Genij Ortopedii. 2022. Vol. 28, no. 3. P. 386-391.

Original article

https://doi.org/10.18019/1028-4427-2022-28-3-386-391

Changes in the antioxidant/prooxidant balance in patients with acquired shortening of the lower leg bones at the stages of the Ilizarov lengthening procedure

A.M. Aranovich, M.V. Stogov✉, N.V. Tushina, E.A. Kireeva

Ilizarov National Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russian Federation

Corresponding author: Maksim V. Stogov, stogo_off@list.ru

Abstract

Introduction Optimization of the conditions of distraction osteogenesis using the method of G.A. Ilizarov remains an important challenge to ensure a reduction in the number of complications and an increase in the effectiveness of this technology. **The aim of the study** was to study the changes in the antioxidant/prooxidant balance in patients with acquired shortening of the lower leg bones at the stages of the Ilizarov lengthening procedure. **Methods** The antioxidant/prooxidant status was studied in 12 patients with shortening of one of the lower legs resulting from hematogenous osteomyelitis (group 1) and in 13 patients with post-traumatic shortening of one of the lower legs (group 2). The level of peroxidation products, the activity of superoxide dismutase and vitamins E and A in the blood of these patients were assessed in the dynamics of monofocal bone lengthening using the Ilizarov method. **Results** It was found that in patients with acquired shortening of the shin bones, regardless of the shortening etiology, during surgical lengthening, an increase in the level of peroxidation products in the blood was observed with a decrease in the level of vitamin E and an increase in superoxide dismutase activity. **Discussion** The data obtained, together with the current findings from fundamental research, allow us to note that the use of antioxidants (AOs) in patients undergoing segment length correction using the Ilizarov technology is possible from two positions. 1. The positive effect of AOs in terms of stimulation of distraction osteogenesis has been proven. 2. AOs have a systemic effect in the regulation of the recovery processes in the paraosseous tissues of the limb segment under lengthening. **Conclusion** Peroxidation is activated in the course of surgical lengthening of the lower leg bones in patients with acquired shortenings, regardless of the shortening etiology. The use of antioxidants in such patients can specifically inhibit peroxidation, thereby reducing the risk of provoking bone formation disorders.

Keywords: shortening of limb bones, distraction osteogenesis, Ilizarov method, antioxidants

For citation: Aranovich A.M., Stogov M.V., Tushina N.V., Kireeva E.A. Changes in the antioxidant/prooxidant balance in patients with acquired shortening of the lower leg bones at the stages of the Ilizarov lengthening procedure. *Genij Ortopedii*, 2022, vol. 28, no 3, pp. 386-391. https://doi.org/10.18019/1028-4427-2022-28-3-386-391

INTRODUCTION

Acquired shortening of the bones of the lower extremities due to various etiologies can be quite effectively corrected with the method of transosseous distraction osteosynthesis developed by G.A. Ilizarov [1–5]. Optimization of the conditions for distraction osteogenesis (DO) remains an important task to reduce the number of complications and improve the efficiency of this technology [6, 7]. At present, methods for stimulating distraction osteogenesis have been intensively investigated and are associated with the development of cell technologies [8–10] and pharmacotherapy [11–13]. However, among the studies in the direction of pharmaceutical stimulation, the works in which the search for direct means of activating DO prevail. Methods for pharmacocorrection of systemic disorders and complications in the application of transosseous distraction osteosynthesis (contracture, pain) have been poorly developed, and studies in this area are scarce [14]. In this regard, there are very few works studying the need for the use of systemic regulators: vitamins, antioxidants, immunomodulators, etc. One of the possible reasons for this is that there is no data to justify the use of such regulators, since most of the research has been devoted to the study of the state of bone metabolism in the course of surgical lengthening of limb bones [15].

The purpose of our study was to investigate the changes in the antioxidant/prooxidant balance in patients with acquired shortening of lower leg bones at the stages of the Ilizarov lengthening procedure.

MATERIALS AND METHODS

The study included 12 patients with shortening of one of the lower legs resulting from hematogenous osteomyelitis (group 1), and 13 patients with post-traumatic shortening of one of the lower legs (group 2). The mean age was 38.5 ± 5.6 and 34.4 ± 8.7 years, respectively, for groups 1 and group 2. The ratio of men to women was 9/3 and 11/2 for groups 1 and 2. The average lengthening magnitude in group 1 was 3.2 ± 0.2 cm, and 2.7 ± 0.1 cm in group 2 (differences between groups are significant at $p = 0.03$).

