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Effect of Preparations Based on Lanthanide Ions and Calcium on the Bone Density in Rats with a Femur Fracture

I.F. Akhtyamov [1], F.V. Shakirova [2], D.A. Korobeynikova [2], Han Hao Zhi [1], R.I. Sadykov [1, 3]

[1] *Kazan State Medical University, Kazan, Russian Federation*

[2] *Bauman Kazan State Academy of Veterinary Medicine, Kazan, Russian Federation*

[3] *City Clinical Hospital No. 7, Kazan, Russian Federation*

Abstract

Purpose of the study — to evaluate bone density at the femur fracture site with local introduction of preparations based on etidronate lanthanide ions and calcium in the experiment. ***Materials and Methods.*** The experiment included 45 male rats divided into three comparable groups equal in quantity. The experiment in control group was performed without stimulation of bone formation, preparation based on etidronate lanthanide ions and calcium were introduced to the animals of the first group and preparation containing etidronate and calcium (without lanthanide ions) were used in the second group. Preparation was introduced twice at the site of femoral fracture in the study groups of animals. The authors performed daily clinical monitoring and measured the density of the cortical bone at the fracture site using computer tomography. ***Results.*** Earlier support on the operated limb was observed after introduction of etidronate lanthanide ions and calcium. In the respective study group after introduction of the preparation the authors reported statistically significant 20% higher cortex density at earlier terms in contrast to the control group, and 24% higher density as compared to group with introduction of etidronates and calcium (without lanthanide ions). By day 30 cortical plate density in the group with introduction of preparation based on lanthanide ions was statistically significantly higher at 37% as compared to two other groups. ***Conclusion.*** The authors observed a positive effect of studied preparations on bone regenerate formation in rats. Data of the present research allows to conclude that periosteal introduction of preparations based on etidronate lanthanide ions and calcium at the osteotomy site provides formation of a cortical plate with improved density properties, and normalization of density indicators in the present group occurred in earlier terms that in the group with preparation based on etidronates and calcium (without lanthanide ions).

Keywords: bone regeneration, bone density, rat femur fracture, intramedullary osteosynthesis, bone regeneration, calcium, lanthanide ions.

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Ildar F. Akhtyamov; e-mail: yalta60@mail.ru

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Introduction

It is known that trauma and surgery have an adverse effect both on microcirculatory parameters and on the immune system. This can lead to the development of various complications, including the inhibition of bone tissue repair [1]. The severity of the process is explained by a combination of bone injury with a soft tissue defect, fragments displacement, and circulatory insufficiency. This leads to inflammatory complications and a large number of nonunions [2, 3, 4], pseudoarthrosis, and posttraumatic tissue defects [5, 6]. The combination of three or more predisposing factors allows the patient to be attributed to the risk group and is an indication for the bone repair stimulation [7].

The prospects for a significant improvement in the treatment of patients with bone injuries and diseases only by improving the methods of reposition and fixation of bone fragments are now practically exhausted [8].

Various materials are used in attempts to influence the bone repair processes. These materials stimulate two bone repair processes: osteoconduction and osteoinduction. Osteoconduction is the property of a material to serve as a scaffold for newly formed bone tissue [9, 10, 11]. Osteoinduction is the property of a material to support proliferation of undifferentiated mesenchymal cells and their transformation into osteoblasts using bone growth factors [12, 10].These materials include autografts and substances, containing growth factors, that stimulate the proliferation and differentiation of bone tissue, and play the scaffold role for a new bone tissue formation [8].

One of such materials is the biocomposite material LitAr. Studies were conducted on dogs with the formation of a semicircular defect of the tibia, which then was filled with a collagen-apatite sponge LitAr. It was found that this material promoted the activation of endosteal, intermediate and periosteal osteogenesis [13]. A composite material Matribon is a synthetic hydroxyapatite with a chitosan gel containing as a bioregulator. The local application of Matribon into the induced femoral defect in rats promoted the active bone repair with restoration of the morphologically normal bone matrix and dense bone tissue [11]. Currently, calcium phosphate compounds are widely used. A group of scientists conducted a comparative study on outbred rats. In this study a tibia defect was formed in the rats. And then calcium phosphate compounds, namely: KollapAn, Ostim, Chronos, Tserosorb, were placed in this defect. It was found that the most rapid formation and remodeling of bone callus occurred after CollapAn application[14].

