ISSN : 2321-9602

Indo-American Journal of Agricultural and Veterinary Sciences




editor@iajavs.com
iajavs.editor@gmail.com

Indo-American Journal of Agricultural and Veterinary Sciences

ISSN 2321–9602 www.iajavs.com

Dynamics of acute-phase and endothelial responses and immune complex development during bone replacement of bone fragment fractures in dogs using germanium-doped calcium-phosphate ceramics

Shamitha G[1], Suneetha [2]

Abstract

Fragmentary bone fractures are associated with a higher rate of postoperative morbidity because of bone defect formation. Therefore, bone replacement, specifically doped calcium-phosphate ceramics, is required to restore bone structure and function. This study aims to biochemically evaluate acute-phase and endothelial responses, as well as immunological complex development, after bone replacement of bone fragment fractures in dogs using germanium-doped calcium-phosphate ceramics. Plate osteosynthesis was used on dogs with long tubular bone fragment fractures. Bone defects in the experimental group (n = 10) were filled with germanium-doped calcium-phosphate ceramics (H/-TCP/l-Ge-700), whereas those in the control group (n = 10) were left unfilled. following the injury and again 3, 7, 14, 30, and 60 days following osteosynthesis, blood samples were collected. Recovery of limb function was 1.3 times quicker (P 0.001) and complete recovery was 1.2 times faster (P 0.01) in the experimental group compared to the control. Bone regenerate in the control animals did not have adequate density and had a strong periosteal response, whereas in the experimental animals the defect was filled with a regenerate of high X-ray density on day 60. Albumin and total protein levels were similarly within the normal range in both samples. Protein C activity in the experimental group was 1.3% higher (P 0.001) on day 3, and 2% higher (P 0.001) on day 7 compared to the control group, before returning to baseline by day 14. By day 60, the concentration of ceruloplasmin in the experimental mice had returned to normal after initially being 1.1-fold (0.001) greater than in the control animals. After injury, small molecular circulating immune complexes (CIC) increased 1.1-fold (0.001), peaking on day 14 for both the control and experimental groups. By day 60, the CIC level in the experimental group had returned to pre-injury levels. Nitric oxide (NO) levels rose from day 3 to day 30 (with a high on day 7) in the experimental group and from day 7 to day 60 (with a peak on day 30) in the control group. When fragments of long tubular bones are implanted with calcium-phosphate ceramics doped with geranium, the inflammatory-resorptive stage of reparative osteogenesis is mildly suppressed and the proliferative stage is strongly stimulated.

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

1. Introduction

Consolidation of a fracture is a lengthy, multi-stage process that is affected by many variables, including but not limited to: the severity 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; the type and technique of osteosynthesis; postoperative infectious and inflammatory complications; the presence of congenital bone defects or clefts; and the patient's age. There have been several studies on this topic (Bosch et al., 1992;Sturmer, 1996; Marsell & Tinhorn, 2011; Dmytriev & Khomyn, 2017; Dmitrijev, 2018; Zhu et al., 2021).Bone flaws caused by complicated fragmented or pathological fractures increase the risk of complications after surgery. The wounded limb may develop problems with its static-dynamic function or even the animal's overall health may change (Chemerovs'kyi, 2020; Oheim, 2022). Replacement of post-traumatic bone defects and stimulation of reparative osteogenesis are necessary for the full restoration of bone structure and function in a number of clinical instances (Rublenko et al., 2015; Shevchenko, 2020; Shevchenko & Rublenko 2022).

1. Assistant professor, Department of Pharmaceutical Analysis, A.S.N College of Pharmacy , Guntur.
2. Assistant professor, Department of Pharmacology, A.S.N College of pharmacy, Guntur.

Indo-American Journal of Agricultural and Veterinary Sciences
ISSN 2321–9602 www.iajavs.com

Thus, zinc activates oxidation enzymes and has a dual effect – it supports osteoblastogenesis, inhibits osteoclasto- genesis, 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 andangiogenic potential. They reduce the frequency of infec- tious and inflammatory processes associated with implants and improve the bone's quality around it, increasing its min- eral 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 metabo- lism. 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 or- ganic 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, particularlyantitumor, analgesic, anti-inflammatory, antioxidant, im- munomodulatory, 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 ef- fects, 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 osteore- placement of various nosological forms of bone defects or osteoplasty remain poorly known.