Patients of both groups underwent surgical lengthening of the bones of the shortened segment by the method of Ilizarov monofocal osteosynthesis. The surgical treatment was aimed at the restoration of the support function and the length of the affected lower limb. Pharmacotherapy in the postoperative period included painkillers, antibiotics and anticoagulant therapy. At the time of removal of the apparatus, all patients of both groups achieved the planned result of treatment. The mechanical axis of the lower limb was aligned, no complications were noted.

The permission of the Ethics Committee at the Federal State Budgetary Institution Ilizarov National Medical Research Center of the Ministry of Health of Russia was obtained.

At the stages of treatment, the antioxidant/prooxidant status of patients was assessed. To assess the intensity of peroxidation (PO) in the blood serum of patients, the level of protein peroxidation products (PPOP) and lipids such as diene conjugates (DC) and malondialdehyde (MDA) was determined. To evaluate the antioxidant system, the activity of the antioxidant enzyme superoxide dismutase (SOD) in erythrocytes and the concentration of vitamins A and E in blood serum were found.

The level of PPOP was determined in the protein precipitate by reaction with 2,4-dinitrophenylhydrazine; the reaction products were recorded at a wavelength of 270 nm. The PPOP concentration was expressed in units of optical density (opt. dens.) per mg of total protein, the level of which in blood serum was determined using reagent kits from Vital Diagnostics (Russia). The concentration of DC in blood plasma was determined in the heptane phase after extraction from a heptane-isopropanol mixture (1:1) at a wavelength of 232 nm. MDA was determined in deproteinized plasma by reaction with thiobarbituric acid. The concentration of DC and MDA was calculated per mg of total lipids, the level of which in the blood serum was determined using reagent kits from LaChema (Czech Republic). SOD activity in erythrocytes was determined by a reaction based on the ability of the enzyme to compete with nitroblue tetrazolium (NBT) for superoxide anions formed as a result of the aerobic interaction of NADH and phenazine methsulfate. The activity of SOD in erythrocytes was expressed in μmol NBT per 10^9 erythrocytes per minute. The concentration of vitamins A and E in blood serum was determined by the fluorescence method on the Fluorat-02-ABLF-T analyzer (Russia). The indicators of 15 practically healthy people (reference group) aged 20 to 40 years (mean age 33.2 ± 5.7 years) were taken as the norm.

The results of the study, shown in the tables, are presented as the arithmetic mean and standard deviation (Xi ± SD). The normality of the distribution of samples was determined using the Shapiro-Wilk test. The significance of differences between the values of patients' indicators before treatment and at the time of treatment was compared with the values of the reference group. In addition, patients' findings at the time of treatment were compared with baseline values (before treatment) and between the groups. The procedure for statistical assessment of the significance of differences in indicators within the study groups (before/after treatment) was performed using the Wilcoxon W-test. The Mann-Whitney T-test was used to assess the statistical significance of the studied parameters with the norm and between groups.

RESULTS

The study of the concentration of PO products showed that in patients of group 2 (post-traumatic shortening etiology), the level of MDA in the blood plasma was significantly higher relative to the corresponding norm before the start of surgery (Table 1). At the stage of distraction, a statistically significant increase in the level of DC and MDA in the blood of patients of both groups was found. In the period of fixation and by the time of the device removal, the concentration of PO products in patients of group 1 did not differ significantly compared to the norm and preoperative values, while in patients of group 2, by the time of the device removal, DC and MDA values remained elevated.