Of great interest is the effect of bisphosphonates on bone tissue regeneration. Until recently, it was believed that bisphosphonates did not directly affect bone formation, but increased bone balance by simple inhibition the bone resorption. However, the results of new studies in vivo showed that this was not entirely true [15, 16, 17]. Bisphosphonates are able to create strong bonds with hydroxyapatite crystals due to selective adsorption on bone surface. Bisphosphonates act through bone resorption inhibition [18]. At the cellular level, it was shown in vitro that bisphosphonates increased osteoblasts proliferation [19, 20].

Currently, the possibility of topical application of bisphosphonates, both as a single substance and in combination with other components, is under discussion [21]. Experiments in vivo demonstrated an osteogenesis increase around the implants after local use of bisphosphonate solution. This observation is of great interest, since the revealed increase in osseointegration at the implant-bone interface after local use of bisphosphonate was superior to its systemic use [21]. However, it was found that bisphosphonates in solution were not able to remain in the injury zone for a long time [22, 23].

In order to keep the components at the place of their application, we used in our study lanthanides. They are able to form colloids and hydroxides under the body pH. The transition of a lanthanide from a dissolved, ionized state, to a colloid state leads to a change in the absorption and penetration through biological barriers, a decrease in excretion from the body, and the formation of a particularly strong bond with internal organs and tissues due to colloidal adsorption [24].

The purpose of this study — to assess in vivo the bone density changes in the fracture area during intramedullary osteosynthesis after the local injection of the mixture of ethidronates, lanthanides and calcium ions.

Material and methods

The study design

This was a prospective study taken place at the Department of Surgery, Veterinary Obstetrics and Pathology of small animals of the Bauman Kazan State Academy of Veterinary Medicine in 2017–2019. All experiments were conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986). The animals were kept in the same conditions and received the same diet.

Ethics of the study

This study was conducted under the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986) and approved by Local Ethics Committee at the Kazan State Medical University (the record No 10 on the 18th of December 2018).

Animals

As an experimental model, 45 male mongrel white rats were taken with the average weight 334.9±22.3 g and 5–6 months age. The animals were selected on the basis of analogues and divided into three groups of 15 animals each.

Surgical technique

Surgery was performed under general potentiated anesthesia (Rometar 2% — 0.15–0.20 ml/kg, Zoletil 100–10–15 mg/kg). All experimental rats underwent osteotomy at the lateral middle third of the femur diaphysis followed by retrograde two nails insertion into the medullary canal. The final stage consisted of an intradermal suture.

The animals of the control group did not receive any stimulating components into the fracture zone. The animals of the experimental group No. 1 received the mixture of ethidronates, lanthanide and calcium ions into the fracture zone. The animals of the experimental group No. 2 received ethidronate and calcium ions, but without lanthanides.

The rats of the experimental groups received the stimulating substances into the fracture zone parosteally, from the lateral and medial surfaces, twice, on the 3rd and 5th days after the surgery at a dose of 0.2 ml to each animal (Fig. 1).

The duration of the experiment was 30 days. Experimental animals were monitored daily throughout the treatment period.

The CT scan was performed on a Siemens Emotion-16 multispiral X-ray computer tomograph before surgery, on the 7th, 14th and 30th days after surgery (parameters: 110 kV, 114 mA, cut thickness 0.6 mm).

Local bone mineral density was determined at 8 control points, 4 points on each side of the cortical plate: at 2 points 1 cm above the fracture zone and at 2 points 1 cm below the fracture zone.

Bone density was automatically calculated in Hounsfield units (HU), Fig. 2.

Subsequently, the obtained digital values were added and divided by the number of points to obtain the average value. At all stages of the studiy, a comparative assessment of the regenerate zones density with the density before surgery was performed.

