Ukrainian Journal of
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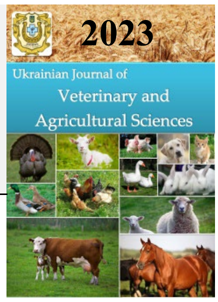

The Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv

Volume 6
Number 2

original article | UDC 636.7.09:616-001.5:666.51 | doi: 10.32718/ujvas6-2.06

Dynamics of acute-phase and endothelial reactions and immune complex formation during bone replacement with germanium-doped calcium-phosphate ceramics of bone fragment fractures in dogs

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Article info
Received 14.06.2023
Received in revised form 24.07.2023
Accepted 25.07.2023

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Contents

Abstract

Bone defects that occur with fragment fractures lead to an increase in the number of postoperative complications. Therefore, to restore the structure and function of the bone, there is a need for bone replacement, in particular doped calcium-phosphate ceramics. The purpose of the work is the biochemical assessment of acute-phase and endothelial reactions and immune complex formation during bone replacement with germanium-doped calcium-phosphate ceramics of bone fragment fractures in dogs. Dogs with fragment fractures of long tubular bones and plate osteosynthesis were included in the study. In the experimental group (n = 10), bone defects were replaced with germanium-doped calcium-phosphate ceramics (HA/β-TCP/l-Ge-700), in the control group (n = 10) – unalloyed (HA/β-TCP-700). Blood samples were taken after the injury and on the 3rd, 7th, 14th, 30th, and 60th days after osteosynthesis. Partial recovery of limb function in the experimental group was faster by 1.3 times ($P < 0.001$) and full by 1.2 times ($P < 0.01$) compared to the control. On the 60th day, in experimental animals, the defect was filled with a regenerate of high X-ray density without a periosteal reaction, while in the control animals, the bone regenerate did not have sufficient density with a significant periosteal reaction. In both groups, total protein and albumin content varied within the normal range. The activity of protein C on the 3rd day in the experimental group was 1.3 times higher ($P < 0.001$), and on the 7th day, it was twice as high ($P < 0.001$) compared to the control group with normalization by the 14th day. The concentration of ceruloplasmin from the 7th day in the control animals was 1.1 times higher ($P < 0.001$) than in the experimental animals, with normalization in the latter by the 60th day. The level of small molecular circulating immune complexes (CIC) after the injury increased by 1.1 times ($P < 0.001$) and reached a peak in the control and experimental groups on the 14th day, with an increase in indicators by 2.1 and 1.4 times ($P < 0.001$), respectively, with normalization in the experimental group on the 60th day. The level of nitric oxide (NO) in the control group increased from the 7th to the 60th day, with a peak on the 30th day, and in the experimental group – from the 3rd to the 30th, with a peak on the seventh day. Osteoreplacement of fragment fractures of long tubular bones in dogs with calcium-phosphate ceramics doped with germanium is accompanied by a moderate level of the acute phase reaction and immune complex formation, an increase in the endothelial reaction and the anticoagulant potential of the blood, which contributes to a decrease in the intensity of the inflammatory-resorptive stage of reparative osteogenesis and an increase in its proliferative phase, which, respectively, accelerates the consolidation of fractures.

Keywords: reparative osteogenesis; bioactive ceramics; nitric oxide; metal ions; immune complexes; protein C.

Citation:
Todosiuk, T. P., & Rublenko, M. V. (2023). Dynamics of acute-phase and endothelial reactions and immune complex formation during bone replacement with germanium-doped calcium-phosphate ceramics of bone fragment fractures in dogs. *Ukrainian Journal of Veterinary and Agricultural Sciences*, 6(2), 30–36.

1. Introduction

From a clinical point of view, the consolidation of a fracture is a rather long, multi-stage process, which is influenced by many factors: the intensity and morpho-functional features of the injury site, the nature and degree of bone and soft tissue damage, the presence of post-traumatic disorders of the peripheral blood supply, methods and methods of osteosynthesis, postoperative infectious and inflammatory complications, the presence of congenital bone defects or concomitant pathology associated with disturbances in the structural and functional state of bone tissue (osteoporosis, neoplasia, endocrine pathology, etc.) (Bosch et al., 1992; Sturmer, 1996; Marsell & Tinhorn, 2011; Dmytriev & Khomyn, 2017; Dmitrijev, 2018; Zhu et al., 2021).