Reparative osteogenesis is initiated by the immune sys- tem's innate response through the mechanisms of the in- flammatory 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 migra-tion 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 concentrationand 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 oste- ogenesis 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 metabo- lism is highly diverse, and the response of acute function,the state of endothelial activity, and immunological reactivi- ty play a significant role in the course of reparative osteo- genesis, 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 osteoreplace- ment, in particular with germanium-doped calcium- phosphate ceramics.

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

2. Materials and methods

The research was conducted based on the interdepart- mental clinic for small domestic animals of the Faculty of Veterinary Medicine of the Bila Tserkva National Agrarian University following the principles of the European Conven- tion on the Protection of Vertebrate Animals Used for Ex- perimental and Scientific Purposes (Official Journal of the European Union L276/33, 2010), as well as under the Lawof 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 Proce- dure for conducting experiments and experiments on ani- mals by scientific institutions”. The Bila Tserkva National Agrarian University Ethics Committee approved the pre-sented research project, protocol

Indo-American Journal of Agricultural and Veterinary Sciences
ISSN 2321–9602 www.iajavs.com

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 crite-ria for selecting animals into the group were the duration of the bone injury, no more than one day, the localization ofthe bone injury in the area of the diaphysis, and the fragmenttype 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 intramus- cular administration of medetomidine (20 μg/kg, “Madison”,Brovafarma), butorphanol tartrate (0.1 mg/kg, “Butolar Zoo”), sodium thiopental (7 mg) was administered intrave-nously to maintain anesthesia/kg, Thiopenat, Brovafarma). Infiltration anesthesia at the incision site was performedwith a 0.5 % lidocaine solution (3 mg/kg).

Non-viable fragments present in the fracture area were removed. In the control and experimental groups, extracorti- cal (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 addi-tionally performed.

The volume of the bone defect was determined gravimet-rically 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 vol- ume 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 ceram- ics (ГТлGer-700), and of the control group – with unalloyedceramics (ГТr-700), synthesized at the Institute of Materials Science Problems named after I. M. Frantsevych (Kyiv). Granules of two-phase calcium phosphate ceramics (ГТr- 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 metaphos- phate – 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 groupswere prescribed antibiotic therapy (“Ceftriaxone” PJSC Borshchagivskyi ChPF) at 20 mg/kg twice a day for seven days.

In the postoperative period, clinical studies were con- ducted according to the criteria of the intensity of the in- flammatory 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 af-ter 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 vac- cination (n = 10). Blood samples were taken from them withthe 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 al- bumin 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 polyeth- ylene 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 interac- tion 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 re- search group began to lean on the injured limb 6–10 days after osteosynthesis. Complete recovery of limb functionwas 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 tothe 24th day. That is, on average, the partial recovery oflimb function in the experimental group

Indo-American Journal of Agricultural and Veterinary Sciences
ISSN 2321–9602 www.iajavs.com

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 visualizedin the place of the bone defect in animals of both groups. Onthe 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 with- in the physiological norm. Changes in the level of albuminin the blood serum of injured animals, as a negative reactant of the acute phase, during all periods of the study also oc- curred 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 osteogene- sis, it decreased by 1.2 times (P < 0.001) in the controlgroup 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 experi- mental 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.