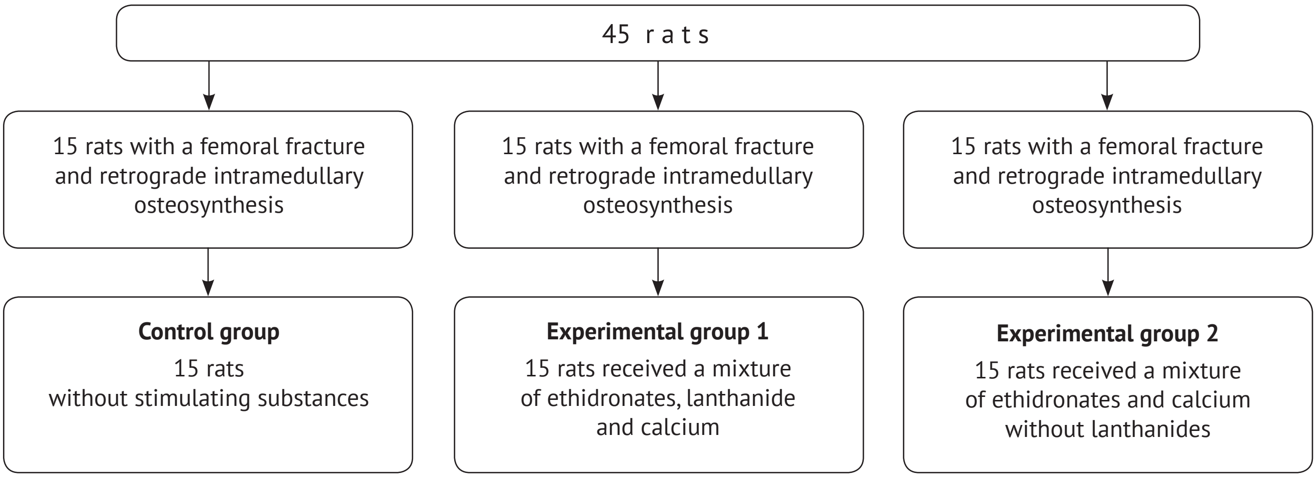

Fig. 1. Study flow diagram

Fig. 2. MSCT of a damaged lower rat extremity. Local bone density assessment:
4 points in the fracture zone on each side of the cortical plate — two points above the fracture zone and two points below it

Statistical analysis

Statistical processing was performed using the SPSS application software package, version 13. The normality of indicators distribution was evaluated using the Kolmogorov–Smirnov test. For paired comparisons, Student's test was used. To compare three or more groups, dispersion analysis was used. Subsequent intergroup comparisons were performed using Student's test with Bonferroni correction. Differences were considered statistically significant at $p<0.05$. Numerical values are presented in the form M±m, where M is the arithmetic mean, m is the standard error of the mean.

Results

Clinical examination of the animals revealed that in the group 1 (ethidronates+calcium+lanthonides) the animals began to lean on the operated limb earlier than in other groups, which was confirmed by the signs of fracture fusion on the tomograms.

According to the multispiral computed tomography, the density of the femoral cortical plate in the middle third of the diaphysis before surgery was 1718±43.1 HU (Table 1).

On the 7th day after the operation, a transverse fracture in the middle third of the femoral diaphysis was visualized on a series of tomograms in animals of all three groups. The density of the cortical plate in animals of experimental group No. 1 averaged 1331.0±16.4 HU. This was significantly higher by 20% than in the control group (p = 0.001) and 24% higher than in animals of experimental group No. 2 (p = 0.001) (Fig. 3).

Table 1

Densitometric indices of the cortical plate, HU

Groups	Before surgery	Days after surgery		
		7th	14th	30TH
Control	1718±43.1	1052.84±9.6	1222.3±10.4	1278.3±27.2
Experimental No. 1		1331.06±16.4*	1587.5±34.7*	2041.9±60.6*
Experimental No. 2		1012.4±3.8	1133.7±15.4	1304.7±13.6

*Statistically significant differences of the mean of experimental group No. 1 in relation to the control group and experimental group No. 2 (p<0.05).