Bone defects arising from complex fragmentary or pathological fractures cause an increase in the number of postoperative complications. In the future, disorders of the static-dynamic function of the injured limb and even changes in the general state of the animal's body as a whole are possible (Chemerovs'kyi, 2020; Oheim, 2022). In this regard, in several clinical cases, for the complete restoration of bone structure and function, there is a need to replace post-traumatic bone defects and stimulate reparative osteogenesis (Rublenko et al., 2015; Shevchenko, 2020; Shevchenko & Rublenko, 2022).

Currently, a relatively large number of bone replacement materials are offered. Recently, calcium phosphate ceramics, which include hydroxyapatite and α- or β-tricalcium phosphate, which is similar in composition to the mineral component of bone and has sufficient osteoconductive properties in the conditions of osteosynthesis, are the most popular in clinical practice. To strengthen the osteoinductive properties, calcium-phosphate ceramics are alloyed with trace elements that play a significant role in bone metabolism – Zn, Si, Ge, St, Cu, Mg (Oryan et al., 2014; Bouler et al., 2017; Bian et al., 2017; Cheng er al., 2017; Li et al., 2017).

Thus, zinc activates oxidation enzymes and has a dual effect – it supports osteoblastogenesis, inhibits osteoclastogenesis, and strengthens osteogenesis due to the stimulation of collagen synthesis and the increase in the activity of the bone isoenzyme of alkaline phosphatase. At the same time, Zn reduces the toxic effects of other metals, particularly cadmium (Macdonald, 2000; Hadley et al., 2010; Qi et al., 2020; O'Connor et al., 2020).

In its turn, magnesium affects the activity of osteoblasts and osteoclasts by reducing the proliferation of osteoclasts and increasing the ability of osteoblasts to proliferate and adhere, thus changing the balance of bone tissue remodeling toward osteogenesis (Wang & Yeung, 2017; Ewald et al., 2019).

Copper ions are known for their antibacterial activity and angiogenic potential. They reduce the frequency of infectious and inflammatory processes associated with implants and improve the bone's quality around it, increasing its mineral density and promoting the formation of a new vascular network (Wan et al., 2007; Wu et al., 2022).

Elements of the IV group of the periodic system of chemical elements – silicon (silicon, Si) and germanium (Ge) turned out to be unique in their effect on bone metabolism. The presence of silicon ions in bioactive materials ensures forming a relatively close chemical bond with the bone. Silicon accelerates the processes of osteogenesis by inducing angiogenesis, stimulating the production of type 1 collagen and the differentiation of osteoblasts, and during early calcification, it precipitates hydroxyapatite in the organic matrix, prevents excessive resorption of bone tissue (Fujii et al., 2007; Huang et al., 2017; Li et al., 2017; Huang et al., 2018).

Germanium has highly diverse properties, particularly antitumor, analgesic, anti-inflammatory, antioxidant, immunomodulatory, fungicidal, antiviral, and antimicrobial effects. In the case of osteogenesis, it affects osteoblasts, their proliferation, and activation and suppresses the activity of osteoclasts. Ge mineral salts can counteract some of the effects of silicon depletion. However, it should be noted that their concentrations, which have positive and harmful effects, are very similar (Ilnitskyi & Smurna, 2007; Li et al., 2017).

Currently, the molecular-biological mechanisms of the influence of these microelements in the composition of calcium-phosphate ceramics on reparative osteogenesis and the clinical-pathogenetic criteria of their use for osteoreplacement of various nosological forms of bone defects or osteoplasty remain poorly known.

Reparative osteogenesis is initiated by the immune system's innate response through the mechanisms of the inflammatory reaction, which has a crucial influence on the coordination of its stages. It arises as a result of the release of chemokines in injured tissues with the subsequent migration to the site of injury of neutrophils, monocytes, and macrophages, which produce numerous mediators (pro-inflammatory cytokines – IL-1, IL-6, and TNF-α) which, in turn, due to cytokinemia, activate receptors hepatocytes and, accordingly, stimulate the synthesis of acute phase proteins (AphP) by the liver (Collo & Pepper 1999; Stoika & Filchenkov, 2001; Oryan et al., 2014). At the same time, the result of pro-inflammatory cytokinemia is the strengthening of endothelial function with an increase in NO concentration and the activation of the natural anticoagulant protein C, which causes the reconstruction of the fibrin matrix in the fracture area. At the same time, the reflection of the state of immunological reactivity in the dynamics of reparative osteogenesis is immune complex formation, the biochemical marker of which is large and small circulating immune complexes (antigen-antibody).

