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Research Article

Pharmacological correction of experimental osteoporosis and fractures related to it by means of rosuvastatin, L-norvaline and their combination

Mikhail S. Sobolev[1], Alexander V. Faitelson[1], Densingh Samuel Raj Rajkumar[1]

1 *Kursk State Medical University, 3 Karl Marx Sr., Kursk 305000 Russia*

Corresponding author: *Densingh Samuel Raj Rajkumar* (densingh7@gmail.com)

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Abstract

Introduction: osteoporosis (OP) is a multifactorial disease which is based on a dynamic decrease in bone mass and, as a result, disruption of bone structure, leading to higher chances of skeletal fractures. Endothelial dysfunction is a key cause of impaired blood supply to the bone, resulting in a decreased perfusion, disrupted osteogenesis and, as a consequence, osteoporotic changes. According to modern literature and available research, L-norvaline and rosuvastatin have a powerful endotheliotropic effect. However, there is no information that the osteoprotective properties of these drugs used as a monotherapy and as their combination have ever been studied.

Materials and Methods: Simulation of experimental osteoporosis was performed on 120 white female Wistar rats. After eight weeks, the operated rats developed hypoestrogenic osteoporosis. L-norvaline and rosuvastatin, both as a monotherapy and in their combination, were used from week 9 to week 12, inclusive. The extent of changes on the background of osteoporosis was estimated twelve weeks after oophorectomy. Simulation of closed osteoporotic fractures of the femurs and their osteosynthesis were performed on 120 white female Wistar rats. Experimental osteoporosis was modeled on all animals (except the control group). Eight weeks after the removal of the ovaries on the background of developing osteoporosis, fractures of the femur were simulated. The test drugs and their combination were applied from week 1 to week 4, inclusive, after the modeling of osteoporotic fractures and their osteosynthesis. Twelve weeks after the start of the experiment, the results of fracture consolidation were analyzed.

Results and Discussion: Rosuvastatin at a dose of 0.86 mg/kg, L-norvaline at a dose of 10 mg/kg, and their combination prevented a decrease in the level of microcirculation in the callus, had an anti-osteoporotic effect, and also contributed to an increased number of healed experimental fractures. The osteoprotective effect of the test drugs is, apparently, due to their endothelial-protective action.

Conclusion: The range of the pleiotropic action of drugs with endothelial-protective properties can be extended by adding an osteoprotective element, which, however, requires additional research.

Keywords

L-norvaline, microcirculation, osteoporosis, fracture, rosuvastatin, endothelial dysfunction

Introduction

Osteoporosis is a multifactorial disease which is based on a dynamic decrease in bone mass and, as a consequence, a disruption of bone structure, leading to higher cganc-es of skeletal fractures (Avrunin 1998, Benevolenskaya 2004).

The key triggers in the development of osteoporosis are a decrease in calcium and vitamin D levels, a sedentary lifestyle, tobacco smoking, alcohol abuse, prolonged intake of glucocorticoids, and rheumatoid arthritis. The older age, genetic determinism, weight loss and menopause can be attributed to the background triggers. The high incidence of fractures against osteoporosis places this pathology among the main problems of modern public health. According to the World Health Organization, osteoporosis is ranked fourth in the world among non-communicable diseases, coming after pathologies of the cardiovascular system, malignant neoplasms and diabetes. Osteoporosis has been diagnosed in 75 million people in Europe and Japan.

Pharmacological drugs for the treatment of osteoporosis can be divided into three groups: drugs that lower bone resorption (estrogens, calcitonin); drugs that stimulate osteogenesis (fluorides, anabolic steroids, strontium ranelate); pharmacological preparations of multifactor action (vitamin D) (Lesnyak 2005, Rozhinskaya 2007). This division is relative, since these groups of drugs affect all bone formation processes. However, an increased number of fractures on the background of skeletal osteoporosis indicates that currently there is no only way of pharmacological treatment of this condition. This fact encourages the search for alternative ways of pharmacological correction of the pathology of bone remodeling, which determined the topic of this study.

