Study of the anti-osteoporotic effect of certain drugs with endothelial protective activity

Oleg S. Gudyrev*, Alexander V. Faitelson, Mikhail S. Sobolev, Mikhail V. Pokrovskii, Tatyana G. Pokrovskaya, Mikhail V. Korokin, Olga A. Efremova

INTRODUCTION

The reason for the development of osteoporosis is the misalignment of the main processes of osteogenesis - resorption and bone formation. When the regional blood supply to the bone is disturbed, the number of osteoblasts decreases and their activity is inhibited, while osteoclasts intensify their activity. Therefore, the blood supply plays a key role in the remodeling and reparative regeneration of bone tissue. The structure of the bone microvessels themselves is significantly different from the vessels of other tissues; they only have an endothelial layer through which all the regulation of metabolic processes between osteoclasts, osteoblasts, and blood are carried out. Endothelial dysfunction and endothelium-associated pathologies are most often the main cause of the deterioration of microcirculatory blood flow in the bone tissue, which in turn leads to disruption of osteogenesis, thereby causing osteoporosis.[1-3]

As is known, modern pharmacology has two main ways of development. The first is the search for innovative molecules,[4] their thorough and comprehensive study,[5] including preclinical[6] and clinical studies,[7] and the costly launch into the pharmaceutical market. The second is the expansion of indications for the use of drugs already used in medical practice due to the discovery of pleiotropic effects in them.

Previous studies have demonstrated the positive osteoprotective effects of such drugs with endotheliotropic properties such as enalapril, losartan, resveratrol, and other. Their effectiveness suggests that other endothelial protectors such as L-arginine, L-norvaline, and rosvastatin will also be effective in osteoporosis. Materials and Methods: In an experiment on female Wistar white rats, the osteoprotective effect of L-arginine, L-norvaline, and rosvastatin was studied on a model of experimental osteoporosis caused by bilateral ovariectomy. To confirm the development of osteoporosis and to evaluate the effectiveness of the studied drugs, a morphological study of the proximal femur metaphysis was carried out; for this purpose, glass slides with histological specimens were subjected to light microscopy. The bone microcirculation level was assessed in the cancellous bone tissue of the proximal metaphysis of the right femur. BIOPAC Systems equipment was used to obtain bone microcirculation data: Polygraph MP100-150 with laser Doppler flowmetry (LDF) module LDF100C and TSD144 sensor. Results: It was found that, after ovariectomy, female rats develop endothelial dysfunction, including vessels of the microvasculature of the bone bed, leading to deterioration of the blood supply to the bone tissue and the occurrence of osteoporosis. It was found that L-arginine, L-norvaline, and rosvastatin, possessing endothelial protective activity, prevent the deterioration of the blood supply to the bone tissue and the thinning of bone trabeculae, thus possessing anti-osteoporotic activity.

Conclusion: Endothelial protectors such as arginine, norvaline, and rosuvastatin have a pronounced ability to improve the morphofunctional state of the bone tissue during experimental osteoporosis in rats.

KEY WORDS: Endothelial dysfunction, L-arginine, L-norvaline, Osteoporosis, Rosuvastatin, Strontium ranelate
osteoporosis, also indicate endothelial protective properties, which indicate the relevance of studying their osteoprotective properties and determine 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 simulate experimental osteoporosis, rats were anesthetized by intraperitoneal administration of a solution of chloral hydrate at a dose of 300 mg/kg and an operation of bilateral ovariectomy was performed. The development of osteoporosis and anti-osteoporotic effect of the studied drugs was evaluated 8 weeks (on day 57) after ovariectomy by evaluating regional microcirculation, vascular tests, and histomorphometric research.

The microcirculation level was assessed in the cancellous bone tissue of the proximal metaphysis of the right femur. BIOPAC Systems equipment was used to obtain bone microcirculation data: Polygraph MP100-150 with laser Doppler flowmetry (LDF) module LDF100C and TSD144 sensor. Registration of LDF results was carried out by the AcqKnowledge program ver.3.8.-4.2., and microcirculation values were expressed in perfusion units (PUs).