Since the spectrum of these metal ions on bone metabolism is highly diverse, and the response of acute function, the state of endothelial activity, and immunological reactivity play a significant role in the course of reparative osteogenesis, their biochemical markers may have a particular diagnostic and prognostic value, as in understanding the mechanisms of the osteoinductive action of microelements, as well as in determining the effectiveness of osteoreplacement, in particular with germanium-doped calcium-phosphate ceramics.

Aim of the work – biochemical evaluation of acute-phase and endothelial reactions and immune complex formation after bone replacement with germanium-doped calcium-phosphate ceramics of bone fragment fractures in dogs.

2. Materials and methods

The research was conducted based on the interdepartmental clinic for small domestic animals of the Faculty of Veterinary Medicine of the Bila Tserkva National Agrarian University following the principles of the European Convention on the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Official Journal of the European Union L276/33, 2010), as well as under the Law of Ukraine "On the Protection of Animals from Cruelty" dated March 28, 2006, p. No. 27, Art. 230 and the Order of the Ministry of Education, Culture and Sports No. 416/20729 as of March 16, 2012 "On approval of the Procedure for conducting experiments and experiments on animals by scientific institutions". The Bila Tserkva National Agrarian University Ethics Committee approved the presented research project, protocol No. 1, as of January 23, 2019.

The study included dogs with complex comminuted forearm fractures, tibia, femur, or humerus admitted to the clinic during 2020–2023. Injured animals were divided into experimental (n = 10) and control (n = 10) groups. The criteria for selecting animals into the group were the duration of the bone injury, no more than one day, the localization of the bone injury in the area of the diaphysis, and the fragment type of fracture with the presence of a bone defect.

Clinical signs established the presence of a fracture and radiologically using a RUM-20 X-ray machine. The images were digitized electronically (AGFA. Healthcare N.V. CR 10-X, Germany). X-ray control of repositioning of bone fragments and consolidation of fractures was carried out on the 14th, 30th, and 60th day of reparative osteogenesis.

Anesthetic support for osteosynthesis included intramuscular administration of medetomidine (20 μg/kg, "Madison", Brovafarma), butorphanol tartrate (0.1 mg/kg, "Butolar Zoo"), sodium thiopental (7 mg) was administered intravenously to maintain anesthesia/kg, Thiopenat, Brovafarma). Infiltration anesthesia at the incision site was performed with a 0.5 % lidocaine solution (3 mg/kg).

Non-viable fragments present in the fracture area were removed. In the control and experimental groups, extracortical (bony) osteosynthesis was performed with a plate made of unalloyed stainless steel from the company "Inmed" (Ukraine). In the case of combined fractures of the forearm bones, intramedullary osteosynthesis of the ulna was additionally performed.

The volume of the bone defect was determined gravimetrically by filling its cavity with a plastic mass of sodium alginate – alginate powder was mixed with a sterile 0.9 % NaCl solution in a ratio of 1 : 2 at a temperature of 23 °C. After hardening, it was removed from the defect and placed in a measuring cylinder with water. According to the volume of displaced water, it was established that the volume of the defect in animals of both groups varied within 2.7 ± 0.07 cm^3.

In animals of the experimental group, bone defects were replaced with germanium-doped calcium-phosphate ceramics (ГТлGer-700), and of the control group – with unalloyed ceramics (ГТг-700), synthesized at the Institute of Materials Science Problems named after I. M. Frantsevych (Kyiv). Granules of two-phase calcium phosphate ceramics (ГТг-700) consist of 65 wt.% of the hydroxyapatite phase (HAP) and 35 wt.% of β-tricalcium phosphate (β-TCF). The size of the granules is 700 μm. Doped calcium-phosphate ceramics (ГТлGer-700) contained 1.0 wt.% of germanium metaphosphate – Ge(PO3)4.

