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Anti-osteoporotic Action Research of Nanoparticulated Resveratrol and Losartan.

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ABSTRACT

The study demonstrated eight weeks after ovariectomy in female white Wistar rats endothelial dysfunction of bone microvasculature and deterioration of regional blood flow in bone develops, leading to the emergence of generalized osteoporosis. Nanoparticulated forms of losartan and resveratrol, possessing endothelium protective action, effectively prevents the reduction of regional microcirculation in bone tissue, keeping it at the level of intact rats. It is allowed to maintain an adequate level of bone remodeling processes, which manifested in slowing of the thinning of bone trabeculae and preventing the occurrence of their microfractures.

Keywords: osteoporosis, endothelial dysfunction, bivalos, losartan, resveratrol.

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INTRODUCTION

Osteoporosis (OP) - is a systemic skeletal disease characterized by low bone mass per unit volume and a destruction of microarchitectonics of bone tissue, leading to increased bone fragility and a high risk of fracture. The core of OP is an imbalance between the two main processes of bone remodelling : bone formation and bone resorption [1].

One of the essential links in the pathogenesis of OP is the deterioration of blood supply to the bone [10], leading to inhibition of the activity of osteoblasts, as well as the activation of osteoclasts. So, in the course of research, we have found the close relationship between the adequacy of blood supply to the bone and its quality, as expressed in the thickness of the trabecular bone and bone stability to external influences [8].

As is known, the structure of the bone microvasculature differs substantially from the morphology of the vascular bed of the remaining tissues. Bone microvessels have only endothelium, which mediates all humoral regulation of exchange between blood and bone cells. In the course of our research it was also shown that generalized hypoestrogen induced OP in female Wistar rats is accompanied by signs of endothelial dysfunction (ED), which leads to deterioration of regional blood flow and can lead to disruption of osteogenesis and osteoreparation causing OP.

In modern pathogenetic therapy of OP, has neglected medication's having endothelium protective and the positive impact on the blood supply to the bone. This indicates us the actuality of studying osteoprotective actions of drugs with proven positive endotheliotropic effects, such as, for example, Losartan, and Resveratrol. At the same time, the actual problems of modern experimental and clinical pharmacology are increasing efficiency and reducing the amount of side effects of the drug, which can be achieved by Nanoparticulated form of drug combined with reduced dosages therefore In this regards, the aim of this study was to investigate the antiosteoporotic properties of nanoparticulated form of Resveratrol and Losartan.

Objectives

The aim of the present study is to investigate the antiosteoporotic properties of nanoparticulated Resveratrol and nanoparticulated Losartan.

MATERIALS AND METHODS

Experiments were carried out on 267 white female Wistar rats weighing 250 ± 25 g. For modelling system OP in rats, they were anesthetized by intra-peritoneal injection of a solution of chloral hydrate at a dose of 300 mg / kg, and the operation was carried out bilateral ovariectomy [5]. The development of generalized OP was evaluated after eight weeks (57 days) after the operation.

Level of microcirculation was evaluated in the proximal metaphysis of the femur. For this, after fixing the animal on the table for surgical manipulation [7] on the femur a mono cortical hole was made by drilling to stabilize the sensor, that is used to measure microcirculation in bone, introduced pin-conductor [6]. For collecting data of microcirculation in bone was used equipment from companies Biopac systems: MP100 and MP150 polygraphs module laser Doppler flowmetry (LDF) LDF100C and sensor TSD144. Registration of LDF results performed using Acknowledge version 3.8.-4.2., The values expressed in microcirculatory perfusion units (PU).

Development of hypoestrogen induced ED was assessed after measuring the intraosseous microcirculation level, wherefore conducted tests on endothelium-dependent vasodilation (EDVD) in response to a bolus intravenous injection of a solution of acetylcholine at a dose of 40 micrograms / kg [16] and endothelium nondependent vasodilation (ENVD) in response to a bolus injection of a solution of sodium nitroprusside in a dose of 30 micrograms / kg [2]. For an objective assessment of endothelial dysfunction in generalized OP calculated coefficient of endothelial dysfunction (CED) on the basis of LDF in the bone [4, 8]. For confirmation of OP and comprehensive assessment of the effectiveness of the studied drugs and their combinations, we performed a histomorphological study of the proximal metaphysis of femoral bones. The

slides with histological preparations were subjected to light microscopy and photographed the trabeculae of bone by fixing the camera lens on the eyepiece of the microscope. For histomorphometry of bone tissue was used pre-calibrated program the ImageJ version 1.39-1.43, which measures the width of the bone trabeculae and is expressed in micrometers.

