medullary cavity in the middle third of the femoral shaft. The animals were divided into the control (n = 24) and three experimental groups (Group I, II, and III, n = 72). After being injured, the animals in experimental groups were given intraperitoneal antitumor chemotherapeutics, which are most often used in antitumor chemotherapy protocols; the therapy was repeated every 21 days of the study. Group I (n = 24) – doxorubicin (60 mg/m²), Group II (n = 24) – 5-fluorouracil (600 mg/m²), Group III (n = 24) – methotrexate (40 mg/m²).

On the 15th, 30th, 45th, and 60th day after the injury, the animals were sacrificed by decapitation under ketamine anesthesia (100 mg/kg) with subsequent removal of the injured long tubular bones.

Bone samples were preliminarily fixed in 10% neutral buffered formalin. For further analysis of tissue-specific structures of the regenerate, a fracture was performed at the site of defect. The obtained samples were kept in PBS buffer (pH 7.4) for 2 hours to remove the formalin. Further, bone material was dehydrated through graded alcohols (50%, 70%, 90%, 96%, 100%; kept for 30 min in each). Then the test material was air-dried to constant weight and sprayed with silver in a standard vacuum device "ВУП-5М" (SELMI, Sumy, Ukraine). To study the morphology of regenerate surface, scanning electron microscopy (SEO-SEM Inspect S50-B Scanning Electron Microscope) was used [12]. Elemental analysis of the studied samples was performed by X-ray energy dispersive spectroscopy (AZtecOne energy dispersive spectrometer with X-MaxN20 detector, Oxford Instruments PLC). Quantitative and qualitative distribution of chemical elements was determined in the following three areas: the bone regenerate, the border between the regenerate and native bone, and the native bone adjacent to the regenerate. Quantitative and qualitative assay of chemical elements was investigated by spot and linear analysis.

Statistical analysis of the obtained digital values was performed with the help of MX Excel XP

statistical computer program using the Student's t-test. The difference was considered significant at $p < 0.05$.

Study results and discussion

On the 15th day of the experiment, raster electron microscopy in the control group showed irregular bone trabeculae with large-loop structure and areas of intertrabecular space at the site of the defect. This indicated the beginning of bone tissue formation in the regenerate. The surface of newly formed trabeculae presented with low calcium and phosphorus concentrations, which were 8.99 ± 0.15% and 4.35 ± 0.14%, respectively. The obtained values indicated the beginning of organic matrix calcification in the regenerate area. The surface of the native bone presented with Ca concentration of 22.57 ± 0.16% and P concentration of 13.05 ± 0.19%, which was lower than physiological parameters and was due to the bone reaction to mechanical trauma. At the border between the regenerate and native bone, the level of these macronutrients was also reduced due to the activation of remodeling processes in the newly formed bone regenerate and the use of endogenous Ca for ossification. Ca and P concentrations were 19.07 ± 0.22% and 9.3 ± 0.14%, respectively (Fig. 1).

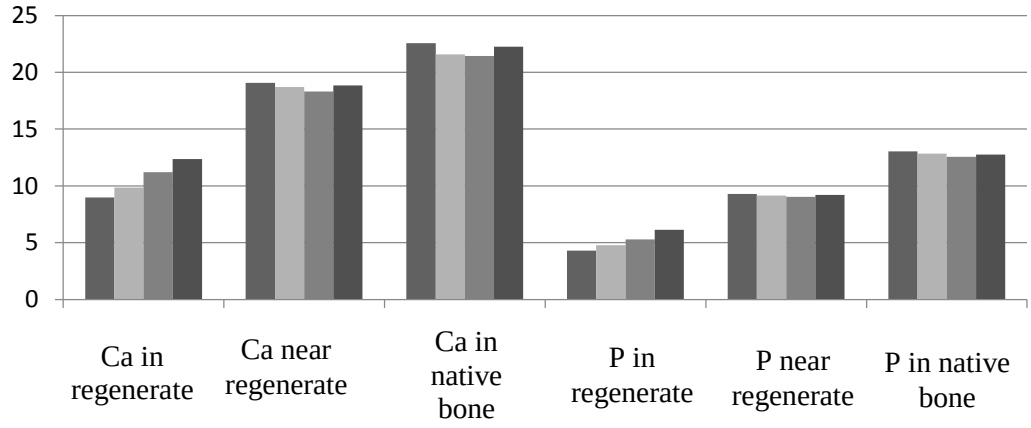


Figure 1 – Changes in calcium and phosphorus content in different areas of the injured long tubular bone in the control mature rats at different points of reparative osteogenesis