The wound was sutured with a knotted suture using tubular drainage, which was removed on the third–fourth day of the postoperative period. Animals of both groups were prescribed antibiotic therapy ("Ceftriaxone" PJSC Borshchagivskyi ChPF) at 20 mg/kg twice a day for seven days.

In the postoperative period, clinical studies were conducted according to the criteria of the intensity of the inflammatory reaction, partial and complete recovery of the function of the injured limb, and radiologically confirmed consolidation of the fracture.

Blood samples for biochemical studies were collected after the injury by 24 hours and on the 3rd, 7th, 14th, 30th, and 60th days after osteosynthesis. In addition, a group of clinically healthy dogs came to the clinic of small pets of Bila Tserkva National Agrarian University for routine vaccination (n = 10). Blood samples were taken from them with the owners' consent since there are no reference values for some of the studied biochemical parameters.

In the blood serum, the amount of total protein and albumin was determined using "Filisit-Diagnostyka" kits (Ukraine), ceruloplasmin was determined using the Ravin method using "Reagent" kits (Dnipropetrovsk), and the concentration of circulating immune complexes (CIC) was determined using the method of precipitation in polyethylene glycol-6000 solutions, with a concentration of 3.75 % for the detection of large CICs (LmIC) and 7 % – small ones (SmIC). The level of its metabolites determined the content of nitric oxide (NO) – nitrites; as a reducing agent, cadmium metal granules were used, which were added to blood serum samples after protein precipitation in it. Due to the interaction of serum nitrites with Gries's reagent, the resulting colored complex was colorimetrically measured using a spectrophotometer (wavelength 540 nm). All measurements were performed with a Stat Fax 4500 spectrophotometer.

Digital indicators were processed using MS Excel using generally accepted methods of variational statistics with the calculation of the arithmetic mean value and the standard error of the mean value (M ± m). Differences between groups P < 0.05 were considered significant.

3. Results and discussion

3.1 Results

Clinical and radiological image. The animals of the research group began to lean on the injured limb 6–10 days after osteosynthesis. Complete recovery of limb function was noted on the 15th–20th day of the postoperative period. The control animals began to resist infection on the 9–12th day, and their limb function was restored from the 18th to the 24th day. That is, on average, the partial recovery of limb function in the experimental group was 1.3 times faster (P < 0.001), and the total recovery was 1.2 times faster (P < 0.01) compared to the control animals.

After repositioning and osteosynthesis, for example, of bone fragments of the forearm (Fig. 1 a, b), calcium-phosphate ceramic granules were radiologically visualized in the place of the bone defect in animals of both groups. On the 60th day of reparative osteogenesis (Fig. 1 c, d), in the animals of the experimental group, the filling of the bone defect with regenerated material of high X-ray density was noted without a pronounced reaction of the periosteum, which indicated the complete consolidation of the fracture and was the reason for the removal of the means of fixation. In control animals, bone regeneration of heterogeneous structures with relatively low X-ray density and spread of periosteal reaction proximally and distally from the injury site was noted.

Biochemical indicators. In the control and experimental groups, the total protein content in blood serum varied within the physiological norm. Changes in the level of albumin in the blood serum of injured animals, as a negative reactant of the acute phase, during all periods of the study also occurred within the physiological norm (Table 1) with its significant decrease compared to the indicator of clinical healthy animals during the first 14 days of the postoperative period. However, on the third day of reparative osteogenesis, it decreased by 1.2 times (P < 0.001) in the control group and by 1.1 times (P < 0.001) in the experimental group, with a significant difference between the groups (P < 0.05). Moreover, on the seventh day in the control animals, its level continued to decrease and reached 33.05 ± 0.37 g/l, 1.2 times lower than in the experimental dogs (P < 0.001). On the 14th day, the albumin concentration in the experimental group did not significantly differ from the indicator of clinically healthy dogs. In contrast, this occurred only on the 30th day in the control group.