To study antiosteoporotic action we chose nanoparticulate form of preparations, endothelium and osteoprotective effects of which has been proven in the course of earlier investigations: Losartan and Resveratrol [9]. In the experiments Losartan (6 mg / kg) and its nanoparticulate form (further onwards-"N-Losartan" at a dose of 0.6 mg / kg) was administered intragastrically once a day every day for eight weeks after simulation osteoporosis. Resveratrol (2 mg / kg) and its nanoparticulated form (further onwards-"N-Resveratrol", 0.2 mg / kg) was administered intraperitoneally on the same lines. As a comparison, the drug in a dose Bivalos 171 mg / kg.

The control group was a group of animals with the experimental OP is not receiving pharmacological correction. The group of intact rats were false-operated animals (false ovariectomy operation without removal of the ovaries).

Statistical analysis of the data was carried out in the program Microsoft Excel. "Descriptive statistics" was used to calculate the average value (M) and error of the mean (m). "Two-Sample t-test with different dispersion" used to compare the performance of animals in the different groups and to determine the significance of differences between them. statistically considered significant differences in the values of $p < 0,05$

RESULTS OF THE STUDY

Results of LDF allowed to establish significantly lower bone microcirculation in rats eight weeks after ovariectomy (61.52 ± 3.74 PE; $n = 42$) compared to the intact animals ($PE 100,51 \pm 4.41$; $n = thirty$).

In response to the systemic administration of acetylcholine and nitroprusside observed a decrease in microcirculation, followed by normalization of blood flow. Thus, reducing the level of microcirculation during EDVD sample in the group of intact animals averaged $46,7 \pm 3,8\%$ from baseline values in the group of rats with experimental OP - $38,9 \pm 3,8\%$. When the reaction ENVD level of microcirculation in the group of intact rats were decreased by an average of $29.0 \pm 3,5\%$ of the original value in the control animal group - by $27,3 \pm 5,3\%$.

In the group of intact animals received $CED = 1,3 \pm 0,2$, the group of rats with experimental OP CED was significantly high and was $2,4 \pm 0,2$.

Osteoporotic changes in the bones of the skeleton were confirmed histologically in all rats eight weeks after ovariectomy: observed thinning of bone trabeculae and expansion intertrabecular spaces. Moreover, in some of histological preparations were observed microfractures of trabeculae. An objective measure of the development of the OP eight weeks after bilateral oophorectomy has been a significant due to decrease in the average width of bone trabeculae in the studied localization. Thus, the average width of the trabecular bone in the proximal metaphysis of the femur in rats with experimental OP ($61,68 \pm 1,24$ mm) was less than in intact animals ($97,69 \pm 1,02$ mm) by 36.8%.

In analyzing the activity of the study drugs was found that Losartan ($n = 35$), Resveratrol ($n = 20$) and their Nanoparticulate form ($n = 20$), medication for comparison Bivalos ($n = 20$) effectively prevents the reduction of regional blood flow in the bone femur (Fig. 1).

LDF results in the groups of rats treated with Bivalos, Losartan, N-Losartan, and N-Resveratrol, Resveratrol, approaching the performance of intact animals and statistically did not differ among themselves, and also significantly higher than the values in the control group.

It was ascertain that all the studied drugs, except Bivalos, resulted in proportion between the area of triangle above the curve of restoration of the level of microcirculation in bone, in response to acetylcholine and nitroprusside to those of intact animals. The studied drugs significantly reduces CED, demonstrating

endothelial protective action to the following values: Losartan - $1,5 \pm 0,2$, N-Losartan - $1,5 \pm 0,2$, Resveratrol - $1,3 \pm 0,2$, N-Resveratrol - $1,3 \pm 0,1$. The value of CED in the group of animals receiving Bivalos amounted to $2,1 \pm 0,2$.

Light microscopy examination of slides of femurs in rats treated discovered preserving bone structure and greater width of bone trabeculae than in rats with OP without treatment. Revealed that Bivalos, Losartan, N-Losartan, and the N-Resveratrol, Resveratrol prevented the decrease in the average width of bone trabeculae to the level of animals with experimental OP, but the average width of trabeculae did not reach the value of intact rats (Fig. 2).