Table1

Concentration of peroxidation products in the blood of patients of the studied groups in the course of lower leg lengthening (Xi ± SD)

Stage	Group	PPOP, unit opt. dens. / mg protein	DC, nmpl/mg lipids	MDA, nmol/mg lipids
	Norm	**198 ± 25**	**2.10 ± 0.26**	**1.20 ± 0.21**
Before operation	1	204 ± 15	2.21 ± 0.74	1.19 ± 0.26
	2	222 ± 41	2.93 ± 1.06	1.59 ± 0.21#
Distraction day 10	1	207 ± 15	2.82 ± 0.74	2.95 ± 0.42*#
	2	229 ± 31	7.37 ± 2.40*#	2.05 ± 0.45*#
End of distraction	1	202 ± 41	7.38 ± 2.11*#	2.27 ± 0.51*#
	2	186 ± 19	6.25 ± 2.00*#	2.51 ± 0.79*#
Fixation day 30	1	197 ± 21	3.09 ± 0.82*	1.37 ± 0.36
	2	215 ± 31	2.72 ± 0.80	2.53 ± 0.55*#
Frame removal	1	210 ± 29	2.92 ± 1.12	1.07 ± 0.26
	2	204 ± 27	4.11 ± 1.01*#	2.44 ± 0.34*#

Note: * – significance of difference with preoperative values at a significance level of $p < 0.05$; # – significant difference with the norm at a significance level $p < 0.05$

Changes in the intensity of peroxidation reactions caused significant changes in the antioxidant system (Table 2). Initially, we found a significantly reduced level of vitamin E in patients of group 1. In the period of distraction, a significant decrease in the level of vitamin E and an increase in SOD activity in erythrocytes were observed in patients of both groups. At the same time, a more significant decrease in vitamin E was noted in patients of group 2. At the time of removal of the device, the antioxidant status in patients of group 2 was restored to the normal range, while a reverse decrease in the level of vitamin E was noted in the patients of group 1.

To assess the antioxidant-prooxidant (AO-PO) balance, we additionally calculated the AO/PO ratio, which is equal to the ratio of the products of all studied antioxidants (SOD*vitamin E*vitamin A) to the product of all prooxidants (DC*MDA*PPOP) (Table 3).

The table shows that the ratio of AO/PO was significantly lower relative to the norm in patients of both groups before the start of treatment. In the distraction stage, even a greater decrease in this ratio was noted, which was more significant in group 2. The AO/PO ratio at the time of the device removal remained reduced relative to the norm, and in the patients of group 2, this decrease was significantly lower relative to the initial preoperative values.

Table 2

Concentration of vitamins in the blood serum and the activity of superoxide dismutase in the erythrocytes of patients in the course of lower limb lengthening (Xi ± SD)

Stage	Group	Vitamin E,mcg/ml	Vitamin A,mcg/ml	SOD, µmol NBT-10^9 erythrocyte/min
	Norm	**3.32 ± 0.76**	**0.28 ± 0.10**	**21 ± 3**
Before operation	1	1.70 ± 0.50#	0.27 ± 0.09	18 ± 5
	2	3.40 ± 1.59	0.24 ± 0.07	16 ± 8
Distraction day 10	1	1.10 ± 0.37#	0.25 ± 0.07	49 ± 7*#
	2	0.80 ± 0.22*#	0.20 ± 0.09	37 ± 15*
End of distraction	1	2.06 ± 0.37#	0.25 ± 0.09	38 ± 4*#
	2	1.20 ± 0.06*#	0.32 ± 0.04	32 ± 11*#
Fixation day 30	1	2.80 ± 0.57*	0.25 ± 0.07	36 ± 6*#
	2	1.46 ± 0.29*#	0.29 ± 0.03	44 ± 6*#
Frame removal	1	1.20 ± 0.22#	0.29 ± 0.04	20 ± 5
	2	2.79 ± 0.65	0.27 ± 0.06	26 ± 9

Note: * – significance of difference with preoperative values at a significance level $p < 0.05$; # – significant difference with the norm at a significance level $p < 0.05$

Table 3

Antioxidant-prooxidant ratio in patients in the course of lower limb lengthening (Xi ± SD)

Lengthening stage	Group	AO(SOD*A*E)*100 / PO(PPOP*DC*MDA)
	Norm	**3.91 ± 0.78**
Before operation	1	1.63 ± 0.41#
	2	1.35 ± 0.30#
Distraction day 10	1	0.81 ± 0.22*#
	2	0.17 ± 0.05*#
End of distraction	1	0.61 ± 0.19*#
	2	0.42 ± 0.17*#
Fixation day 30	1	2.98 ± 0.61*
	2	1.26 ± 0.33#
Frame removal	1	1.22 ± 0.41#
	2	0.95 ± 0.17*#