Microcirculatory blood supply is essential for bone tissue repair processes (Nikolova et al. 2006, Faitelson et al. 2010). The structure of capillary vessels in bones is significantly different from the structure of those in other tissues of the body. Their wall is composed exclusively of the endothelium, through which the processes of regulation between the bone cells and the body are carried out (Gudyrev et al. 2011).

Endothelial dysfunction is a key cause of impaired blood supply to the bone, resulting in impaired perfusion, impaired osteogenesis and, as a result, osteoporotic changes (Kloen et al. 2003).

Previously conducted experimental studies proved significant osteoprotective effects of substances that minimize endothelial dysfunction, such as angiotensin-converting-enzyme (ACE) inhibitor enalapril, angiotensin (AT) receptor blocker losartan and phytoalexin resveratrol (Faitelson et al. 2010, Gudyrev et al. 2011, Kloen et al. 2003).

According to modern literature and available research, L-norvaline and rosuvastatin have a powerful endotheliotropic effect (Coffin et al. 1995, Molchanova 2016). However, there is no information that the osteoprotective properties of these drugs used as a monotherapy and as their combination have ever been studied.

Objective of the study

To assess the osteoprotective activity of L-norvaline, rosuvastatin and their combination in comparison with Bivalos® (strontium ranelate) when correcting experimental osteoporosis and fractures related to it ,caused by bilateral ovariectomy.

Materials and research methods

The conduct of this study was approved by the local ethical committee for working with laboratory animals. Experimental osteoporosis was simulated on 120 white female Wistar rats weighing from 200 to 300g. To simulate experimental osteoporosis, the rats were anesthetized by intraperitoneal administration of a 300 mg/kg aqueous solution of chloral hydrate, after which an operation – bilateral ovariectomy – was performed. After eight weeks, the operated rats hypoestrogenic developed osteoporosis (Stabrovskaya 2011, Faitelson et al. 2010). The test pharmacological preparations, both as a monotherapy and as their combination, were used from week 9 to week 12, inclusive. For the purpose of control, a group of rats was formed, in which the animals underwent anesthesia for an abdominal opening, without having the ovaries removed and with layered wound closure (false ovariectomy).

The extent of the changes on the background of osteoporosis was assessed twelve weeks after ovariectomy by histomorphometry.

Modeling of closed osteoporotic fractures of the femoral bones and their osteosynthesis were performed on 120 white female Wistar rats with a body weight from 200 to 300g. In all the animals (except the control group), experimental osteoporosis was simulated. After eight weeks, in the operated rats developed systemic osteoporosis.

Eight weeks after the removal of the ovaries against the background of developing osteoporosis, femoral fractures were modeled (osteoporotic fractures).

In order to simulate experimental osteoporotic fractures, an external impact was applied to the metaphysis with a load perpendicular to the bone axis, until a transverse, oblique, or comminuted fracture appeared in it (Certificate for rationalization proposal No. 1975-11 11.23.11). In order to stabilize the fracture zone, a minimally invasive, closed intramedullary pin osteosynthesis was performed. Absence of pathological mobility in the fracture zone served as a criterion for the adequacy of osteosynthesis (Certificate for rationalization proposal No. 1974-11 11.23.11).

The test drugs and their combination were applied from week 1 to week 4, inclusive, after modeling osteoporotic fractures and their osteosynthesis. Twelve

weeks after the start of the experiment, the results of fracture consolidation were analyzed macroscopically and radiographically.

Measurement of microcirculation in the bone tissue was assessed in the proximal metaphyseal part of the femur, and in the case of modeling osteoporotic fractures – in the early callus. For this, the rats were anesthetized by intraperitoneal injection of an aqueous solution of chloral hydrate at a dose of 300 mg/kg; then an operative access was achieved to the proximal metaphyseal region of the femur. In the cortical bone layer or on the surface of the callus, a perforation hole was being formed, into which a needle sensor was inserted to determine a level of local microcirculation.