On day 30 of the experiment, electron diffraction patterns showed that the main part of the defect was represented by reticulofibrous bone trabeculae and small areas of lamellar tissue located mainly on the periphery of the regenerate. There was some space between the regenerate and the native bone. A gradual increase in Ca and P concentrations in the newly formed regenerate was determined (9.87 ± 0.19% and 4.8 ± 0.18%, respectively). This indicated further active remodeling processes in the bone defect. In the native bone and on its border with the regenerate, the concentrations of Ca and P were almost unchanged vs. the preceding results.

On day 45 of the experiment, scintigram findings revealed that the vast majority of the bone defect was represented by lamellar and, to a lesser extent, by reticulofibrous bone tissue. There was no space between the regenerate and the native bone. Ca and P concentrations in the defect area were 11.2 ± 0.21% and 5.3 ± 0.18%, i.e. higher vs. the preceding results by 13.47% ($p < 0.005$) and 10.42% ($p < 0.005$), respectively. The increase in these values indicated active mineralization in the area of bone regeneration. Ca and P concentrations in the native bone (21.45 ± 0.19% and 12.55 ± 0.19%) and on its border with the regenerate (18.32 ± 0.17% and 9.04 ± 0.16%) remained almost unchanged. There was a decrease vs. the preceding results in Ca concentration in the native bone by 0.6% ($p = 0.34$) and on its border with the regenerate by 1.7% ($p = 0.003$); P concentration decreased by 2.33% ($p = 0.019$) and 1.09% ($p = 0.33$), respectively.

On day 60 of the experiment, electron diffraction patterns showed that the bone defect was represented by lamellar bone tissue tightly adhered to the native bone. Ca and P concentrations in the newly formed regenerate equaled 12.37 ± 0.21% and 6.15 ± 0.19%, i.e. higher by 10.45% ($p < 0.005$) and 16.04% ($p <$

0.005) vs. the preceding results and by 37.59% ($p < 0.005$) and 43.02% ($p < 0.005$) vs. the 15-th day results. This indicated intensive mineralization in bone marrow and active processes of reparative regeneration. There was a further increase in Ca concentration in the native bone by 3.73% ($p < 0.005$) and on the border with the regenerate by 2.89% ($p < 0.005$); P concentration increased by 1.59% ($p = 0.09$) and 1.99% ($p = 0.14$), respectively. These changes indicated a pronounced remodeling activity in the native bone areas adjacent to the defect (Fig. 2).

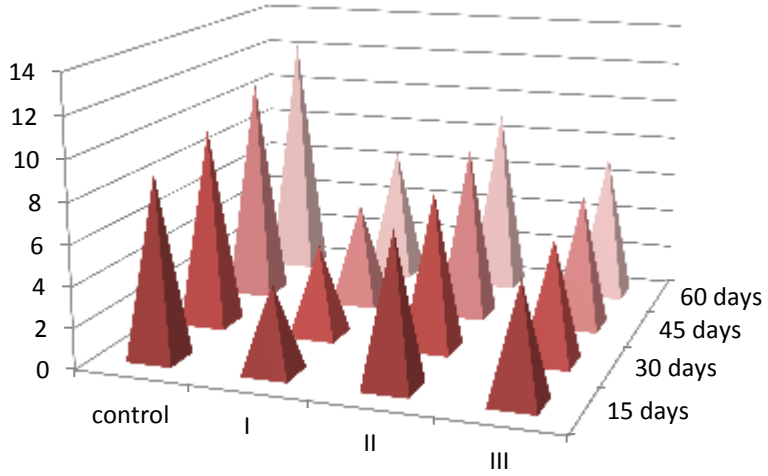
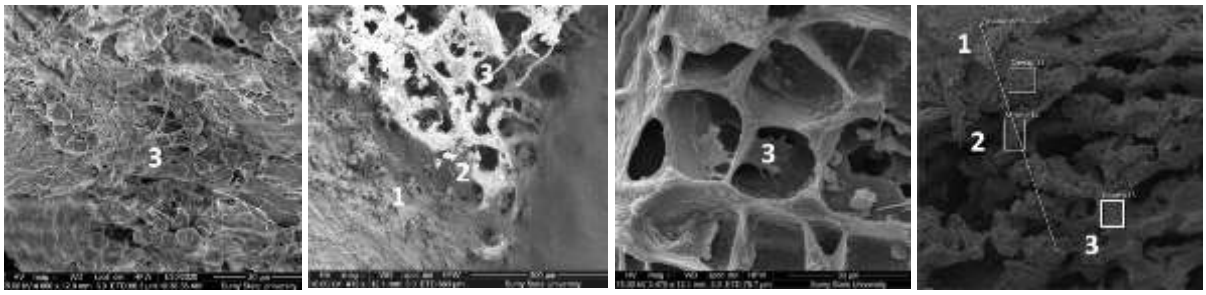