Note: * – significance of difference with preoperative values at a significance level $p < 0.05$; # – significant difference with the norm at a significance level $p < 0.05$. Significant difference between the groups at < 0.05

DISCUSSION

Our findings show that in patients with acquired shortening of the tibia, regardless of the shortening etiology, a decrease in the AO/PO ratio was observed in the course of surgical lengthening. It was also observed in the case of patients who underwent lengthening for esthetic reasons [16]. At the same time, it was found that the values of the concentration of PO products in patients with post-traumatic shortening were higher than in patients of the group with the consequences of hematogenous osteomyelitis at all periods of the examination. The reason for this is either the initially reduced antioxidant activity in patients with post-traumatic shortening, or these changes are the result of surgical treatment, where the main difference between the studied groups was the lengthening magnitude (in group 1 it was higher than in group 2). The first reason is supported by the above data, which demonstrate that in patients with post-traumatic shortening, the prooxidant activity was indeed higher relative to the control group (AO/PO ratio was lower in group 2 relative to group 1). In turn, our correlation analysis of the level of PO products by the end of distraction with the lengthening magnitude did not reveal significant values (correlation coefficients did not exceed 0.3 at $p > 0.05$). Therefore, it is impossible to state that the magnitude of elongation could have affected the decrease in AO/PO balance, especially since the maximum decrease in the AO/PO ratio in group 2 was observed not by the end of distraction but on its tenth day. Therefore, it can be concluded that the intensity of PO growth in patients with acquired shortenings of the bones of the lower extremities did not increase with an increase in the lengthening value, but to a greater extent depended on the initial preoperative state of the AO/PO status in the examined patients.

Obviously, at the stages of surgical lower limb lengthening, the activation of PO is an indispensable element of the body's systemic response to surgery. Considering that it is now widely recognized that oxidative stress, which causes PO growth, underlies the stimulation of apoptosis of osteoblasts and osteocytes, simultaneously activating osteoclastogenesis [17], it can be concluded that developing oxidative stress during surgical limb bone lengthening may be a factor if not influencing, then creating conditions for DO inhibition. Under such conditions, adequate antioxidant support, which provides PO inhibition, can have a positive impact on DO. Indeed, to date, there are works showing the positive effect of the use of vitamin E and other antioxidants, in particular, on DO [18, 19] and bone metabolism in general [20].

In this regard, the prospects for the use of various antioxidants (AOs) of natural and synthetic origin to stimulate osteogenesis have been discussed in many current studies. Thus, numerous works show the ability of various compounds with antioxidant properties to suppress oxidative stress reactions, thereby stimulating osteogenesis by inhibiting osteoclastogenesis while simultaneously stimulating osteoblastogenesis [21–26]. Moreover, the use of AOs in cases of their impregnation into implantable products for the tasks of increasing their osseointegration looks promising [27, 28]. Such studies look promising and also support the wider use of compounds with AO properties.

Thus, the use of antioxidant support to optimize the conditions for DO, at least theoretically, looks like a fairly reasonable and promising technology. However, for the further development of this direction, it is important to determine the indications for the use of AO in order to optimize DO. This problem is rather complicated, because even the experience of using osteogenesis stimulators (hormones, bisphosphonates) for the purpose of DO stimulation requires special indications [29]. In our study, the issue of indications for the use of AOs requires further clarification, because despite a significant decrease in AO/PO balance, clinical outcomes in all the patients examined by us were assessed as positive.

It should be said that an additional point in favor of the use of AOs in distraction osteogenesis may be the fact that the effect of AO is systemic, providing optimization of not only bone repair, but of paraosseous tissues. Thus, a positive effect of PO inhibition on the state of the skeletal muscles of the elongated limb segment has been shown [30, 31].

Therefore, the use of AOs in orthopedic patients undergoing surgical correction of segment length is justified from at least two points of view: 1) a positive effect of AOs in terms of osteogenesis stimulation has been proven; 2) AOs have a systemic effect. An additional advantage to this is the fact that the number of potential osteogenesis stimulators with AO activity has been constantly growing [32]. Obviously, the limitation for the use of AOs is the lack of clear indications for their use in clinical practice. In this direction, in order to obtain evidence-based results, a comparative study is required, in which the effect of AOs (individually or as part of compounds) on the outcomes, efficacy and safety of surgical treatment of target patients would be studied in homogeneous groups.