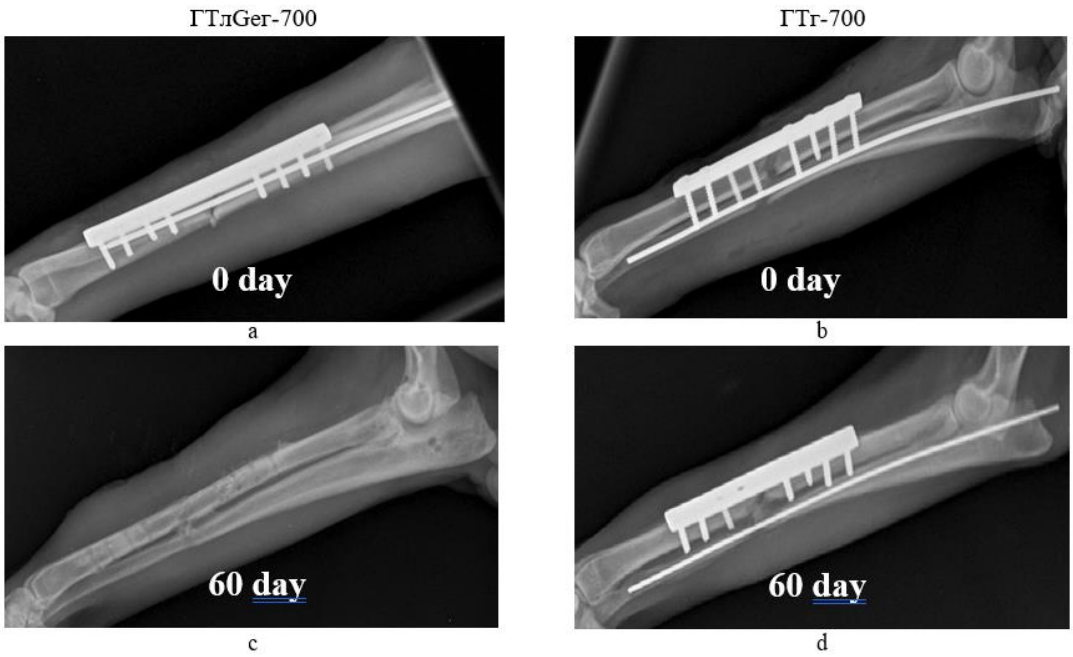

Fig. 1. Radiographs of the bones of the forearm of dogs of the control and experimental groups at different times of their consolidation

Indo-American Journal of Agricultural and Veterinary Sciences

ISSN 2321–9602 www.iajavs.com

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
	ГТдГер. (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
	ГТдГер. (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
	ГТдГер. (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
	ГТдГер. (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
	ГТдГер. (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 antico- agulant, 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 de- crease, 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, mod- erate loss of the anticoagulant potential of the blood. Changes in the acute phase protein concentration and simultaneously the antioxidant ceruloplasmin in blood se- rum 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 experi- mental animals, which normalized by the 60th day. For osteoreplacement with germanium-doped calcium- phosphate ceramics, the acute phase response is more mod- erate 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 molecu- lar 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 levelof 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 osteoreplacementwith 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 ni- tric 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 osteo- genesis 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, in- creasing 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 bloodfrom 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, according- ly, about the formation of conditions for neoangiogenesis.

Indo-American Journal of Agricultural and Veterinary Sciences
ISSN 2321–9602 www.iajavs.com

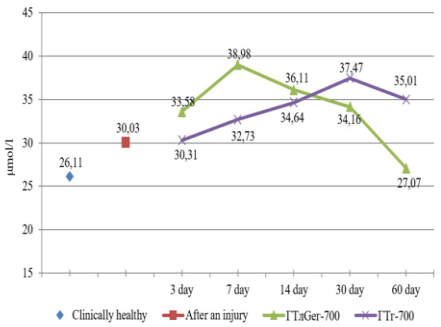

Fig. 2. Nitric oxide level dynamics during bone osteoreplacement defects in dogs with different types of calcium-phospha ceramics

3.2 Discussion

Although the molecular-biological mechanisms of repar-ative osteogenesis, which is quite complex and relatively lengthy in time, but unique among the rest of the regenera- tive processes, great attention is paid; however, the use of bioengineering principles for the treatment of complex path- ogenetic, biomechanical, operational-technical, and specific aspects of traumatic and pathological fractures, when the regenerative potential of bone tissue is lost, as well as duringcorrective 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 frag- ments, presence of infectious agents), which in turn deter- mine 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 sev- eral positive characteristics: biocompatibility, affinity with bone tissue, biodegradability, and high osteoconductive and osteointegration properties. At the same time, there is a needfor 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 materi- als 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 vary- ing degrees. At the same time, the spectrum of influence of these ions on bone metabolism and, accordingly, on repara- tive osteogenesis is exceptionally diverse; therefore, compo- site ceramics doped with ions of trace elements requires complex experimental studies followed by extensive clinicaltesting.