Bone perfusion was investigated on equipment manufactured by Biopac Systems: MP100/150 polygraph and Laser Doppler Flowmetry module (LDF) LDF100C with an invasive needle-shaped TSD144 sensor for inserting into the proximal metaphyseal part of the femur.

At the time of the study of experimental osteoporosis, after measuring the level of bone perfusion, without changing the position of the sensor, vascular tests were performed for endothelium-dependent vasodilation (EDVD) (response to a bolus intravenous administration of a solution of acetylcholine at a dose of 40 μg/kg) and endothelium-nondependent vasodilatation (ENVD) (intravenous administration of a solution of sodium nitroprusside at a dose of 30 μg/kg) in order to calculate the coefficient of endothelial dysfunction (CED) and to assess the severity of endothelial dysfunction.

The registration and processing of the results of Laser Doppler Flowmetry with functional vascular sampling was performed using AcqKnowledge software versions 3.8.1-4.2.0. Microcirculation values are expressed in perfusion units (PU).

To assess changes in bone tissue against the background of experimental osteoporosis and its pharmacotherapy with the test drugs and their combinations, a histological examination of bone tissue in the proximal metaphysis of the femur was performed.

After the animals were removed from the experiment, hematoxylin-eosin (HE) stained histological specimens were made from their femurs. The histological preparations were studied using light microscopy (Leica CME microscope, magnification x100: objective lens x10, eyepiece x10) with photofixation.

To study bone microarchitecture, the previously calibrated ImageJ software, versions 1.39-1.43, was used. The width of the bone trabeculae, expressed in micrometers, was used as a quantitative measure to assess the development of osteoporosis and the effecacy of its medical correction.

All the animals were divided into groups through stratified randomization according to their body weight, husbandry and nutrition conditions, as well as to operations and manipulations performed. During the experiment, the animals were kept in a standard experimental biologically clean room, the air temperature was 22-2)° C, illumination – the light/dark cycle (12 h/12 h), all the rats received granulated feed and filtered water.

In this paper, all the experimental data obtained were analyzed using descriptive statistics (Microsoft Excel analysis package). For group indicators, mean values (M) and error of mean (m) were determined, data in the text and tables are presented as M±m. The analysis of statistically significant differences in intergroup comparisons was performed by the heteroscedastic t-test. When analysing a large number of comparisons, a Student-Newman-Keuls test was used.

Results and Discussion

In accordance with the design of the experiment, eight weeks after the operation of bilateral ovariectomy in female Wistar rats, therapy was started with a reference drug, strontium ranelate (Bivalos) at a dose of 171 mg/kg. This animals received this preparation in a 1% starch solution once a day intragastrically for four weeks. Bivalos is the most common and frequently prescribed drug for the prevention and pharmacological correction of osteoporosis.

Based on the data obtained, it was found that the chosen drug had no effect on the average level of bone perfusion in the proximal metaphyseal zone of the rat femur.

The LDF values in the group of animals treated with Bivalos were statistically significantly different from those in the group of sham-operated animals, but were not statistically significantly different from those in the group of rats with osteoporosis.

Determination of the coefficient of endothelial dysfunction in the rats treated with Bivalos made it possible to confirm that this drug had no endotheliotropic activity.

Microscopic examination of the sections in the studied site of the rats treated with Bivalos pharmacotherapy revealed the absence of microfractures of trabeculae, clearer contours of the bone trabecular network and wider bone trabeculae than in the animals with an osteoporosis model.

In the group of the rats treated with the test drug, it was found and confirmed that there was a direct relationship between the level of microcirculation in the bone tissue of the proximal femur metaphysis and the average width of the bone trabeculae in the studied site. The data obtained are shown in Table 1.

In accordance with the experimental protocol, eight weeks after the operation of bilateral oophorectomy, the female rats were administered rosuvastastin at a dose of 0.86 mg/kg intragastrically once a day in form of a suspension in 1% starch solution for four weeks.