CONCLUSION

Activation of peroxidation occurs in the course of surgical lengthening of the lower leg bones in patients with acquired shortenings, regardless of the etiology. This may be a factor provoking a slowdown in distraction osteogenesis. The use of antioxidants can specifically inhibit the growth of peroxidation reactions, thereby decreasing the risks of bone formation disorders in the target patients. The theoretical prerequisites for the use of antioxidants to stimulate osteogenesis are quite obvious.

REFERENCES

1. Gubin A.V., Borzunov D.Yu., Marchenkova L.O., Smirnova I.L. Academician G.A. Ilizarov's scientific heritage: a look into the future from the past (part II) (devoted to 95th anniversary of Academician G.A. Ilizarov, 65th anniversary of the transosseous osteosynthesis method according to Ilizarov). *Genij Ortopedii*, 2016, no. 3, pp. 6-13. DOI 10.18019/1028-4427-2016-3-6-13. (in Russian)
2. Soldatov Yu.P., Stogov M.V., Ovchinnikov E.N., Gubin A.V., Gorodnova N.V. Evaluation of clinical efficacy and safety of the Ilizarov apparatus for external fixation (literature review). *Genij Ortopedii*, 2019, vol. 25, no. 4, pp. 588-599. DOI 10.18019/1028-4427-2019-25-4-588-599.
3. Aktuglu K., Erol K., Vahabi A. Ilizarov bone transport and treatment of critical-sized tibial bone defects: a narrative review. *J. Orthop. Traumatol*., 2019, vol. 20, no. 1, pp. 22. DOI: 10.1186/s10195-019-0527-1.
4. Borzunov D.Y., Kolchin S.N., Malkova T.A. Role of the Ilizarov non-free bone plasty in the management of long bone defects and nonunion: Problems solved and unsolved. *World J. Orthop*., 2020, vol. 11, no. 6, pp. 304-318. DOI: 10.5312/wjo.v11.i6.304.
5. Bukva B., Vrgoč G., Rakovac I., Dučić S., Sindik J., Čoklo M., Marinović M., Bakota B. Complications in leg lengthening using an Ilizarov external fixator and intramedullary alignment in children: comparative study during a fourteen-year period. *Injury*, 2015, vol. 46, no. Suppl. 6, pp. S48-S51. DOI: 10.1016/j.injury.2015.10.058.
6. Gayuk V.D., Kliushin N.M., Burnashov S.I. Pin site soft tissue infection and osteomyelitis: literature review. *Genij Ortopedii*, 2019, vol. 25, no. 3, pp. 407-412. DOI 10.18019/1028-4427-2019-25-3-407-412.
7. Li Y., Pan Q., Xu J., He X., Li H.A., Oldridge D.A., Li G., Qin L. Overview of methods for enhancing bone regeneration in distraction osteogenesis: Potential roles of biometals. *J. Orthop. Translat*., 2021, vol. 27, pp. 110-118. DOI: 10.1016/j.jot.2020.11.008.
8. Imam M.A., Holton J., Ernstbrunner L., Pepke W., Grubhofer F., Narvani A., Snow M. A systematic review of the clinical applications and complications of bone marrow aspirate concentrate in management of bone defects and nonunions. *Int. Orthop*., 2017, vol. 41, no. 11, pp. 2213-2220. DOI: 10.1007/s00264-017-3597-9.
9. Kim J.B., Lee D.Y., Seo S.G., Kim E.J., Kim J.H., Yoo W.J., Cho T.J., Choi I.H. Demineralized Bone Matrix Injection in Consolidation Phase Enhances Bone Regeneration in Distraction Osteogenesis via Endochondral Bone Formation. *Clin. Orthop. Surg*., 2015, vol. 7, no. 3, pp. 383-391. DOI: 10.4055/cios.2015.7.3.383.
10. Morcos M.W., Al-Jallad H., Hamdy R. Comprehensive Review of Adipose Stem Cells and Their Implication in Distraction Osteogenesis and Bone Regeneration. *Biomed. Res. Int*., 2015, vol. 2015, 842975. DOI: 10.1155/2015/842975.
11. Fourman M.S., Borst E.W., Bogner E., Rozbruch S.R., Fragomen A.T. Recombinant human BMP-2 increases the incidence and rate of healing in complex ankle arthrodesis. *Clin. Orthop. Relat. Res*., 2014, vol. 472, no. 2, pp. 732-739. DOI: 10.1007/s11999-013-3261-7.
12. Kiely P., Ward K., Bellemore C.M., Briody J., Cowell C.T., Little D.G. Bisphosphonate rescue in distraction osteogenesis: a case series. *J. Pediatr. Orthop*., 2007, vol. 27, no. 4, pp. 467-471. DOI: 10.1097/01.bpb.0000271326.41363.d1.