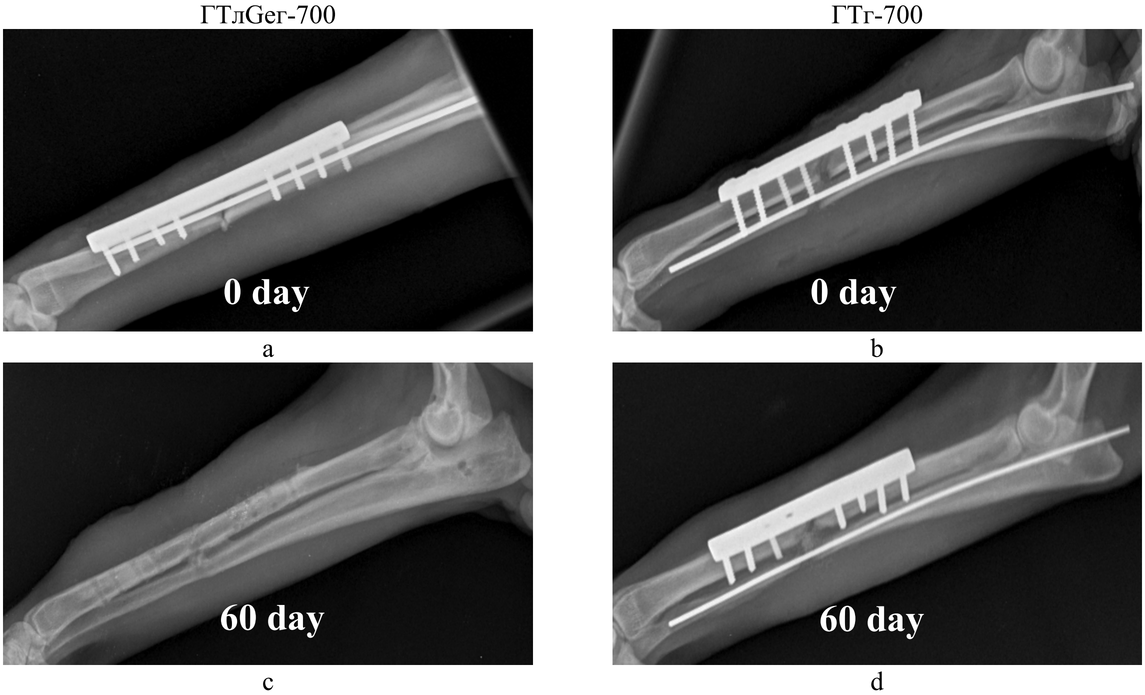

Fig. 1. Radiographs of the bones of the forearm of dogs of the control and experimental groups at different times of their consolidation

Table 1
Dynamics of biochemical indicators for bone replacement in dogs with different types of calcium-phosphate ceramics

Day		Albumin, g/l	Protein C, NV	Ceruloplasmin, mg/l	CIC units	
					large molecular	small molecular
Clinically healthy, (n = 10)		40.74 ± 0.58	1.38 ± 0.05	87.72 ± 0.33	8.1 ± 0.48	18.3 ± 0.42
After an injury, (n = 10)		39.27 ± 0.59	1.32 ± 0.08	95.35 ± 0.68▼▼▼	8.7 ± 0.45	20.1 ± 0.46▼
3	ГТг, (n = 10)	34.18 ± 0.39	0.76 ± 0.04	115.41 ± 0.8	9.0 ± 0.37	31.7 ± 0.78
	ГТлGer, (n = 10)	37.0 ± 0.69**	1.0 ± 0.04***	100.34 ± 0.6***	8.7 ± 0.42	24.8 ± 0.61***
7	ГТг, (n = 10)	33.05 ± 0.37	0.65 ± 0.04	112.14 ± 0.94	9.6 ± 0.45	33.4 ± 0.58
	ГТлGer, (n = 10)	38.92 ± 0.57***	1.27 ± 0.07***	100.91 ± 0.8***	9.0 ± 0.47	25.6 ± 0.69***
14	ГТг, (n = 10)	35.69 ± 0.61	0.85 ± 0.06	110.23 ± 1.05	9.4 ± 0.52	37.8 ± 0.61
	ГТлGer, (n = 10)	39.14 ± 0.78**	1.4 ± 0.03***	101.85 ± 1.08***	8.5 ± 0.54	26.0 ± 0.31***
30	ГТг, (n = 10)	39.22 ± 0.41	1.11 ± 0.05	102.64 ± 1.06	9.3 ± 0.47	34.8 ± 0.76
	ГТлGer, (n = 10)	40.32 ± 0.48	1.34 ± 0.05**	93.41 ± 0.79***	8.0 ± 0.39**	22.1 ± 0.72***
60	ГТг, (n = 10)	41.0 ± 0.59	1.22 ± 0.07	97.53 ± 0.629	8.3 ± 0.45	21.4 ± 0.56
	ГТлGer, (n = 10)	40.63 ± 0.6*	1.39 ± 0.05	88.09 ± 0.7***	8.1 ± 0.41	17.9 ± 0.48***

Note: 1) P: * – < 0.05; ** – < 0.01; *** – < 0.001, compared to the indicators of the control group;
2) P: ▼ – < 0.05; ▼▼ – < 0.01; ▼▼▼ – < 0.001, compared with indicators of clinically healthy animals.