After 12 weeks, the group of animals to which this drug was injected, the indicators of bone perfusion in the cancellous bone tissue of the proximal part of the right femur were recorded. Rosuvastatin at the studied dosage was found to prevent a decrease in the level of regional microcirculatory blood flow in the studied area, keeping

Table 1. Dynamics of Indicators of Microcirculation, CED and the Width of Bone Trabeculae on the Model of Experimental Osteoporosis and Its Correction with Bivalos at a Dose of 171 mg/kg

Group of animals	Microcirculation value, (PU)	CED	Width of the bone trabeculae, (μm)
Control (n=20)	99.91±3.41	1.28±0.18	96.64±1.01
Osteoporosis (n=20)	58.75±3.76*	2.57±0.23*	64.61±0.54*
OP+Bivalos (n=20)	67.48±2.98	2.44±0.19	80.19±0.95**

Note: * – p<0.05 compared to control; ** – p<0.05 compared to the pathology group

it at the level of the control rats – 81.88±3.39 PU. Rosuvastatin at a dose of 0.86 mg/kg increased by 39.4% the level of regional microcirculation in the bone tissue of the proximal femur, compared with the parameters in the animals with a model of hypoestrogenic osteoporosis.

Microscopy data from the proximal metaphyseal femurs of the animals that had received rosuvastatin pharmacotherapy showed a preserved microarchitecture and wider bone trabeculae than in the rats with experimental osteoporosis. The value of the average width of the bone trabeculae exceeded by 16.6% the same indicator in the untreated rats with an osteoporosis model and amounted to 75.31±0.97 μm (Table 2).

In accordance with the study protocol, L-norvaline at a dose of 10 mg/kg was administered intragastrically daily as a suspension in 1% starch solution for four weeks, eight weeks after bilateral oophorectomy.

After 12 weeks, the microcirculation indicators in the cancellous bone of the proximal right femur were recorded. It was established that L-norvaline at the studied dosage prevented a decrease in the level of regional microcirculation in the studied area while maintaining the parameters of bone perfusion at the level of the control group of animals – 92.46±2.29 PU. Thus, it was reliably established that L-norvaline at a dose of 10 mg/kg increased the level of microcirculation in the bone tissue of the studied site by 52.4% compared with the indicators in the animals with a model of hypoestrogenic osteoporosis.

The functional vascular test for EDVD and ENVD and the calculation of the coefficient of endothelial dysfunction in the animals treated with L-norvaline against the background of osteoporosis showed that L-norvaline brought CED values closer to the values of the control group of animals, reducing it to 1.64±0.21.

A morphometric study found that L-norvaline in the studied dosage prevented a decrease in the average width of bone trabeculae to the level of the animals with experimental osteoporosis. However, the average width of trabeculae did not reach the values of the control rats. This indicator was 81.24±0.68 μm, which was 25.7% higher than that in the rats with an osteoporosis model. Thus, twelve weeks after modeling osteoporosis in the rats, L-norvaline at a dose of 10 mg/kg had an osteoprotective effect of improving bone microarchitecture and preventing the thinning of bone trabecula (Table 3).

According to the experimental protocol, eight weeks after the operation of bilateral ovariectomy, the female rats were administered rosuvastatin at a dose of 0.86 mg/kg and L-norvaline at a dose of 10 mg/kg intragastrically once a day in form of a suspension of 1% starch solution for four weeks.

An assessment of intraosseous microcirculation in the trochanteric region of the femur showed that rosuvastatin in combination with L-norvaline (at the studied dosages) increased the level of bone perfusion of the proximal femur metaphysis against the background of experimental osteoporosis. The average value of the microcirculation

Table 2. Dynamics of Indicators of Microcirculation, CED and the Width of the Bbone Trabeculae on the Model of Experimental Osteoporosis and Its Correction with Rosuvastatin at a dose of 0.86 mg/kg compared with Bivalos

Group of animals	Microcirculation value, (PU)	CED	Width of the bone trabeculae, (μm)
Control (n=20)	99.91±3.41	1.28±0.18	96.64±1.01
Osteoporosis (n=20)	58.75±3.76*	2.57±0.23*	64.61±0.54*
OP+Bivalos (n=20)	67.48±2.98	2.44±0.19	80.19±0.95**
OP+ Rosuvastatin (n=20)	81.88±3.39**	1.72±0.18**	75.31±0.97**