13. Wagner F., Vach W., Augat P., Varady P.A., Panzer S., Keiser S., Eckardt H. Daily subcutaneous Teriparatide injection increased bone mineral density of newly formed bone after tibia distraction osteogenesis, a randomized study. *Injury*, 2019, vol. 50, no. 8, pp. 1478-1482. DOI: 10.1016/j.injury.2019.06.001.
14. Lee D.H., Ryu K.J., Shin D.E., Kim H.W. Botulinum toxin a does not decrease calf pain or improve ROM during limb lengthening: a randomized trial. *Clin. Orthop. Relat. Res*., 2014, vol. 472, no. 12, pp. 3835-3841. DOI: 10.1007/s11999-014-3546-5.
15. Novikov K.I., Stogov M.V., Muradisinov S.O., Kolesnikova E.S., Kireeva E.A. The change in blood serum biochemical parameters for leg proximal third lengthening by transosseous osteosynthesis method according to Ilizarov. *Genij Ortopedii*, 2015, no. 1, pp. 40-42. (in Russian)
16. Tushina N.V., Stogov M.V., Novikov K.I. Antioksidantnaia sistema i perekisnoe okislenie lipidov v syvorotke krovi patsientov s kosmeticheskim udlineniem kostei goleni po Ilizarovu. *Uspekhi sovremennogo estestvoznaniia*. 2015. no. 4, pp. 78-80. (in Russian)
17. Domazetovic V., Marcucci G., Iantomasi T., Brandi M.L., Vincenzini M.T. Oxidative stress in bone remodeling: role of antioxidants. *Clin. Cases Miner. Bone Metab*., 2017, vol. 14, no. 2, pp. 209-216. DOI: 10.11138/ccmbm/2017.14.1.209.
18. Borhanuddin B., Mohd Fozi N.F., Naina Mohamed I. Vitamin e and the healing of bone fracture: the current state of evidence. *Evid. Based Complement Alternat. Med*., 2012, vol. 2012, 684510. DOI: 10.1155/2012/684510.
19. Kurklu M., Yildiz C., Kose O., Yurttas Y., Karacalioglu O., Serdar M., Deveci S. Effect of alpha-tocopherol on bone formation during distraction osteogenesis: a rabbit model. *J. Orthop. Traumatol*., 2011, vol. 12, no. 3, pp. 153-158. DOI: 10.1007/s10195-011-0145-z.
20. Zhou P., Shao R., Wang H., Miao J., Wang X. Dietary vitamin A, C, and E intake and subsequent fracture risk at various sites: A meta-analysis of prospective cohort studies. *Medicine* (Baltimore), 2020, vol. 99, no. 35, pp. e20841. DOI: 10.1097/MD.0000000000020841.
21. Acikan I., Mehmet G., Artas G., Yaman F., Deniz G., Bulmus O., Kom M., Kirtay M., Dundar S. Systemic melatonin application increases bone formation in mandibular distraction osteogenesis. *Braz. Oral Res*., 2018, vol. 32, pp. e85. DOI: 10.1590/1807-3107bor-2018.vol32.0085.
22. Cepeda S.B., Sandoval M.J., Crescitelli M.C., Rauschemberger M.B., Massheimer V.L. The isoflavone genistein enhances osteoblastogenesis: signaling pathways involved. *J. Physiol. Biochem*., 2020, vol. 76, no. 1, pp. 99-110. DOI: 10.1007/s13105-019-00722-3.
23. Li Y., Chen G., He Y., Zhang X., Zeng B., Wang C., Yi C., Yu D. Ebselen rescues oxidative-stress-suppressed osteogenic differentiation of bone-marrow-derived mesenchymal stem cells via an antioxidant effect and the PI3K/Akt pathway. *J. Trace Elem. Med. Biol*., 2019, vol. 55, pp. 64-70. DOI: 10.1016/j.jtemb.2019.06.002.
24. Wardhana A.S., Nirwana I., Budi H.S., Surboyo M.D.C. Role of Hydroxyapatite and Ellagic Acid in the Osteogenesis. *Eur. J. Dent*., 2021, vol. 15, no. 1, pp. 8-12. DOI: 10.1055/s-0040-1714039.
25. Wong S.K., Chin K.Y., Ima-Nirwana S. Quercetin as an Agent for Protecting the Bone: A Review of the Current Evidence. *Int. J. Mol. Sci*., 2020, vol. 21, no. 17, pp. 6448. DOI: 10.3390/ijms21176448.
26. Yamada M., Watanabe J., Ueno T., Ogawa T., Egusa H. Cytoprotective Preconditioning of Osteoblast-Like Cells with N-Acetyl-L-Cysteine for Bone Regeneration in Cell Therapy. *Int. J. Mol. Sci*., 2019, vol. 20, no. 20, pp. 5199. DOI: 10.3390/ijms20205199.
27. Savvidis M., Papavasiliou K., Taitzoglou I., Giannakopoulou A., Kitridis D., Galanis N., Vrabas I., Tsiridis E. Postoperative Administration of Alpha-tocopherol Enhances Osseointegration of Stainless Steel Implants: An in vivo Rat Model. *Clin. Orthop. Relat. Res*., 2020, vol. 478, no. 2, pp. 406-419. DOI: 10.1097/CORR.0000000000001037.