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 infor- mation on osteoreplacement with germanium-doped calci- um-phosphate ceramics, which is one of the urgent tasks ofensuring 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 vari-ous materials for replacing bone defects in companion ani- mals is substantiated, in particular, hydroxyapatite ceramics in the composition of hydroxyapatite and β-tricalcium phos-phate, 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 neoangi- ogenesis, a full-fledged lamellar bone tissue at the site of a bone defect, with which the dynamics of biochemical mark- ers of bone metabolism are consistent (Rublenko et al., 2014; 2015; Shevchenko & Rublenko, 2022).

In the modern understanding, osteoreplacement shouldbe considered from the standpoint of clinical effectiveness, degree of ensuring osteoconductivity and osteoinductivity ofosteoreplacement materials,

Indo-American Journal of Agricultural and Veterinary Sciences
ISSN 2321–9602 www.iajavs.com

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 ce- ramics. 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 mod- erate and shorter, which is evidence of a less intense reac- tion of the acute phase and, accordingly, a lower level of cytokinemia. Attention is drawn to the dynamics of the levelof 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 re- generates.

It is known (Huang et al., 2017; Rublenko et al., 2023) that the immune system controls the molecular-biological and histomorphological mechanisms of reparative osteogen- esis 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 con- clusion about the anti-inflammatory properties of germani-um ions.

Therefore, the established dynamics of several biochem- ical indicators characterizing the acute phase reaction, endo-thelial and anticoagulant functions, and the degree of im- mune complex formation, to some extent, reflect the estab- lished clinical and radiological effectiveness of bone re-placement with germanium-doped calcium-phosphate ce- ramics for bone fractures in dogs. However, the molecular biological mechanisms of the effect of germanium ions on reparative osteogenesis require further research.

1. 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.

References

Bian, D., Zhou, W., Den, J., Liu, Y., Li, W., Chu, X., Xiu, P., Cai, H., Kou, Y., Jiang, B., & Zheng, Y. (2017). Development of magnesium-based biodegradable metals with dietary trace ele- ment germanium as orthopaedic implant applications. *Acta Bi- omaterialia*, 64, 421–436. [Crossref] [Google Scholar]

Bosch, U. T., Pohlemann, T., & Hass, N. (1992). Klassifikation und Management des komplexen Beckentraumans. *Umfallechi-rurg*, 95, 189–196. [Google Scholar]

Bouler, J. M., Pilet, P., Gauthier, O., & Verron, E. (2017). Biphasic calcium phosphate ceramics for bone reconstruction: A review of biological response. *Acta Biomaterialia*, 53, 1–12. [Crossref] [Google Scholar]

Chemerovskyi, V. O. (2020). Renthenohrafichna, makromorfolo- hichna i hematolohichna otsinka hidroksyapatytnoi keramiky z riznymy fizyko-khimichnymy vlastyvostiamy. *Naukovyi visnyk veterynarnoi medytsyny*, 1, 140–152 (in Ukrainian). [Crossref] [Google Scholar]

Cheng, L. J., Yu, T., & Shi, Z. (2017). Osteoinduction Mechanismof Calcium Phosphate Biomaterials In Vivo: A Review. *Jour-nal of biomaterials and tissue engineering*, 7(10), 911–918. [Crossref] [Google Scholar]

Collo, G., & Pepper, M. S. (1999). Endothelial cell integrin alpha 5beta 1 expression is modulated by cytokines and during migra-tion in vitro. *Journal of Cell Science*, 112(4), 569–578. [Crossref] [Google Scholar]

Dmitrijev, V. (2018). Features of Dogs Treatment at Fractures of Peripheral Skeleton. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences*, 20(83), 279–281. [Crossref] [Google Scholar]