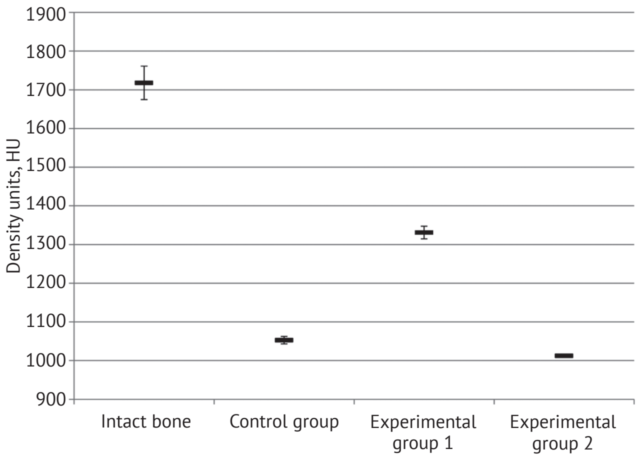

Fig. 3. Scatter plot for density of femoral cortex at the fracture site on day 7 after procedure

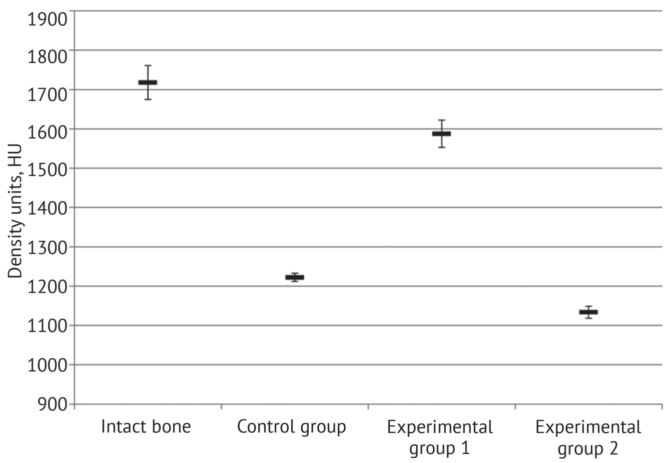

Fig. 4. Scatter plot for density of femoral cortex at the fracture site at day 14 after procedure

On the 14th day, a periosteal reaction was observed in the fracture area in the experimental group No. 1 (ethidronates+lanthanide +calcium). At this moment, the statistically significant differences were revealed between the groups. The cortical plate density of the control group animals (without any substances) was significantly lower by 23% (p = 0.001) than in the animals of the experimental group No. 1, and by 29% (p = 0.042) lower in the animals of the experimental group No. 2 (Fig. 4).

On the 30th day, the computed tomography showed that the density of the cortical plate in the rats of experimental group No. 1 was higher by 37% than in the control group (p = 0.001) and 36% than in the experimental group No. 2 (p = 0.001), Fig. 5.

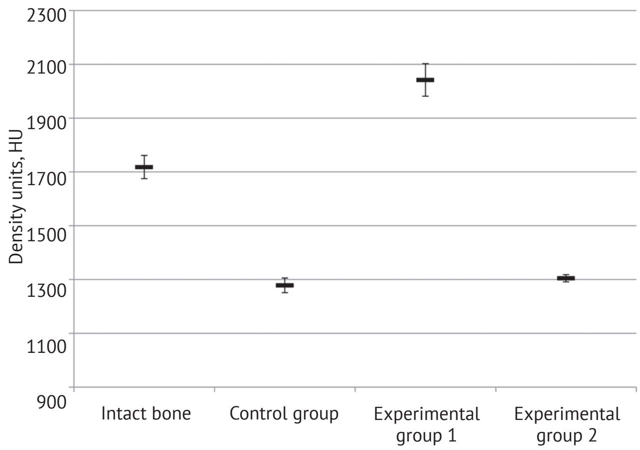

Fig. 5. Scatter plot for density of femoral cortex at the fracture site at day 30 after procedure

Discussion

Under bisphosphonates, the bone resorption was reduced, mineralization processes were normalized, resulted in the bone mass increased [25]. However, it was found that constant inhibition of bone resorption processes over a long period of time could lead to the bone strength decrease despite the fact that their mineral density was increased.

