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STUDY OF ANTI-OSTEOPOROTIC ACTION OF DRUGS FROM THE GROUP OF STATINS

Oleg S. Gudyrev[1*], Alexander V. Faitelson[2], Mikhail V. Pokrovskii[1], Tatyana G. Pokrovskaya[1], Mikhail V. Korokin[1], Alexander P. Grigorenko[1], Olga A. Efremova[1]

[1]Belgorod State University, 85, Pobedy St., Belgorod, 308015, Russia

[2]Kursk State Medical University, 3, K. Marx St., Kursk, 305041, Russia

Abstract:

In an experiment on female Wistar white rats, the osteoprotective effect of atorvastatin, simvastatin and rosuvastatin was studied in a model of experimental osteoporosis caused by bilateral ovariectomy. It was found that after ovariectomy in female rats develops endothelial dysfunction, including the vessels of the microcirculatory of the bone, leading to a deterioration of blood supply to bone tissue and the occurrence of osteoporosis. It was found that atorvastatin, simvastatin and rosuvastatin, possessing endothelioprotective activity, prevent deterioration of blood supply to bone tissue and thinning of bone trabeculae, thus having anti-osteoporotic activity.

Key words: *osteoporosis, endothelial dysfunction, statins, atorvastatin, simvastatin, rosuvastatin, strontium ranelate.*

Corresponding author:

Oleg S. Gudyrev,
Department Of Pharmacology And Pharmaceutical Sciences,
Belgorod State University, 85,
Pobedy St., Belgorod, 308015,
Russia
e-mail: gudyrev@bsu.edu.ru

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INTRODUCTION:

The reason for the development of osteoporotic bone changes is the misalignment of the main processes of osteogenesis – bone resorption and remodeling. If regional blood supply to the bone is broken, the number of osteoblasts decreases and their activity slows down, while osteoclasts activate their activity. Consequently, the blood supply plays a key role in the processes of remodeling and reparative regeneration of bone tissue. The structure of the bone microvessels themselves differs significantly from the vessels of other tissues, they have only the endothelial layer, through which all the regulation of metabolic processes between osteoclasts, osteoblasts and blood take place. Endothelial dysfunction and endothelium-associated pathologies are most often the main cause of worsening of microcirculatory blood flow in bone tissue, which in turn leads to a violation of osteogenesis, thereby causing osteoporosis [1, 2, 3].

As is known, in modern pharmacology, there are two basic ways of development. The first is the search for innovative molecules [4, 5], their thorough in-depth study [6, 7], including preclinical [8-12] and clinical studies [13], and expensive outcome to the pharmaceutical market. The second is the expansion of indications for the use of medicines already used in medical practice due to the discovery of pleiotropic effects [14].

Previous studies have demonstrated the positive osteoprotective effects of such drugs with endotheliotropic properties like enalapril, losartan, resveratrol, L-arginine and others. Along with these drugs, endothelioprotective properties are also possessed by statins, under the influence of which the normalization of the course of angiogenesis leading to a slowdown in the development of osteoporosis [3]. At the same time, in the available literature there is no information that anyone should evaluate the osteoprotective properties of these drugs, which indicates the relevance and determines the purpose of this study.

MATERIALS AND METHODS:

The experiments were carried out on 152 females of white Wistar rats weighing 250±50 g. To model experimental osteoporosis, rats were anesthetized with intraperitoneal administration of a solution of chloral hydrate at a dose of 300 mg/kg and bilateral ovariectomy was made. The development of osteoporosis and anti-osteoporotic action of the studied drugs was evaluated after eight weeks (at day 57) after ovariectomy by evaluating regional microcirculation, vascular tests and histomorphometric examination.

The level of microcirculation was evaluated in the spongy bone of the proximal metaphysis of the right femur. To obtain microcirculation data in the bone, Biopac systems equipment was used: polygraph MP100-150 with LDF100C laser doppler flowmetry (LDF) module and a TSD144 sensor. LDF results were recorded by AcqKnowledge ver. 3.8.-4.2., microcirculation values were expressed in perfusion units (PU).

Development of hypoestrogeninduced endothelial dysfunction was evaluated after measurement of intraosseous level of microcirculation, for which was carried vascular assays on endothelium-dependent vasodilation in response to bolus intravenous injection of a solution of acetylcholine in a dose of 40 μg/kg and endothelium-independent vasodilation in response to bolus administration of sodium nitroprusside in a dose of 30 μg/kg with subsequent calculation of the coefficient of endothelial dysfunction (CED), as the ratio of the area of the triangle above the microcirculation recovery curve in response to the introduction of nitroprusside to the area of the triangle above the microcirculation recovery curve in response to the introduction of acetylcholine.