Note: * – p<0.05 compared to control; ** – p<0.05 compared to the pathology group

Table 3. Dynamics of Indicators of Microcirculation, CED and the Width of Bone Trabeculae on the Model of Experimental Osteoporosis and Its Correction with L-norvaline at a Dose of 10 mg/kg Compared with Bivalos

Group of animals	Microcirculation value, (PU)	CED	Width of the bone trabeculae, (μm)
Control (n=20)	99.91±3.41	1.28±0.18	96.64±1.01
Osteoporosis (n=20)	58.75±3.76*	2.57±0.23*	64.61±0.54*
OP+Bivalos (n=20)	67.48±2.98	2.44±0.19	80.19±0.95**
OP+ L-norvaline (n=20)	92.46±2.29**	1.64±0.21**	81.24±0.68**

Note: * – $p<0.05$ compared to control; ** – $p<0.05$ compared to the pathology group

level was 88.02±3.03 PU, which was 49.8% higher than in the animals with experimental osteoporosis without pharmacological correction.

After measuring the values of intraosseous perfusion, the coefficient of endothelial dysfunction was calculated. It was found that the combination of rosuvastatin and L-norvaline approximated the proportions between the areas of the triangles above the recovery curves of the bone microcirculation level when performing functional vascular tests to such in the control animals. Thus, the combination therapy with rosuvastatin and L-norvaline statistically significantly reduced CED to the values of 1.68±0.25. The indicators of CED in the combination pharmacotherapy with the test drugs were statistically significantly different from those in the group of animals with osteoporosis without treatment, as well as in the rats receiving the reference drug Bivalos ($p<0.05$).

Rosuvastatin at a dose of 0.86 mg/kg in combination with L-norvaline at a dose of 10 mg/kg prevented a decrease in the average width of bone trabeculae to the level of the animals with experimental osteoporosis. However, the average width of the trabeculae did not reach the values obtained in the sham-operated rats. This indicator was 30% higher than in the animals with osteoporosis without ongoing treatment, and amounted to 84.02±0.89 μm (Table 4).

According to the design of the experiment, eight weeks after bilateral removal of the ovaries in the female rats, osteoporotic fractures of the right femur were simulated followed by its osteosynthesis.

According to the study protocol, eight weeks after the removal of the ovaries, two groups of rats were formed in order to simulate an osteoporotic fracture of the femur and its subsequent osteosynthesis, as well as the start of monotherapy with the test L-norvaline at a dose of 10 mg/kg and the reference drug Bivalos at a dose of 171 mg/kg These drugs were administered daily intragastrically for four weeks after modeling a fracture of the proximal femur metaphysis with its subsequent osteosynthesis. Four weeks later, the level of bone perfusion in the callus of the consolidated fracture, a visual and radiological analysis of the quality and number of bone healings, as well as morphometric determination of the width of the bone trabeculae were performed.

L-norvaline at a dose of 10 mg/kg had a positive effect on the microcirculation indicators in the conditions of experimental osteoporotic fractures of the proximal femur

Table 4. Dynamics of Indicators of Microcirculation, CED and the Width of Bone Trabeculae on the Model of Experimental Osteoporosis and Its Correction with a Combination of L-norvaline at a Dose of 10 mg/kg and Rosuvastatin at a Dose of 0.86 mg/kg Compared with Bivalos

Group of animals	Microcirculation value, (PU)	CED	Width of the bone trabeculae, (μm)
Control (n=20)	99.91±3.41	1.28±0.18	96.64±1.01
Osteoporosis (n=20)	58.75±3.76*	2.57±0.23*	64.61±0.54*
OP+Bivalos (n=20)	67.48±2.98	2.44±0.19	80.19±0.95**
OP+ L- norvaline + Rosuvastatin (n=20)	88.02±3.03**	1.68±0.25**	84.02±0.89**

Note: * – $p<0.05$ compared to control; ** – $p<0.05$ compared to the pathology group

metaphysis, unlike the reference drug Bivalos, which did not have such an effect.