In our experiment, an assessment of bone density showed significant differences at various stages of the femoral fracture healing in all three groups. The obtained data may indicate a significant increase in the cortical plate density in the fracture zone under the mixture of ethidronates + calcium + lanthanide.

The bone density increase was also observed in the study of a bisphosphonate such as risedronate, administered orally in rats with experimental metadiaphyseal fractures [26]. Although, there are also opposite data. In the treatment of metadiaphysis radial bone fracture with aledronate in an experiment on dogs, an increase in the regenerate volume was observed, but without the fracture healing, bone mineralization, and bone strength [27].

In our study, the bone density increase in the group with ethidronates + lanthanide + calcium administration was found already on the 7th day after the fracture with an upward trend that kept by the 30th day. At that moment, the bone density in the experimental group No. 1 corresponded to the level before the surgery. A similar increase in bone density, Li et al. observed with oral administration of aledronate in femoral fracture. However, the process of bone callus remodeling was delayed [28]. Manabe et al. and Goodship et al. found a delay in compact bone remodeling under ibandronate administration in an experimental femoral fracture in rats and pamidronate after femoral diaphysis osteotomy, however, these bisphosphonates contributed to the formation of large bone calluses around osteotomy site [29, 30]. It has been established that bisphosphonates did not affect biomechanical indicators.Therefore, it can be said that in animal models, various medications of this group do not inhibit consolidation of the fracture, but they slow down the endochondral ossification [31].

A clinically important point is that bisphosphonates affect the mucous membrane. It has long been known that bisphosphonates can cause gastrointestinal distress, and therefore their oral use was limited [32]. Thus, the development of methods for topical application of bisphosphonates is relevant. In an experiment on a closed fracture model in rats, it was proved that bisphosphonates (zoledronic acid was used in this study), when administered locally, had no effect on mechanical parameters [33].

Conclusion

Thus, on the basis of our study, it can be asserted that it is the lanthanide ions that contribute to the retention of bisphosphonates at the site of their application. This can explain their positive effect on the remodeling process.

The results of the study allow us to conclude that the parosteal use of mixture of ethidronates + lanthanide + calcium in the osteotomy zone is accompanied by the formation of a cortical plate with not only the highest density characteristics, but also much earlier in comparison with the use of ethidronates+calcium alone.

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Authors' contributions

I.F. Akhtyamov — research concept and design, analysis and interpretation of the data, editing.

F.V. Shakirova — interpretation and analysis of the data.

D.A. Korobeinikova — collection of material and its processing, direct research conducting, text preparation.

Kh. Ch. Khan'— collection of material and its processing, direct research conducting, text preparation.

R.I. Sadykov — collection of material and its processing.

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AUTHORS' INFORMATION:

Ildar F. Akhtyamov — Dr. Sci. (Med.), Professor, Head of Traumatology, Orthopedics and Surgery of Extreme States Department, Kazan State Medical University, Kazan, Russian Federation

Shakirova Faina V. — Dr. Sci. (Vet.), Associate Professor, Professor, Surgery, Obstetrics, and Pathology of Companion Animals Department, Bauman Kazan State Academy of Veterinary Medicine, Kazan, Russian Federation

Darya A. Korobeynikova — PhD Student, Surgery, Obstetrics, and Pathology of Companion Animals Department, Bauman Kazan State Academy of Veterinary Medicine, Kazan, Russian Federation

Han Hao Zhi — PhD Student, Traumatology, Orthopedics and Surgery of Extreme States Department, Kazan State Medical University, Kazan, Russian Federation

Rustem I. Sadykov — Assistant, Traumatology, Orthopedics and Surgery of Extreme States Department, Kazan State Medical University; Head of the Trauma Center, City Clinical Hospital No. 7, Kazan, Russian Federation