To confirm the development of osteoporosis and to evaluate the efficacy of the studied drugs, a morphological study of the proximal metaphysis of the femurs was carried out, for this, slide glasses with histological preparations were subjected to light microscopy. For carrying out histomorphometry of bone tissue, a pre-calibrated program ImageJ ver. 1.39-1.43 was used, in which the width of bone trabeculae was measured and expressed in micrometers.

For the study of osteoprotective action, we selected representatives of the group of statins, namely atorvastatin, simvastatin and rosuvastatin at a dose of 0.86 mg/kg. As a comparator, a rather widespread drug on the pharmaceutical market and an effective drug for prevention and correction of osteoporotic disorders was selected – strontium ranelate at a dose of 171 mg/kg. The test preparations were administered to the animals intragastrically daily once a day for eight weeks after ovariectomy as a suspension in 1% starch paste. Animals with experimental osteoporosis received intragastrically 1% starch paste as a placebo. The control rats included false-operated animals (false ovariectomy without ovarian excision), which for eight weeks were also given as placebo intragastrically 1% starch paste.

The experimental data obtained in the work were subjected to analysis using descriptive statistics (Microslot Excel analysis package). The group indices determined the mean values (M) and the error

of the mean (m). An analysis of statistically significant differences in intergroup comparisons was carried out using a heteroscedastic t-test (a two-sample Student t-test with different variances). When analyzing a large number of comparisons, Student's criterion was used with the Newman-Keils correction.

RESULTS:

On day 57 after bilateral ovariectomy, the level of microcirculation in the proximal metaphysis of the right femur was evaluated. Results of the study of blood supply to bone tissue in rats allowed to identify reliably ($p<0.001$) a lower level of microcirculation in the bone tissue of the femur in rats with osteoporosis (n=30) – 61.52±3.74 PU, compared to control animals (n=42) – 100.51±4.41 PU.

After measuring the microcirculation in the bone tissue of the femur functional vascular tests of endothelium-dependent and endothelium-independent vasodilation were performed, and the coefficient of endothelial dysfunction was calculated for the microcirculatory unit of the proximal metaphysis of the femur in rats. Thus, in the group of control animals, CED =1.30±0.19, in the group of rats with experimental osteoporosis CED=2.38±0.23 ($p=0.002$), which indicates the development of endothelial dysfunction in animals with osteoporosis.

For further morphological studies, bone biomaterial was sampled. Histological sections of the proximal parts of the femurs were subjected to microscopy and histomorphometry. Osteoporotic changes in the bones of the skeleton were histologically confirmed in all rats eight weeks after ovariectomy. During the microscopy, pathological changes in spongy bone tissue of the femur in rats with experimental osteoporosis were identified. Thinning of the latticed network of bone trabeculae, as well as thinning and perforation of bone plates were found. In individual histological specimens, micro-fractures of bone trabeculae were determined.

Identified a significant decrease in the average width of bone trabeculae in the spongy tissue of the proximal metaphysis of the femur. Thus, the average width of bone trabeculae in a given location in rats with osteoporosis was 61.68±1.24 μm, that is reliably ($p<0.001$) less than that in control animals – 97.69±1.02 μm.

Thus, endothelial dysfunction developing in female rats due to ovariectomy, including the microcirculatory of bone tissue, leads to a marked worsening of the regional blood flow, which in turn leads to unbalance of bone remodeling processes and the appearance of osteoporotic changes in bone tissue.

It was found that the study drugs - the members of the statin group, as well as the comparison preparation strontium ranelate prevented a decrease in the level of regional blood flow in the bone tissue of the femur in rats with osteoporosis (Figure 1).