In the animals treated with L-norvaline at a dose of 10 mg/kg, the average width of bone trabeculae was higher compared to the animals with osteoporotic femoral fractures (not receiving pharmacotherapy) which had received Bivalos, and exceeded the similar indicator in the rats with femoral fractures without osteoporosis.

An X-ray and visual evaluation of the results of osteoporotic fracture healing revealed that, against the background of administering Bivalos and L-norvaline to the rats for four weeks after modeling and osteosynthesis of osteoporotic fractures,, there were no unsatisfactory results – consolidation of osteoporotic fractures was observed in 100% of cases.

According to the experimental design, a group of rats received a daily monotherapy with rosuvastatin at a dose of 0.86 mg/kg for four weeks after modeling and osteosynthesis of experimental osteoporotic fractures of the proximal metaphyseal part of the femur.

Rosuvastatin at a dose of 0.86 mg/kg positively influenced the level of bone perfusion in the callus of experimental fractures of the proximal metaphyseal region of the femur compared with the animals treated with Bivalos.

In addition, the microcirculation value in the group of animals treated with a rosuvastatin monotherapy was higher than that in the group of control animals with experimental fractures without osteoporosis, but it was slightly lower than the microcirculation values in the group of the rats treated with L-norvaline.

In the animals treated with a rosuvastatin monotherapy at a dose of 0.86 mg/kg, the average width of bone trabeculae was higher than in the group of animals with fractures due to osteoporosis and receiving a reference drug Bivalos, but did not exceed such in the animals from the control group. It can be noted that the width of bone trabeculae in the group of rats treated with a rosuvastatin monotherapy was slightly lower than in the animals treated with a L-norvaline monotherapy.

The visual and X-ray monitoring of the results of consolidation of experimental osteoporotic fractures showed that, with a rosuvastatin monotherapy at a dose of 0.86 mg/kg for four weeks after modeling and osteosynthesis of experimental osteoporotic fractures, no unsatisfactory bone healing results were observed: consolidation of osteoporotic fractures was recorded in 100% of cases.

The design of the experiment also provided for the formation of a group of rats receiving combined L-norvaline pharmacotherapy at a dose of 10 mg/kg and rosuvastatin at a dose of 0.86 mg/kg for four weeks from the moment of modeling and osteosynthesis of osteoporotic fractures of the proximal metaphysis of the femur.

The combined pharmacotherapy with rosuvastatin at a dose of 0.86 mg/kg and L-norvaline at a dose of 10 mg/kg had a positive effect on the level of perfusion in an interfragmental callus of osteoporotic fractures of the proximal femur metaphysis, compared with the animals treated with Bivalos.

In the animals treated with a combination pharmacotherapy with L-norvaline at a dose of 10 mg/kg and rosuvastatin at a dose of 0.86 mg/kg, the average width of bone trabeculae was higher than in the group of rats without pharmacotherapy, and also in the group treated with Bivalos, and was as close as possible to a similar indicator in the rats with femoral fractures without osteoporosis. The width of bone trabeculae on the background of the combined pharmacotherapy was higher than that in the animals treated with L-norvaline and rosuvastatin as a monotherapy.

A visual and X-ray evaluation of the results of consolidation of experimental osteoporotic fractures showed that the combination pharmacotherapy with rosuvastatin at a dose of 0.86 mg/kg and L-norvaline at a dose of 10 mg/kg for four weeks after modeling and osteosynthesis of experimental osteoporotic fractures had no unsatisfactory bone healing results: consolidation of osteoporotic fractures was observed in 100% of cases. The results of this experimental study are shown in Table 5.