Figure 2 – Changes in calcium content in bone defect area in mature rats from control and experimental groups at different points of reparative osteogenesis

Thus, starting from the 15th day and throughout the experiment, the animals of the control group presented with an active increase in calcium and phosphorus concentrations in the area of the newly formed bone defect and with a slight decrease in these trace elements at the border of regenerate and native bone, as well as in the native bone itself which was due to the compensatory reaction to injury. These changes indicated intensive mineralization processes in the newly formed bone regenerate and active remodeling processes in the native bone itself.

In the experimental groups, analysis of scintigrams on the 15th day of the experiment showed delayed formation of bone trabeculae in the defect area, reduced thickness of trabeculae, increased intertrabecular space, microcracks and empty osteocyte lacunae, lack of adhesion between the native bone and newly formed regenerate (Fig. 3).



A B C D

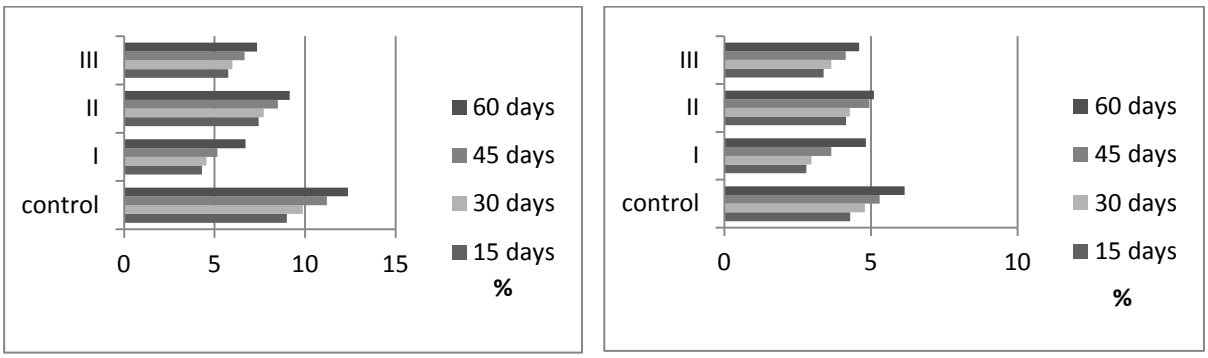
Figure 3 – Scintigrams of the surface of the injured femur of rats on the 15th day of the experiment with the use of: A – doxorubicin, B – 5-fluorouracil, C, D – methotrexate

1 – native bone, 2 – border between native bone and regenerate, 3 – bone regenerate

Microprobe analysis of the injured long tubular bone surface revealed that on the 15th day of the experiment, Ca and P concentrations in the area of the newly formed regenerate were lower than in the controls: Group I – by 52.17% ($p < 0.005$) and 34.88% $p < 0.005$), respectively; Group II – by 17.24% ($p < 0.005$) and 3.48% ($p = 0.15$); Group

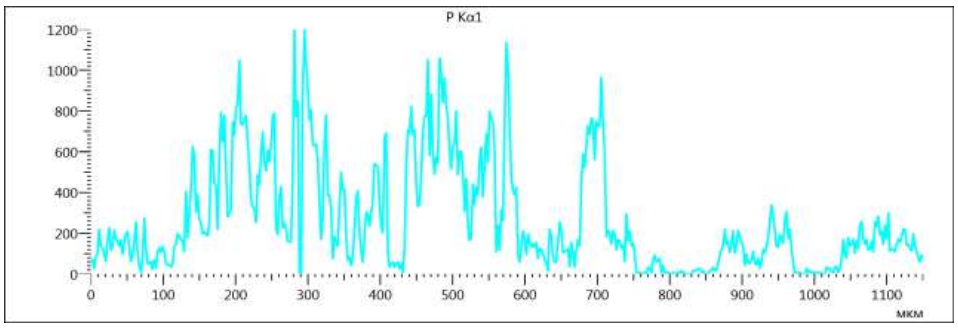
III – by 36.04% ($p < 0.005$) and 21.16% ($p < 0.005$) (Fig. 4).