The activity of protein C, as the leading natural anticoagulant, had a tendency to decrease, which further deepened, reaching a minimum value in the control group compared to the level of healthy dogs on the seventh day (2.1-fold decrease, P < 0.001), and in the experimental group – on the third day (1.4-fold decrease, P < 0.001) (Table 1). At the same time, on the third day of reparative osteogenesis, its activity in the experimental group was 1.3 times higher (P < 0.001), and on the seventh day – twice as much (P < 0.001) compared to the control group. On the next day, on the 14th day, the activity of protein C in the experimental animals normalized, which in the control animals became a trend only from the 30th day. That is, during osteoreplacement with germanium-doped calcium-phosphate ceramics, there is only a short-term, in the early postoperative period, moderate loss of the anticoagulant potential of the blood.

Changes in the acute phase protein concentration and simultaneously the antioxidant ceruloplasmin in blood serum were unidirectional and reliable throughout the study period. Moreover, in the research group, the increase in its content was more moderate. Therefore, in general, in all animals, it increased after the injury by 1.1 times (P < 0.001) compared to the indicator of clinically healthy animals. On the third day of reparative osteogenesis, the ceruloplasmin concentration in control dogs reached peak values and was increased by 1.2 times (P < 0.001). In the following periods, the concentration in blood serum of ceruloplasmin was 1.1 times higher (P < 0.001) in control animals than in experimental animals, which normalized by the 60th day. For osteoreplacement with germanium-doped calcium-phosphate ceramics, the acute phase response is more moderate and significantly shorter in terms of ceruloplasmin level.

Changes in the serum level of large-molecular-weight CICs during the entire period of research in animals of both groups had no significant difference, except the 30th day after osteosynthesis and osteoreplacement when its indicator in control animals was 1.2 times (P < 0.001) higher than in experimental animals. However, the level of small molecular CICs changed dynamically. Thus, after the injury, it increased by 1.1 times (P < 0.001) and subsequently reached its peak in the control and experimental groups on the 14th day, when their indicators increased by 2.1 and 1.4 times (P < 0.001), respectively, with the normalization of the level of small molecular CICs in the experimental group on the 60th day. Moreover, in the remaining terms in the control group, it was 1.2–1.6 (P < 0.001) times greater than in the experimental group.

Therefore, during the reparative osteogenesis of long tubular bones under osteosynthesis and osteoreplacement with calcium-phosphate ceramics, an enhanced reaction of immune complex formation occurs in a certain way due to small molecular CICs. Still, in the case of using germanium-doped ceramics, it is more moderate.

As a biochemical marker of endothelial function, the nitric oxide level dynamically increased in both groups on the third day after the fracture by 1.1 times (P < 0.001). At the same time, from the third day (Fig. 2) of reparative osteogenesis in the experimental group, its concentration was already increased by 1.3 times (P < 0.001) compared to the level of NO in clinically healthy animals, reaching a peak value on the seventh day with its reliable fluctuations (1.5-fold increase) on the 14th and 30th days and normalization on the 60th. In control animals, the maximum concentration of NO was recorded only on the 30th day of research, increasing by 1.4 times (P < 0.001) compared to its level in clinically healthy animals. In the control group, there is a regularity of an increase in the level of NO in the blood from the 7th to the 60th day, with a peak on the 30th day, and in the experimental group – from the 3rd to the 30th, with a peak on the seventh day, which indicates about an early and more intense endothelial reaction and, accordingly, about the formation of conditions for neoangiogenesis.