Table 5. Dynamics of Indicators of Microcirculation, Width of Bone Trabeculae and the Number of Healed Fractures on the Model of Experimental Osteoporotic Fractures and Their Correction with L-norvaline at a Dose of 10 mg/kg, Rosuvastatin at a Dose of 0.86 mg/kg, and Their Combination Compared to Bivalos

Group	Indicators under study			
	Microcirculation value, (PU)	Width of the bone trabeculae, (μm)	Number of healed fractures	Number of unhealed fractures
Control	89.30±4.75	92.93±1.57	15	5
Osteoporosis	66.59±3.61*	59.13±1.65*	11	9
Bivalos	70.39±2.39	79.16±1.43**	20	0
Rosuvastatin	94.34±2.54**	84.13±1.24**	20	0
L-norvaline	107.14±3.37**	86.25±1.31**	20	0
Rosuvastatin + L-norvaline	104.01±3.90**	90.43±1.48**	20	0

Note: * – $p<0.05$ compared to control; ** – $p<0.05$ compared to the pathology group

Conclusion

The proper microcirculatory bed and its condition are a key condition for maintaining bone homeostasis. The deterioration of the blood supply to the bone tissue leads to the development of such pathological conditions of the musculoskeletal system as: osteonecrosis, osteomyelitis (Curban et al. 1993) and osteoporosis (Coffin et al. 1995). The processes of neoangiogenesis and the normal blood supply to the bone fragments in the osteoreparation process play a key role. A trauma to the musculoskeletal system leads to inevitable damage to the supply vascular network and, consequently, to tissue hypoxia, which causes poor fracture consolidation (Kloen et al. 2003).

The vascular endothelium performs the main regulatory function, provides a link with other layers of the vascular wall, responding to their needs by isolating mediators (Madeddu 2005). Thus, the endothelial layer of blood vessels in the bone tissue is an integral part of the bone and is responsible for the level of regional microcirculation.

This fact has been confirmed by a number of studies claiming, for example, that VEGF (the key regulator of the cascade of events leading to the formation and development of the vascular system) plays a key role in the remodeling and repair of bone damage (Gerstenfeld et al. 2003). Inhibition of VEGF leads to an increase in the width of the tibial and femoral growth zones (Faitelson et al. 2010), slower angiogenesis processes in the growth plates, depletion of the vascular network of the metaphyseal zone, as well as deterioration of the formation of trabecular bone structure and resorption of cancellous bone tissue (Meury et al. 2006). Inhibition of VEGF against the background of a femoral fracture in mice leads to a decrease in blood vessel invasion, callus mineralization and impaired bone trabeculae remodeling (Gerstenfeld et al. 2003; Meury et al. 2006). Stimulation of the endosteal circulation in the damaged bone, with skeletal bone fractures (Nikolova and Strilic 2006) allows mesenchymal cells to penetrate into the damaged area (Madeddu 2005) and differentiate themselves into osteoblastic cells with the subsequent formation of cancellous bone.

The processes of remodeling and reparative regeneration of bone tissue are directly dependent on the quality of functioning of the vascular endothelium, the purpose of which is to regulate the level of regional microcirculation in bone tissue.

Endothelial dysfunction – an imbalance of oppositely acting processes: vasorelaxing and vasoconstricting, procoagulant and anticoagulant, growth factors and their inhibitors – is the cause of disorders of the blood supply to the bone tissue, and, therefore, disorders of remodeling and repair of bone damage.

The drugs with endothelioprotective properties are currently being actively srudied. So far, there are no means for the specific correction of endothelial dysfunction, and therefore the search is under way in various groups of drugs. The representatives of statins – rosuvastatin and arginase inhibitor L-norvaline – as well as their combined use – seem to be the most worthwhile.

One of the possible mechanisms of the endotheliprotective effect of rosuvastatin, L-norvaline and their combination is an increase in production and accumulation of NO.

Thus, rosuvastatin and L-norvaline, and their combination, have an endothelioprotective effect on the bone tissue vessels, effectively prevent a decrease in the regional blood flow in the bone tissue against the background of osteoporosis and experimental osteoporotic fractures, and, therefore, have an osteoprotective effect, consisting in the positive influence of the test drugs on bone remodeling and osteoreparation processes.