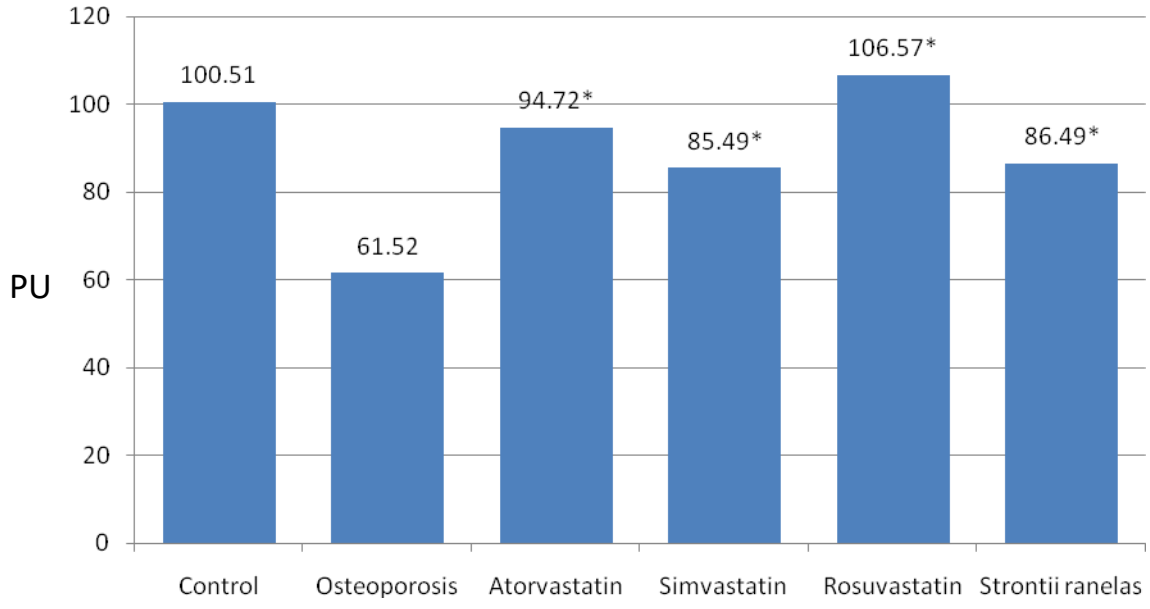

Fig 1: Results of the effects of atorvastatin, simvastatin, rosuvastatin and strontium ranelate on the blood supply of the proximal metaphysis of the femur 8 weeks after bilateral ovariectomy. * – p<0.05 in comparison with animals with osteoporosis.

LDF results in the group of rats treated with atorvastatin (n=20) significantly (p<0.001) were greater than those in the group of rats with osteoporosis without treatment, but statistically significantly did not differ from the indices in the comparison drug group (p=0.432) (n=20).

Results of LDF in animals treated with simvastatin (n=20) also significantly (p<0.001) exceeded the LDF values in the group of rats with osteoporosis without treatment and did not differ significantly (p=0.892) from the results of LDF in animals treated with strontium ranelate.

The LDL results in the group of rats treated with rosuvastatin (n=20) were the highest of all studied drugs and significantly exceeded both the indices of the group of rats with osteoporosis without treatment (p<0.001) and those in the comparison drug group (p=0.008), and were also comparable to those of control animals (p=0.412).

At the same time, all studied statins possessed endothelioprotective activity, significantly preventing an increase in the coefficient of endothelial dysfunction. In rats treated with atorvastatin CED=1.39±0.17 (p=0.015), simvastatin – CED=1.61±0.10 (p=0.026), rosuvastatin – CED=1.35±0.12 (p=0.017). The preparation of comparison of strontium ranelate did not authentically possess endothelioprotective activity, CED=2.14±0.11 (p=0.532).

When microscopic sections of the femur bones in rats treated with statins found the preservation of the bone structure of the proximal metaphysis of the femur. When morphometric studies were carried out, it was noted the prevention of a decrease in the average width of bone trabeculae in the proximal metaphysis of the femur in laboratory animals under the influence of all studied drugs, as well as the reference preparation (Figure 2). Rosuvastatin had the most pronounced anti-osteoporotic activity amongst statins.

CONCLUSION:

The endothelial layer of the intraosseous vessels is an integral part of the bone, plays a central regulatory role, possessing significant metabolic activity, while performing various functional actions, which include: regulation of leukocyte adhesion, regulation of vascular growth, thrombogenicity of vascular wall and immune functions. Endothelial cells themselves, producing a variety of biologically active substances, participate in the regulation of the vascular tone.