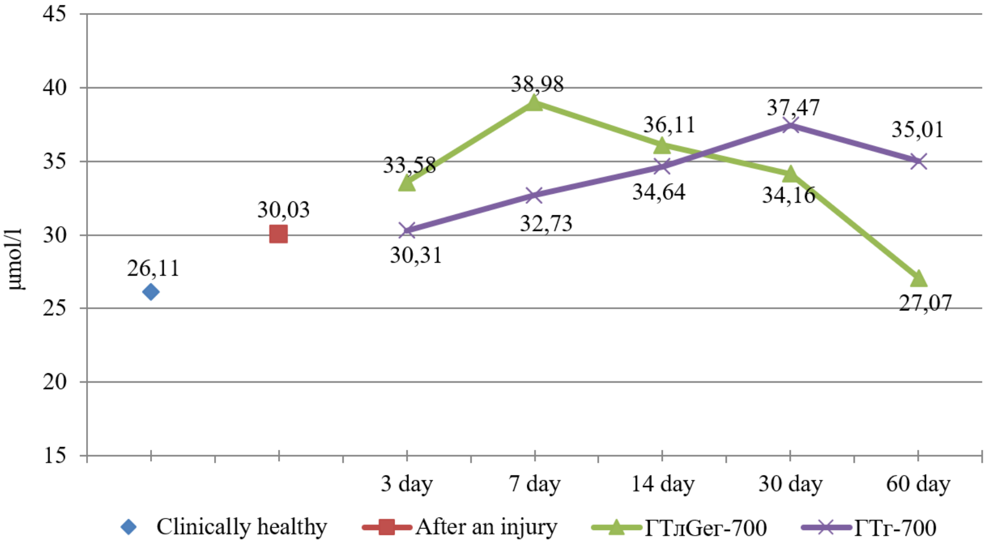

Fig. 2. Nitric oxide level dynamics during bone osteoreplacement defects in dogs with different types of calcium-phosphate ceramics

3.2 Discussion

Although the molecular-biological mechanisms of reparative osteogenesis, which is quite complex and relatively lengthy in time, but unique among the rest of the regenerative processes, great attention is paid; however, the use of bioengineering principles for the treatment of complex pathogenetic, biomechanical, operational-technical, and specific aspects of traumatic and pathological fractures, when the regenerative potential of bone tissue is lost, as well as during corrective osteoplasty, is only at the initial stage.

Based on the molecular and biological patterns of bone metabolism, the critical phase in the implementation of all stages of reparative osteogenesis is its inflammatory-resorptive phase, which depends on many factors (type of fracture, type of bone tissue, method of fixation of fragments, presence of infectious agents), which in turn determine the nature, degree, intensity of the cytokine reaction, and subsequently the intensity of the synthesis of acute phase proteins. However, these pathogenetic reactions do not yet have sufficient clinical and nosological reasoning.

In turn, the problem of osteoreplacement is critical since its comprehensive justification allows for maximizing the optimization of post-traumatic reparative osteogenesis and correcting dysregeneration processes. Among bone-substitute materials, calcium-phosphate ceramics have several positive characteristics: biocompatibility, affinity with bone tissue, biodegradability, and high osteoconductive and osteointegration properties. At the same time, there is a need for its improvement by providing it with osteoinductive properties and a predictable rate of biodegradation with the formation of full-fledged bone tissue for any fracture.

Even a slight modification of calcium phosphate materials can significantly change their properties. In particular, doping these materials with ions of sodium, potassium, zinc, aluminum, silicon, germanium, silver, copper, magnesium, and strontium gives them osteoinductive properties of varying degrees. At the same time, the spectrum of influence of these ions on bone metabolism and, accordingly, on reparative osteogenesis is exceptionally diverse; therefore, composite ceramics doped with ions of trace elements requires complex experimental studies followed by extensive clinical testing.

In particular, doping calcium-phosphate ceramics with germanium can give it new properties, change the nature of biological interaction with bone tissue, and give ceramic implants osteoinductive, antibacterial, immunomodulating, and antitumor properties. It should be noted that despite the large number of works devoted to the biological effects of germanium and its compounds, there is almost no information on osteoreplacement with germanium-doped calcium-phosphate ceramics, which is one of the urgent tasks of ensuring the optimal course of reparative osteogenesis in animals with post-traumatic and pathological bone fractures.