The range of the pleiotropic action of drugs with endothelial-protective properties can be extended by adding an osteoprotective element, which, however, requires additional research.

Findings

1. On the model of osteoporosis caused by bilateral oophorectomy, it was established that L-norvaline at a dose of 10 mg/kg had an endothelium protective effect, reducing the coefficient of endothelial dysfunction to 1.64±0.21, prevents a decrease in blood flow indicators in the femoral bone tissue (92.46±2.29 PU), and also increases the width of the bone trabeculae to 81.24±0.68 μm and prevents trabecula microfractures, proving its osteoprotective effect.
2. On the model of osteoporosis caused by bilateral oophorectomy, it was established that rosuvastatin at a dose of 0.86 mg/kg had an endothelioprotective effect, reducing the coefficient of endothelial dysfunction to 1.72±0.18, prevents a decrease in blood flow indicators in the femoral bone tissue (81.88±3.39 PU), and also increases the width of the bone trabeculae to 75.31±0.97 μm and prevents trabecula microfractures, proving its osteoprotective effect.
3. On the model of osteoporosis caused by bilateral oophorectomy, it was established that the combined use of L-norvaline at a dose of 10 mg/kg and rosuvastatin at a dose of 0.86 mg/kg had an endothelium protective effect, reducing the endothelial dysfunction coefficient to 1.68±0.25, preventing a decrease indicators of blood flow in the bone tissue of the femur (88.02±3.03 PU), and also increases the width of the bone trabeculae to 84.02±0.89 μm and prevents trabecula microfractures, proving its osteoprotective effect.
4. On the model of experimental osteoporotic fractures, it was established that L-norvaline at a dose of 10 mg/kg increased the microcirculation indicators in the fracture zone of the proximal femoral metaphysis to 107.14±3.37 PU, increases the width of bone trabeculae to 86.25±1.31 μm and contributes (in 100% of cases) to the consolidation of fractures, thereby proving its

osteoprotective effect.

5. On the model of experimental osteoporotic fractures, it was established that rosuvastatin at a dose of 0.86 mg/kg increases the microcirculation indicators in the zone of fracture of the proximal metaphysis of the femur to 94.34±2.54 PU, increases the width of bone trabeculae to 84.13±1.24 μm and contributes (in 100% of cases) to the consolidation of fractures, thereby proving its osteoprotective effect.
6. On the model of experimental osteoporotic fractures, it was found that the combination of L-norvaline at a dose of 10 mg/kg and rosuvastatin at a dose of 0.86 mg/kg increases the microcirculation indicators in the zone of fracture of the proximal femoral metaphysis to 104.01±3.90 PU, increases the width of bone trabeculae up to 90.43±1.48 μm and contributes (in 100% of cases) to the consolidation of fractures, thereby proving its osteoprotective effect.

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Author Contributors

- **Mikhail S. Sobolev,** post-graduate student, Department of Traumatology and Orthopedics, Kursk State Medical University, Kursk, Russia, e-mail: mixon_86@mail.ru, ORCID ID 0000-0001-7839-2049. The author defined the purpose and objectives of the study, conducted research on animals, was engaged in obtaining and interpreting the primary results of the study.

- **Alexander V. Faitelson,** Doctor of Medical Sciences, Associate Professor, Professor of the Department of Traumatology and Orthopedics, Kursk State Medical University, Kursk, Russia, e-mail: vladimirfaitelson@gmail.com, ORCID ID 0000-0003-3759-6373. The author was engaged in the choice of the optimal model of pathology and the assessment of the dynamics of the development of a reproducible pathological condition, including electrophysiological and histomorphometric methods of research.

- **Densingh Samuel Raj Rajkumar,** Candidate of Medical Sciences, Teaching Assistant,Department of Traumatology and Orthopedics, Kursk State Medical University, Kursk, Russia, e-mail: densingh7@gmail.com, ORCID ID 0000-0003-2868-0776. The author conducted research on animals, was engaged in statistical processing of the primary results of the study.