On the 15th day of the experiment, there was also a decrease in Ca and P concentrations on the native bone surface vs. the controls: Group I – by 14.79% ($p < 0.005$) and 24.52% ($p < 0.005$), respectively; Group II – by 18.52% ($p < 0.005$) and 22.60% ($p < 0.005$), Group III – by 22.24% ($p < 0.005$) and 13.64% ($p < 0.005$). Thus, the obtained data of microanalysis in all experimental groups indicated a delay in the formation of bone regenerate in the area of the defect and reduced mineralization intensity, as well as slowdown of the remodeling activity of the native bone in response to injury (Fig. 5).

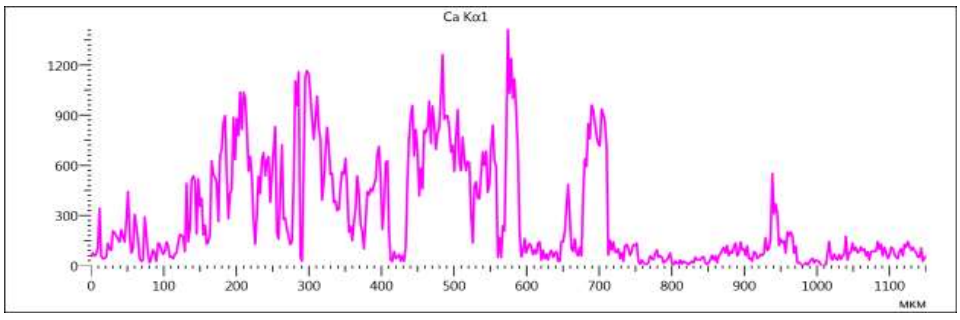


A B

Figure 4 – Changes in the content of calcium (A) and phosphorus (B) in the area of bone defect of mature rats of control and experimental groups at different points of reparative osteogenesis



A



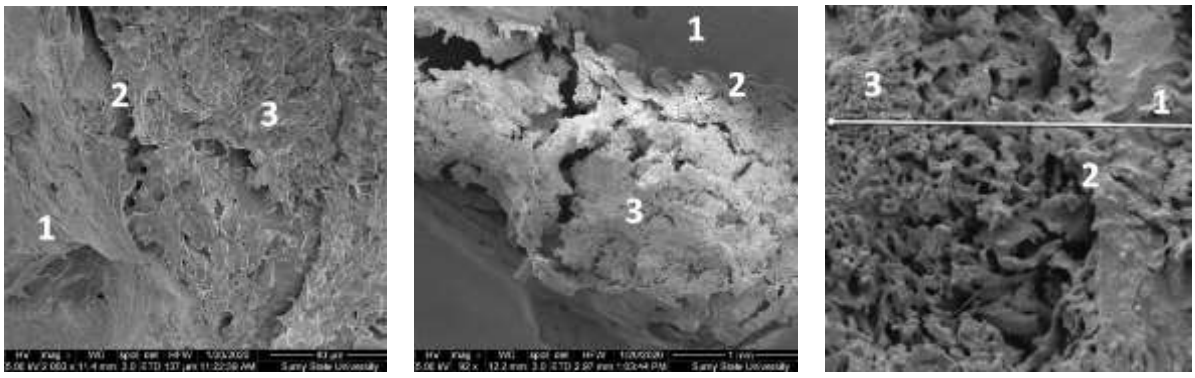
B

Figure 5 – Distribution of phosphorus and calcium content on the surface of the injured long bone (native bone, border between native bone and regenerate, regenerate) on the 15th day of the experiment after methotrexate use: A – phosphorus, B – calcium

On the 30th day of the experiment, after two injections of appropriate antitumor chemotherapy with an interval of 21 days, most of the regenerate was represented by thinned irregular trabeculae of coarse-fibrous bone tissue with small areas of lamellar bone tissue (Fig. 6).