Dmytriev, V. S., & Khomyn, N. M. (2017). Application of extra- cortical osteosynthesis for diaphyseal fractures of limb bones indogs. *Scientific Messenger*

Indo-American Journal of Agricultural and Veterinary Sciences ISSN 2321–9602 www.iajavs.com

of LNU of Veterinary Medicine and Biotechnologies, 19(77), 15–17. [Crossref] [Google Scholar]

Ewald, A., Kreczy, D., Brückner, T., Gbureck, U., Bengel, M., Hoess, A., & Fuchs, A. (2019). Development and bone regen- eration capacity of premixed magnesium phosphate cement pastes. *Materials*, 12(13), 2119. [Crossref] [Google Scholar]

Fujii, A., Kuboyama, N., Yamane, J., Nakao, S., & Furukawa, Y. (1993). Effect of organic germanium compound (Ge-132) on experimental osteoporosis in rats. *General Pharmacology: The Vascular System*, 24(6), 1527–1532. [Crossref] [Google Scholar]

Hadley, K. B., Newman, S. M., & Hunt, J. R. (2010). Dietary zinc reduces osteoclast resorption activities and increases markersof osteoblast differentiation, matrix maturation, and mineraliza-tion in the long bones of growing rats. *The Journal of nutri- tional biochemistry*, 21(4), 297–303. [Crossref] [Google Scholar]

Huang, Y., Wu, C., Zhang, X., Chang, J., & Dai, K. (2018). Regulation of immune response by bioactive ions released from silicate bioc- eramics for bone regeneration. *Acta biomaterialia*, 66, 81–92. [Crossref] [Google Scholar]

Ilnitskyi, M. H., & Smurna, O. V. (2007). Osoblyvosti osteohenezuta reparatyvnoi reheneratsii kistok taza u sobak. *Veterynarna medytsyna Ukrainy*, 7, 35–37 (in Ukrainian). [Google Scholar]

Li, L., Ruan, T., Lyu, Y., & Wu, B. (2017). Advancesin Effect of Germanium or Germanium Compoundson Animals. *Journal of Biosciences and Medicines*, 5(7), 56–73. [Crossref] [Google Scholar]

Macdonald, R. S. (2000). The role of zinc in growth and cell pro- liferation. *The Journal of Nutrition*, 130(5), 1500–1508. [Crossref] [Google Scholar]

Marsell, R., & Tinhorn, T. A. (2011). The biology of fracturehealing. *Injuru*, 42(6), 551–555. [Crossref] [Google Scholar]

O'Connor, J. P., Kanjilal, D., Teitelbaum, M., Lin, S.S., & Cottrell, J. A. (2020). Zinc as a Therapeutic Agent in Bone Regenera- tion. *Materials (Basel, Switzerland)*, 13(10), 2211. [Crossref] [Google Scholar]

Oheim, R., Tsourdi, E., Seefried, L. et al. (2022). Genetic Diagnos- tics in Routine Osteological Assessment of Adult Low Bone Mass Disorders. *The Journal of Clinical Endocrinology & Metabolism*, 107(7), e3048–e3057. [Crossref] [Google Scholar]

Oryan, A., Alidadi, S., Moshiri, A., & Maffulli, N. (2014). Boneregenerative medicine: classic options, novel strategies, and fu-ture directions. *Journal of Orthopaedic Surgery*, 9(1), 18. [Crossref] [Google Scholar]

Qi, S., He, J., Zheng, H., Chen, C., Jiang, H., & Lan, S. (2020). Zinc supplementation increased bone mineral density, im-proves bone histomorphology, and prevents bone loss in dia-betic rat. *Biological trace element research*, 194(2), 493–501. [Crossref] [Google Scholar]

Rublenko, M. V., Semenyak, S.A., & Ul'yanchich, N. V. (2014). Dynamika biomarkeriv reparatyvnoho osteohenezu za umov zamishchennia kistkovykh defektiv. *Naukovyi visnyk Lvivskoho natsionalnoho universytetu veterynarnoi medytsyny ta biotekhnolohii imeni S. Z. Gzhyc'kogo*, 16(3(60)), 287–294 (inUkrainian). [Article] [Google Scholar]