The development of hypoestrogen-induced endothelial dysfunction was evaluated after measurement of intraosseous level of microcirculation. For it 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 effectiveness of the studied drugs, a morphological study of the proximal femur metaphysis was carried out; for this purpose, glass slides with histological specimens were subjected to light microscopy. For bone tissue histomorphometry, 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.

To study the osteoprotective action, we selected the following substances: L-arginine - L-isomer of the basic amino acid arginine, from which nitric oxide (NO) is produced with the participation of the enzyme endothelial NO-synthase, in a dose of 200 mg/kg; L-norvaline - an arginase inhibitor - an enzyme that catalyzes the splitting of arginine into ornithine and urea, at a dose of 10 mg/kg; and a representative of the group of statins - rosuvastatin, at a dose of 0.86 mg/kg. An effective drug for the prevention and correction of osteoporotic disorders was chosen as a reference drug - strontium ranelate at a dose of 171 mg/kg. The study drugs were administered to the animals intragastrically daily once a day for 8 weeks after oophorectomy as a suspension in 1% starch paste. Experimental osteoporosis animals received intragastrically 1% starch paste as a placebo. The group of control rats included sham-operated animals (sham ovariectomy without removing the ovaries), which also received 1% starch paste for 8 weeks as a placebo intragastrically.

Experimental data obtained in the work were analyzed using descriptive statistics (Microsoft Excel analysis package). For group indicators, mean values (M) and mean error (m) were determined. Analysis of statistically significant differences in intergroup comparisons was carried out according to the heteroscedastic t-test (two-sample Student’s t-test with different dispersions). When analyzing a large number of comparisons, Student’s criterion with the Newman–Keuls amendment was used.

RESULTS

On the 57th day after the operation of bilateral oophorectomy, the level of microcirculation was assessed in the proximal metaphysis of the right femur. The results of studying the blood supply to the bone tissue of rats revealed a significantly (P < 0.001) lower level of microcirculation in the bone tissue of the thigh in rats with osteoporosis (n = 30) –61.52 ± 3.74 PU, compared with control animals (n = 42) –100.51 ± 4.41 PU.

After measuring the microcirculation in the femoral bone tissue, functional vascular tests of endothelium-dependent and endothelium-independent vasodilation were performed, and the values of the CED for the microcirculatory link of the proximal metaphysis of the femur in rats were also calculated. So, in the group of control animals received CED =1.30 ± 0.19, in a group of rats with experimental osteoporosis CED =2.38 ± 0.23 (P = 0.002), which indicates the development in animals with osteoporosis of endothelial dysfunction.

For further morphological studies, bone material was collected. Histological sections of the proximal femur of animals were subjected to microscopy and histomorphometry. Osteoporotic changes in the bones of the skeleton were histologically confirmed in all rats 8 weeks after oophorectomy. During microscopy, pathological changes were found in the cancellous
bone of the thigh in rats with experimental osteoporosis and found thinning of the network of bone trabeculae, as well as thinning and perforation of bone plates. Microfractures of bone trabeculae were determined in individual histological specimens.

Revealed a significant decrease in the average width of the bone trabeculae in the spongy tissue of the proximal femur metaphysis. Thus, the average width of bone trabeculae in this localization in rats with osteoporosis was 61.68 ± 1.24 µ, which is significantly (P < 0.001) less than this indicator in control animals - 97.69 ± 1.02 µ.

Thus, endothelial dysfunction, including microcirculatory bed of bone tissue, developing in female rats as a result of oophorectomy leads to a noticeable deterioration of regional blood flow, which, in turn, leads to an imbalance of bone remodeling processes and osteoporotic changes in bone tissue.

It was found that the studied drugs, as well as the reference agent strontium ranelate, prevented a decrease in the level of regional blood flow in the bone tissue of the thigh in rats with osteoporosis [Figure 1].

The LDF results in the group of rats treated with L-arginine (n = 20) significantly exceeded those in the group of rats with osteoporosis without treatment (P < 0.001) and were not statistically significantly different from those in the comparison drug group (P = 0.091, n = 20) and control rats (P = 0.736).