Microprobe analysis of the regenerate showed a gradual increase in the Ca and P concentrations, but they were still lower than in the control group. Thus, Ca and P values in Group I as compared to the controls were lower by 53.90% ($p < 0.005$) and 37.91% ($p < 0.005$), respectively; Group II – by

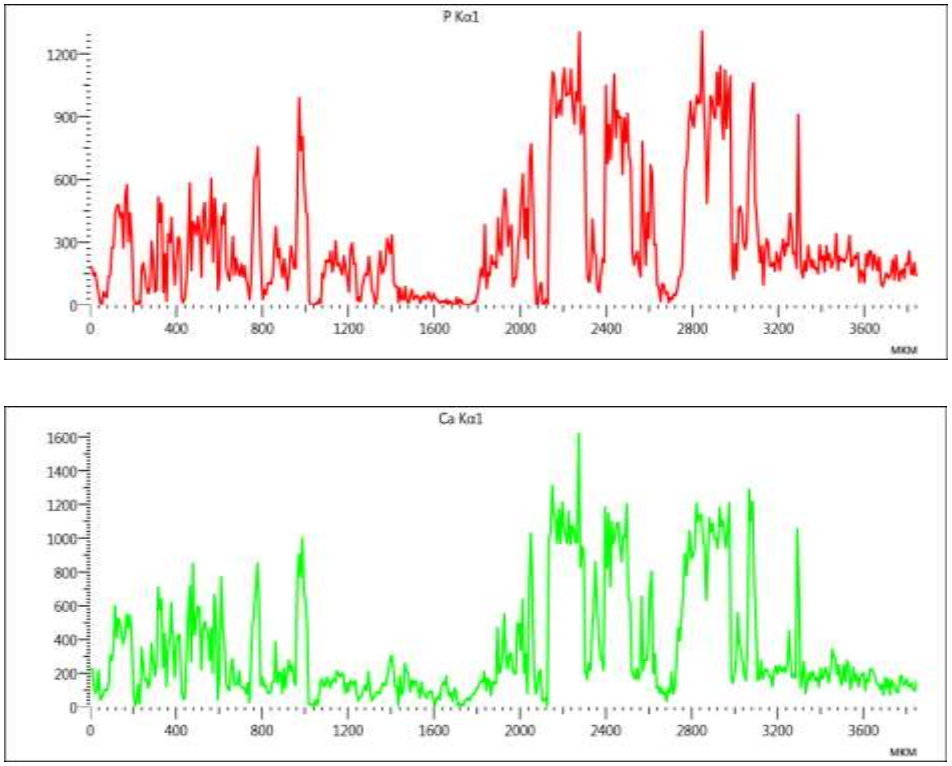
21.78% ($p < 0.005$) and 10.62% ($p < 0.005$), Group III – by 39.31% ($p < 0.005$) and 23.96% ($p < 0.005$). There was a trend to further reduction in Ca concentration in the native bone compared to the preceding results: Group I – by 3.06% ($p < 0.005$), Group II – by 3.86% ($p < 0.005$), Group III – by 3.87% ($p < 0.005$); and in P concentration: Group I – by 4.26% ($p < 0.005$), Group II – by 5.05% ($p < 0.005$), Group III – by 3.73% ($p < 0.005$) (Fig. 7).



A B C

Figure 6 – Scintigrams of the surface of the injured femur of rats on the 30th day of the experiment with the use of: A – doxorubicin, B – 5-fluorouracil, C – methotrexate

1 – native bone, 2 – border between native bone and regenerate, 3 – bone regenerate



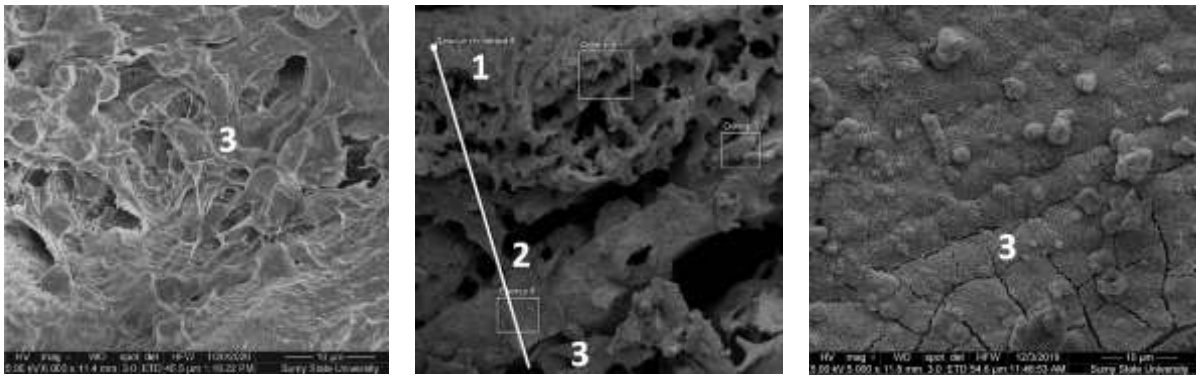
A

B

Figure 7 – Distribution of phosphorus and calcium on the injured surface of the long bones (native bone, border between native bone and regenerate, regenerate) on the 30th day of the experiment after methotrexate use: A – phosphorus, B – calcium