28.Zheng K., Torre E., Bari A., Taccardi N., Cassinelli C., Morra M., Fiorilli S., Vitale-Brovarone C., Iviglia G., Boccaccini A.R. Antioxidant mesoporous Ce-doped bioactive glass nanoparticles with anti-inflammatory and pro-osteogenic activities. *Mater. Today Bio*, 2020, vol. 5, 100041. DOI: 10.1016/j.mtbio.2020.100041.
29.Hvid I., Horn J., Huhnstock S., Steen H. The biology of bone lengthening. *J. Child. Orthop*., 2016, vol. 10, no. 6, pp. 487-492. DOI: 10.1007/s11832-016-0780-2.
30.Kaynar A., Civelek S., Kasymova Z., Keklikoglu N., Toklu S., Uzun H. Effects of hyperbaric oxygen therapy on biochemical and histological parameters of muscle groups in proximity to the distracted rat tibia. *Int. J. Oral. Maxillofac. Surg*., 2014, vol. 43, no. 12, pp. 1514-1521. DOI: 10.1016/j.ijom.2014.06.019.
31.Stogov M.V., Emanov A.A., Stepanov M.A. Muscle metabolism during tibial lengthening with regular and high distraction rates. *J. Orthop. Sci*., 2014, vol. 19, no. 6, pp. 965-972. DOI: 10.1007/s00776-014-0627-y.
32.Gambari L., Grigolo B., Grassi F. Hydrogen Sulfide in Bone Tissue Regeneration and Repair: State of the Art and New Perspectives. *Int. J. Mol. Sci*, 2019, vol. 20, no. 20, 5231. DOI: 10.3390/ijms20205231.

The article was submitted 04.03.2021; approved after reviewing 08.04.2021; accepted for publication 28.03.2022.

Information about the authors:

1. Anna M. Aranovich – Doctor of Medical Sciences, Professor, aranovich_anna@mail.ru;
2. Maksim V. Stogov – Doctor of Biological Sciences, stogo_off@list.ru;
3. Natalia V. Tushina;
4. Elena A. Kireeva – Candidate of Biological Sciences.

Source of funding *The work was carried out within the framework of the state assignment for the implementation of scientific research and developments at the Federal State Budgetary Institution Ilizarov National medical Research Centre for Traumatology and Orthopaedics of the Ministry of Health of Russia on the topic "Bioactive osteosynthesis of long bone injuries".*