The results of LDF in animals treated with L-norvaline (n = 20) also significantly (P < 0.001) exceeded the values of LDF in the group of rats with osteoporosis without treatment and animals treated with strontium ranelate (P < 0.001) but did not differ significantly from the control group (P = 0.051).

The LDF results in the group of rats treated with rosvustatin (n = 20) also significantly exceeded both the indices of the group of rats with osteoporosis without treatment (P < 0.001) and the indicators in the group of the comparator drug (P = 0.008) and were also comparable with control animals (P = 0.412).

At the same time, all the studied drugs had endothelial protective activity, reliably preventing an increase in the CED. In rats treated with L-arginine, CED = 1.34 ± 0.21 (P = 0.012), L-norvaline - CED = 1.37 ± 0.10 (P = 0.003), and rosvustatin - CED = 1.35 ± 0.12 (P = 0.017). The comparison drug strontium ranelate did not significantly have endothelial protective activity, CED = 2.14 ± 0.11 (P = 0.532).

Microscopic examination of sections of the femur in rats treated was found to preserve the structure of the bone tissue of the proximal metaphysis of the femur. When conducting morphometric studies, prevention of a decrease in the average width of bone trabeculae in the proximal femur metaphysis in laboratory animals under the influence of all the studied drugs, as well as the comparator drug, was noted [Figure 2]. Among the studied drugs, L-norvaline was the most pronounced anti-osteoporotic activity.

CONCLUSION

The endothelial layer of intraosseous vessels is an integral part of the bone, plays a central regulatory role, and has significant metabolic activity, while performing various functional actions, which include regulation of leukocyte adhesion, regulation of vascular growth, atrombogenicity (coefficient of thrombogenetic seam), and thrombogenicity of the vascular wall and immune functions. Endotheliocytes themselves, producing a variety of biologically active substances, are involved in the regulation of vascular tone.

Among the significant number of mediators that are produced by the endothelial layer, vasoconstrictors are distinguished such as angiotensin II and endothelium I and vasodilators such as NO, endothelial
hyperpolarizing factor, and prostacyclin. Endothelial dysfunction is an imbalance between vasodilators and vasoconstrictor mediators, which is characterized by a decrease in the production of vasodilators with the intensification of the synthesis of vasoconstrictors. The main vasodilator mediator is NO, which as a result of biosynthesis is produced from the amino acid L-arginine with the participation of the enzyme endothelial synthase NO (NO synthase). Metabolism of L-arginine in cells proceeds in two ways: First, under the influence of arginase, L-arginine is hydrolyzed to ornithine and urea; and second, the conversion of L-arginine to NO and citrulline is catalyzed by NO-synthase.\(^8\) At the same time, arginase and NO-synthase enzymes compete with each other for a common substrate - L-arginine. A number of studies have revealed an increase in arginase activity with the development of endothelial dysfunction. Furthermore, arginase inhibits the activity of NO synthase, prevents the production of NO, and reduces the effect of arginase leading to an increase in the production of NO, which has a positive effect on the normalization of vascular function.\(^9\)

The pharmacological effects of statins are based on the inhibition of HMG-CoA reductase, and the mechanism of this influence is agonism toward HMG-CoA reductase, which leads to a decrease in the concentration of low-density lipids and triglycerides and, consequently, to a decrease in cholesterol. But the effect of statins on function endothelium is more extensive than just lowering cholesterol. It was experimentally proved that some drugs are able to stimulate the transcription of the NO synthase gene in human endothelial cells, which leads to an improvement in the expression of NO synthase, the result of which is an increase in the secretion of NO in the endothelium. Moreover, the use of modern statins, which include rosuvastatin, has a positive effect on the elastic properties of the vascular wall, reduces the indices of endothelial dysfunction, and increases the functional activity of endothelial cells. Stimulation of NO production in the endothelium is characteristic of all statins and does not depend on their effect on cholesterol synthesis.\(^10\) These properties positively affect the state of intrasosseous microcirculation, thereby indirectly improving bone tropism, including a positive effect on osteoregeneration.

REFERENCES


Source of support: Nil; Conflict of interest: None Declared