Analysis of scintigrams on the 45th day of the experiment after three injections of appropriate antitumor chemotherapeutics (after injury, on Days 21 and 42) showed a delay in the formation of lamellar bone tissue in the regenerate and low intensity mineralization of the organic matrix (Fig. 8). This was evidenced by decreased levels of Ca and P in the area of the defect vs. the controls: Group I – by 54.02% ($p < 0.005$) and 31.13% ($p < 0.005$), Group II – by 24.11% ($p < 0.005$) and

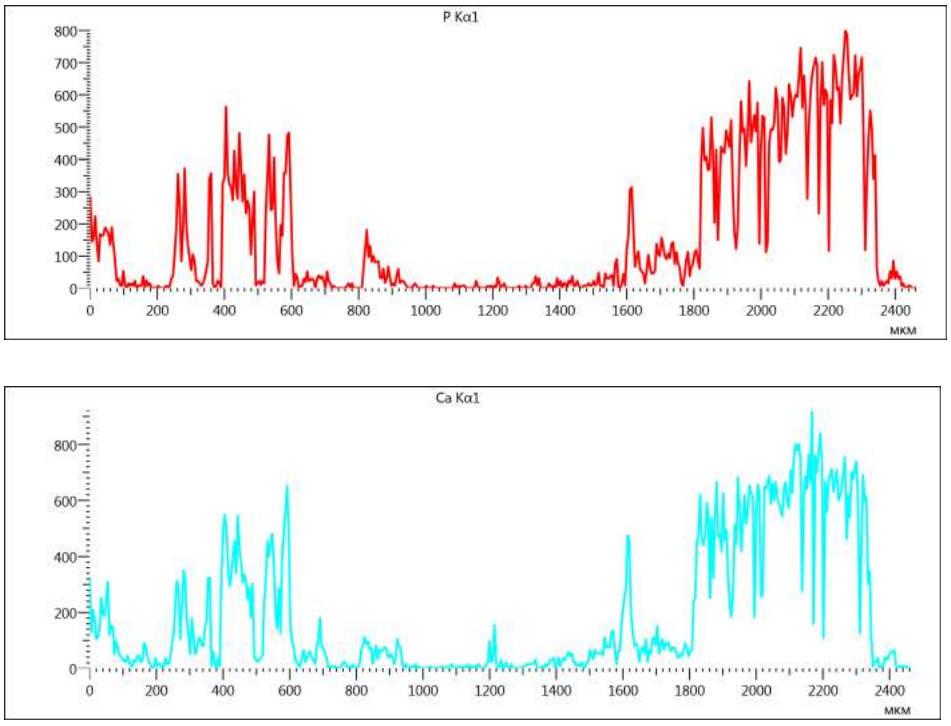
6.60% ($p < 0.005$), Group III – by 40.63% ($p < 0.005$) and 21.87% ($p < 0.005$). A slight decrease in these minerals as compared to both the preceding results and the controls was observed in the areas adjacent to the bone defect and in the native bone (Fig. 9).



A B C

Figure 8 – Scintigrams of the surface of the injured femur of rats on the 45th day of the experiment with the use of: A – doxorubicin, B – 5-fluorouracil, C – methotrexate

1 – native bone, 2 – border between native bone and regenerate, 3 – bone regenerate