Rublenko, M. V., Todosiuk, T. P., Chemerovskyi, V. O., Ulianchych, N. V., Firstov, S. O., & Kolomiiets, V. V. (2023). *Osteozamishchennia kaltsii-fosfatnoiu keramikoiu, lehovanoiukremniiem i hermaniiem, za perelomiv kistok u tvaryn*: naukovo- praktychna monohrafiia. Bilotserkivskyi natsionalnyi ahrarnyi universytet. Harnitura “Taims” Bila Tserkva (in Ukrainian). [Abstract]

Rublenko, M. V., Andriiets, V. H., Semeniak, S. A., Ulianchych, N. V. ta in. (2015). *Vykorystannia kompozytnykh materialiv za perelomiv trubchastykh kistok u tvaryn:* naukovo-metodychnyi posibnyk. Bilotserkivskyi natsionalnyi ahrarnyi universytet. Bi-la Tserkva (in Ukrainian). [Abstract]

Sahandra, I. V. (2014). Preparati Germaniyu ta ïh zastosuvannya v medicini. *Ukrainskyi naukovo-medychnyi molodizhnyi zhurnal*, 4(84), 83–86 (in Ukrainian). [Google Scholar]

Shevchenko, S. M. (2020). Dynamika hematolohichnykh pokaznykiv, makromorfolohichna i renthenolohichna kartyny reparatyvnoho osteohenezu v kroliv za vykorystannia trombot- sytarnykh kontsentrativ ta hidroksyapatytnoi keramiky. *Naukovyi visnyk veterynarnoi medytsyny*, 1, 153–164 (in Ukrainian). [Crossref] [Google Scholar]

Shevchenko, S., & Rublenko, M. (2022). The effect of osteosubsti- tution by platelet-rich autofibrin and hydroxyapatite ceramic with β-tricalcium phosphate on biochemical parameters of blood in rabbits. *Turkish Journal of Veterinary and Animal Sciences*, 46(4), 599–608. [Crossref] [Google Scholar]

Stoika, R. S., & Filchenkov, O. O. (2001). Bifunktsionalna diia transformuiuchoho faktoru rostu b v rehuliatsii proliferatsii ta apoptozu klityn imunnoi systemy. *Imunolohiia ta alerholohiia*, 3, 5–16 (in Ukrainian). [Google Scholar]

Sturmer, K. M. (1996). Pathophysiology disrupted bone healing. *Orthopaede*, 25(5), 386–393. [Crossref] [Google Scholar]

Indo-American Journal of Agricultural and Veterinary Sciences
ISSN 2321–9602 www.iajavs.com

Todosiuk, T.P. (2020). X-ray and macromorphological evaluation of reparative osteogenesis after implantation of hydroxyapatite composite doped with germanium. *Scientific Journal of Veterinary Medicine*, 2, 183–194.
[Crossref] [Google Scholar]

Wan, Y. Z., Xiong, G. Y., & Liang, H., Raman, S., He, F., & Huang, Y. (2007). Modification of medical metals by ion im- plantation of copper. *Applied Surface Science*, 253(24), 9426– 9429.
[Crossref] [Google Scholar]

Wang, W., & Yeung, K. W. (2017). Bone grafts and biomaterials substitutes for bone defect repair: A review. *Bioactive materi- als*, 2(4), 224–247.
[Crossref] [Google Scholar]

Wu, Y., Zhou, H., & Zeng, Y. (2022). Recent Advances in Copper- Doped Titanium Implants. *Materials*, 15(7), 2342.
[Crossref] [Google Scholar]

Zhu, G., Zhang, T., Chen, M., Yao, K., Huang, X., Zhang, B., Li, Y., Liu, J., Wang, Y., & Zhao, Z. (2021). Bone physiological microenvironment and healing mechanism: Basis for future bone-tissue engineering scaffolds. *Bioactive Materials*, (6)11, 4110–4140.
[Crossref] [Google Scholar]