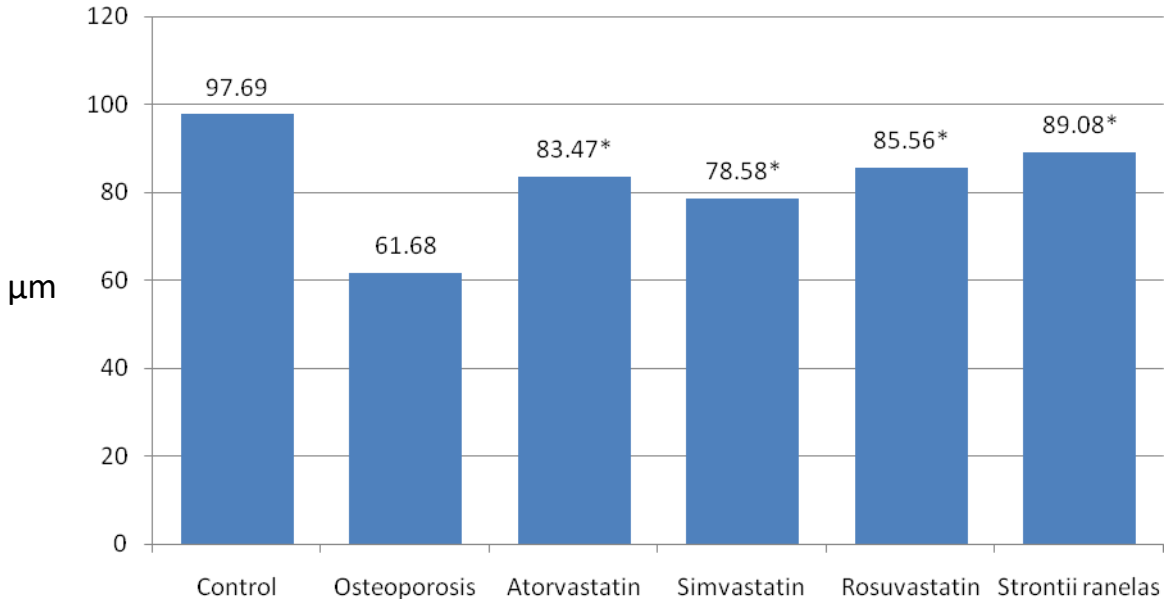

Fig 2: Results of the effects of atorvastatin, simvastatin, rosuvastatin and strontium ranelate on the average width of the bone trabeculae of the proximal metaphysis of the femur 8 weeks after bilateral ovariectomy. * – p<0.05 in comparison with animals with osteoporosis.

Among a significant number of mediators that are produced by the endothelial layer, vasoconstrictors are: angiotensin II, endothelin I and vasodilators are: nitric oxide (NO), endothelial hyperpolarizing factor, prostacyclin. Endothelial dysfunction is an imbalance between vasodilating and vasoconstrictor mediators, which is characterized by a decrease in vasodilator production with activation of vasoconstrictor synthesis. The main vasodilator mediator is nitric oxide, which as a result of biosynthesis is produced from the amino acid L-arginine with the participation of the enzyme endothelial nitric oxide synthase (NO-synthase). The metabolism of L-arginine in cells proceeds along two routes: 1st – under the influence of arginase L-arginine hydrolyzes into ornithine and urea; 2nd – the conversion of L-arginine to nitric oxide and citrulline – is catalyzed by NO-synthase [15, 16]. In this case, the enzymes arginase and NO-synthase compete for a common substrate – L-arginine. In a number of studies, an increase in the activity of arginase with the development of endothelial dysfunction was revealed. Also, arginase inhibits the activity of nitric oxide synthase, preventing the production of nitric oxide, and the decrease in the effect of arginase leads to an increase in the production of nitric oxide, which favorably affects the normalization of vascular function.

The pharmacological effects of statins are based on the inhibition of HMG-CoA reductase, the mechanism of this effect consists in the agonism to the reductase of HMG-CoA, which leads to a decrease in the concentration of low-density lipids and triglycerides and, as a consequence, to a decrease in cholesterol, but the effect of statins on endothelial function is more extensive than just lowering cholesterol. It has been experimentally proved that some drugs are capable of stimulating the transcription of the gene NO-synthase in human endotheliocytes, which leads to an improvement in the expression of NO-synthase, the result is an increase in the secretion of nitric oxide in the endothelium. Moreover, the use of modern statins, including rosuvastatin, positively affects the elastic properties of the vessel wall, reduces endothelial dysfunction, increasing the functional activity of endotheliocytes. Stimulation of NO production in the endothelium is characteristic of all statins and does not depend on their effect on the synthesis of cholesterol. These properties have a positive effect on the state of intraosseous microcirculation, thereby indirectly improving the trophism of bone tissue, including positively affecting osteoregeneration.

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