A

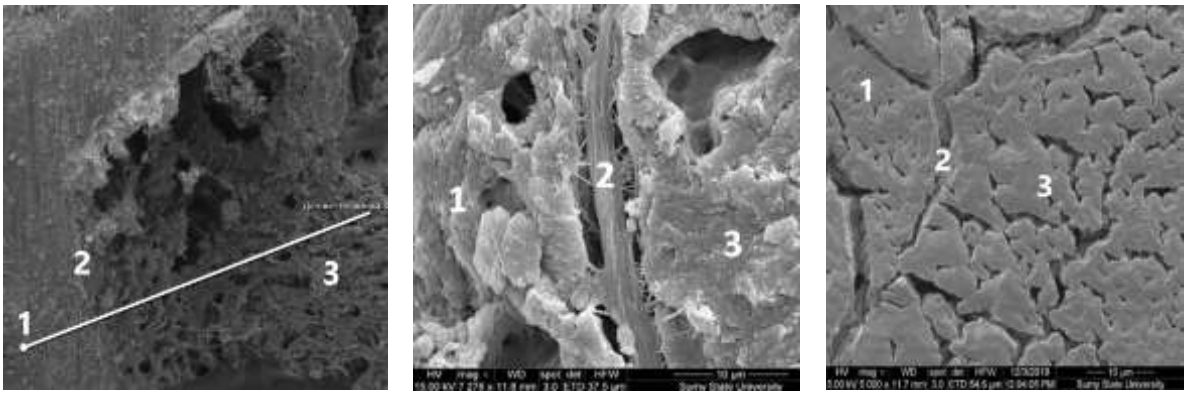
B

Figure 9 – Distribution of phosphorus and calcium levels on the surface of the injured long bone (native bone, border between native bone and regenerate, regenerate) on the 45-th day of the experiment after 5-fluorouracil use: A – phosphorus, B – calcium

Raster electron microscopy of rat bone samples on the 60th day of the experiment showed delayed formation of lamellar tissue in the defect area, the areas of thinned newly formed bone trabeculae, gaps between them, increased intertrabecular space and lack of close adhesion between the regenerate and native bone (Fig. 10). This indicates a delay in callus formation in the area of the defect under the action of chemotherapeutics.

The mineral content on the 60th day of the injury began to increase gradually, but was much lower than in the control group. Thus, there was a decrease in Ca and P concentrations in the area of the defect: Group I – by 45.68% ($p < 0.005$) and

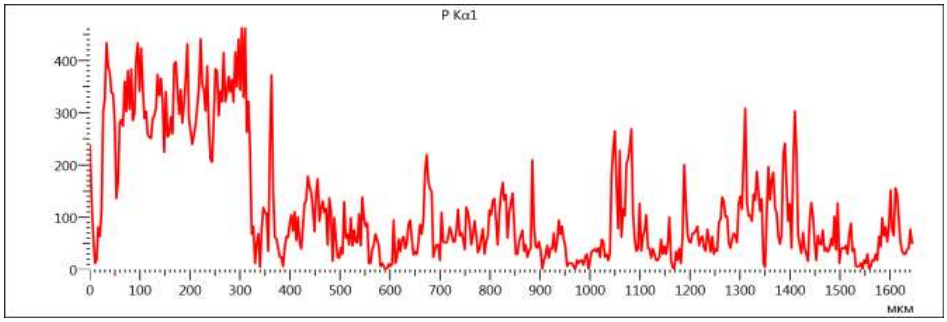
21.46% ($p < 0.005$) vs control; Group II – by 26.03% ($p < 0.005$) and 17.07% ($p < 0.005$), Group III – by 40.66% ($p < 0.005$) and 25.20% ($p < 0.005$). In the native bone and areas adjacent to the regenerate, in contrast to the preceding outcomes, there was a gradual increase in the level of these trace elements within 1–2%, which was still lower than in the control group. Thus, Ca and P levels in the native bone were lower than in the control group: Group I – by 15.60% ($p < 0.005$) and 18.58% ($p < 0.005$); Group II – by 20.45% ($p < 0.005$) and 17.33% ($p < 0.005$), Group III – by 22.24% ($p < 0.005$) and 14.59% ($p < 0.005$) (Fig. 11).



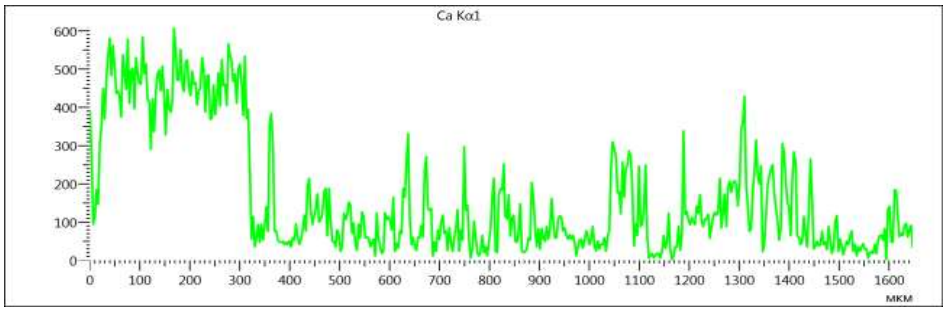
A B C

Figure 10 – Scintigrams of the surface of the injured femur of rats on the 60th day of the experiment with the use of: A – doxorubicin, B – 5-fluorouracil, C – methotrexate