In some research (Rublenko et al., 2014; Chemerovskyi, 2020; Shevchenko, 2020; Todosiuk, 2020), the use of various materials for replacing bone defects in companion animals is substantiated, in particular, hydroxyapatite ceramics in the composition of hydroxyapatite and β-tricalcium phosphate, synthesized at the Institute of Materials Science named after I. N. Frantsevych (Kyiv).

We have previously histomorphologically established the formation of an early osteoblastic reaction and neoangiogenesis, a full-fledged lamellar bone tissue at the site of a bone defect, with which the dynamics of biochemical markers of bone metabolism are consistent (Rublenko et al., 2014; 2015; Shevchenko & Rublenko, 2022).

In the modern understanding, osteoreplacement should be considered from the standpoint of clinical effectiveness, degree of ensuring osteoconductivity and osteoinductivity of osteoreplacement materials, possible reactions to the implant of the immune system, and the nature of other pathogenetic reactions of reparative osteogenesis.

In particular, osteoreplacement with germanium-doped hydroxyapatite ceramics causes a more dynamic course of reparative osteogenesis with a moderate manifestation of the inflammatory-resorptive phase compared to unalloyed ceramics. This is evidenced by the dynamics of a complex of biochemical indicators reflecting its course. Thus, only on the third day was there a decrease in the level of albumin in the blood, and the concentration of ceruloplasmin was moderate and shorter, which is evidence of a less intense reaction of the acute phase and, accordingly, a lower level of cytokinemia. Attention is drawn to the dynamics of the level of NO in the blood, which in the case of osteoreplacement with germanium calcium-phosphate ceramics turned out to be early, more intense with normalization already on the 60th day, and the strengthening of endothelial function is a reflection of early neoangiogenesis as a critical factor in ensuring bone tissue repair.

To some extent, changes in the activity of protein C are correlated with the dynamics of NO, as it is activated on the surface of the endothelium and, thanks to its anticoagulant effect, ensures its proliferation in the fibrin matrix and regenerates.

It is known (Huang et al., 2017; Rublenko et al., 2023) that the immune system controls the molecular-biological and histomorphological mechanisms of reparative osteogenesis or is implemented through its reactions; in general, immune complex formation is a permanent natural process in the body. LmIC are formed through the activation of the complement system and are eliminated by macrophages. Such a reaction proved reliable only after osteoreplacement with unalloyed ceramic on the 30th day, which is evidence, most likely, of additional involvement of pro-inflammatory factors in the formation of bone regeneration. In the case of long-term persisting in the tissues of small molecular CICs, their level after osteoreplacement with doped ceramics turned out to be reliably moderate, which leads to the conclusion about the anti-inflammatory properties of germanium ions.

Therefore, the established dynamics of several biochemical indicators characterizing the acute phase reaction, endothelial and anticoagulant functions, and the degree of immune complex formation, to some extent, reflect the established clinical and radiological effectiveness of bone replacement with germanium-doped calcium-phosphate ceramics for bone fractures in dogs. However, the molecular biological mechanisms of the effect of germanium ions on reparative osteogenesis require further research.

4. Conclusions

Osteoreplacement of fragment fractures of long tubular bones in dogs with calcium-phosphate ceramics doped with germanium is accompanied by a moderate level of the acute phase reaction and immune complex formation, an increase in the endothelial reaction and the anticoagulant potential of the blood, which contributes to a decrease in the intensity of the inflammatory-resorptive stage of reparative osteogenesis and an increase in its proliferative phase and, accordingly, accelerates the consolidation of fractures.

Conflict of interest

The authors report no conflict of interest in the presented work.

Acknowledgments/Funding

The presented research was carried out in accordance with the approved topic of the dissertation work "Clinical-experimental substantiation of bone replacement with calcium phosphate ceramics, doped with germanium, for complex bone fragment fractures in dogs" (protocol No. 8 dated June 1, 2023) and implementation of the State Budget topic "Preclinical studies of products with of made biomaterials" No. 48/1 dated 08/27/19 within the framework of the scientific research work "Development and bringing to clinical practice bone implants of various purposes from the latest biomaterials for the restoration of bone tissue and bone function after wounds in combat" (Agreement No. 515 dated April 17, 2019) in accordance with the targeted scientific and technical program of the National Academy of Sciences of Ukraine "Research and development on the problems of increasing the defense capability and security of the state" and the order of the Presidium of the National Academy of Sciences of Ukraine No. 255 dated April 16, 2019.

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