1 – native bone, 2 – border between native bone and regenerate, 3 – bone regenerate



A



B

Figure 11 – Distribution of phosphorus and calcium levels on the surface of the injured long bone (native bone, border between native bone and regenerate, regenerate) on the 60th day of the experiment after doxorubicin use: A – phosphorus, B – calcium

Thus, all experimental groups during the study presented with a delay in callus formation in the defect area, mineralization intensity reduction in the newly formed tissues of the bone regenerate and remodeling activity slowdown in the native bone. The data obtained was consistent with the results of Nisha Y. at al. (2021) and confirmed the outcomes of Fan J. at al. (2012) Geor and Georgi et al. (2012)

who had found that chemotherapy with methotrexate inhibited bone formation by inhibiting the activation of Wnt/β-catenin signaling pathway. As a result, the differentiation of osteoblastic cells providing mineralization of the bone matrix [13, 14, 15] was inhibited. King (2012) also found that methotrexate treatment promoted the formation of osteoclasts in the long bones of rats by increasing the level of proinflammatory cytokines and enhancing the activation of NF-kβ transcription factor. This disrupted the processes of bone remodeling due to increased bone tissue loss, which reduced the activity of reparative regeneration [16].

The reduction in mineralization found in our experiment with the use of doxorubicin was consistent with the findings of Fan C., Georgiou K.R. at al. (2017) stating that treatment with doxorubicin induced osteoclast-mediated bone resorption and, by causing oxidative stress, inhibited osteoblast differentiation and survival. This, in turn, led to a decrease in the level of 25-hydroxyvitamin D_3 and alkaline phosphatase in the serum, which affected the process of calcium deposition in bone tissue. Bone specific alkaline phosphatase (BAP) is synthesized by osteoblasts, which provide bone mineralization, and reflects their activity. These changes in bone remodeling lead to a decrease in the volume of trabecular bone in metaphase and low bone mineralization, which with long-term use of doxorubicin chemotherapy increases the risk of fractures in such patients [17].

In our study, it was found that treatment with 5-fluorouracil adversely affects bone mineralization. This is confirmed by the work of Vyas D. at al. and Raghu Nadhanan et al. (2012) showing that 5-fluorouracil chemotherapy led to increased levels of proinflammatory cytokines such as NF-κB, TNF-α, IL-1β, and IL-6, as well as reduced osteoblast density and caused a significant increase in osteoclast activity. As a result, low bone mineral density developed and bone loss was enhanced [18, 19]. However, it was found that after 5-fluorouracil cessation, there was a gradual improvement in bone metabolism, in contrast to the use of doxorubicin and methotrexate [20].

The use of chemotherapeutics in our experiment led to the development of hypophosphatemia. This is confirmed by the studies of Wigner N., where a decrease in phosphorus levels during chemotherapy caused the resistance to BMP-2 with subsequent inhibition of differentiation of the chondrogenic cells, namely chondrocytes and osteoblasts. This led to a decrease in the volume of mineralized tissue and the development of low bone mineral density, which increased the risk of fractures [21].

Although chemotherapeutic drugs are necessary for the treatment of cancer, they adversely affect mineral metabolism by causing hypocalcemia and hypophosphatemia, which disrupts mineralization processes and causes osteopenia and osteoporosis.

Conclusions/Висновки

Antitumor chemotherapy slows down the formation of callus in the area of the defect and causes disorders of phosphorus-calcium metabolism both in the native bone and regenerate. This is manifested by a decrease in the intensity of mineralization of the newly formed bone matrix and a slowdown in the remodeling activity of the native bone. Doxorubicin and methotrexate provide the most negative impact on mineralization process among antitumor chemotherapeutic agents.

Prospects for future research/Перспективи подальших досліджень

In the future, it is planned to study the dependence of changes in the strength and structural properties of bones on mineral metabolism disorders with the use of different groups of antitumor chemotherapeutics.

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Conflict of interest/Конфлікт інтересів

The authors declare no conflict of interest.

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