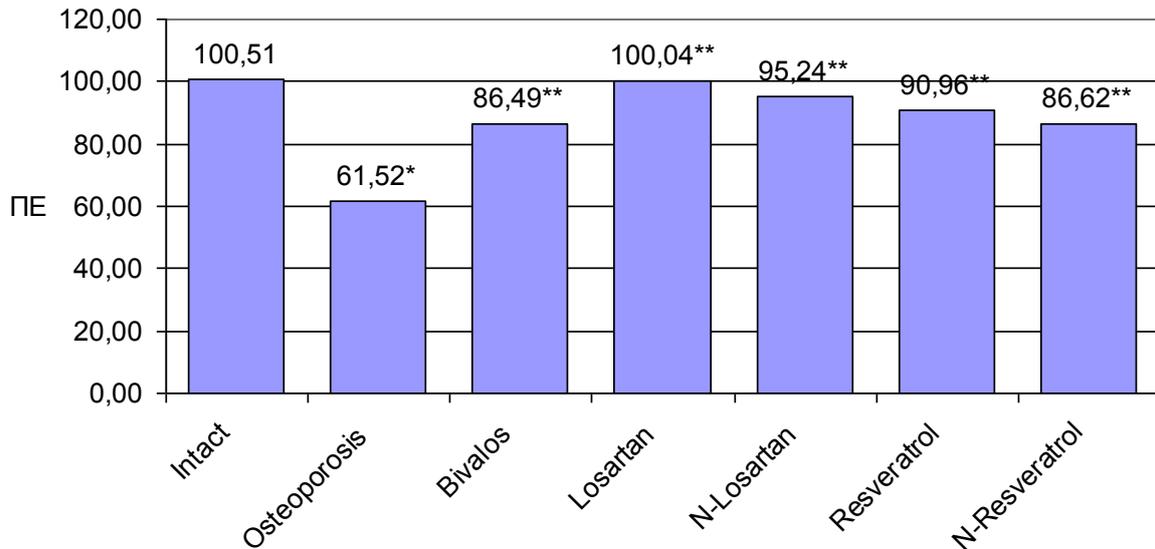


Figure 1: The results of the effect of Losartan, Resveratrol, their Nanoparticulate forms and Bivalos on blood supply to the proximal metaphysis of femur 8 weeks after bilateral ovariectomy.

Note. * - $P < 0.05$ compared to the group of intact animals; ** - $P < 0.05$ compared with the group of rats having osteoporosis.

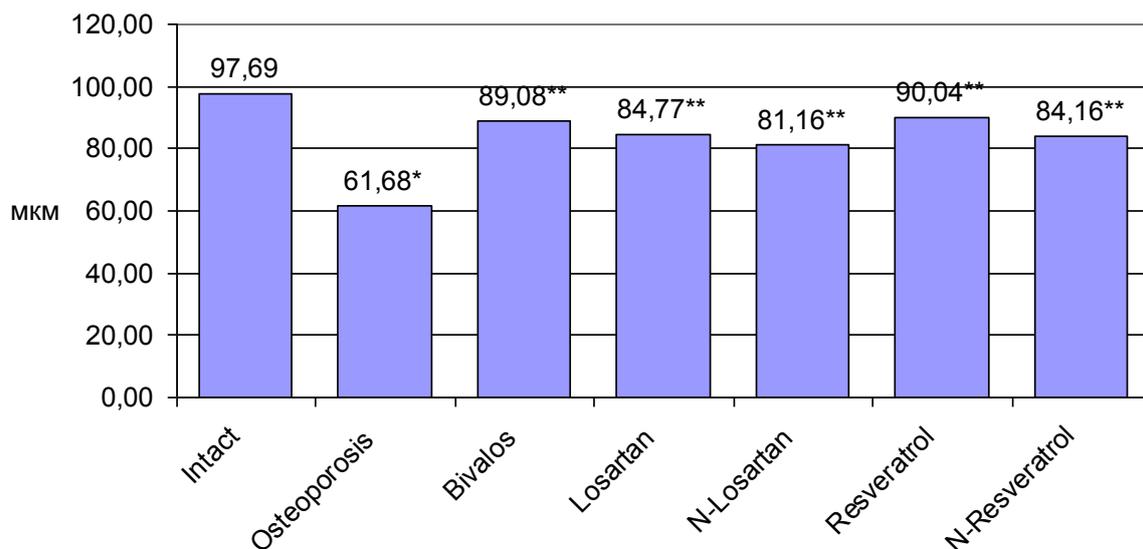


Figure 2: Results of the effect of Losartan, Resveratrol, their Nanoparticulate forms and Bivalos on the average width of bone trabeculae in the proximal metaphysis of femur 8 weeks after bilateral ovariectomy.

Note. * - $P < 0.05$ compared to the group of intact animals; ** - $P < 0.05$ compared with the group of rats having osteoporosis.

When studying the activity of combinations of studied medication, has been found that the combination of Losartan in a dose of 6 mg / kg with Resveratrol in a dose of 2 mg / kg (n = 20), N-Losartan 0.6 mg / kg with Resveratrol in a dose of 2 mg / kg (n = 20), Losartan 6 mg / kg with N-Resveratrol in a dose of 0.2 mg / kg (n = 20) and N-Losartan 0.6 mg / kg with N-Resveratrol in a dose of 0.2 mg / kg (n = 20) has more effectively prevented reduction of regional blood flow in the hip bone as compared to monotherapy (Fig. 3).

Results LDF in the groups of rats treated with combination therapy were not significantly different from that of intact animals, and sometimes even exceed them, did not differ significantly among themselves, and also significantly exceeded not only the values in the control group, but also in the group of animals treated with the drug of comparison Bivalos.

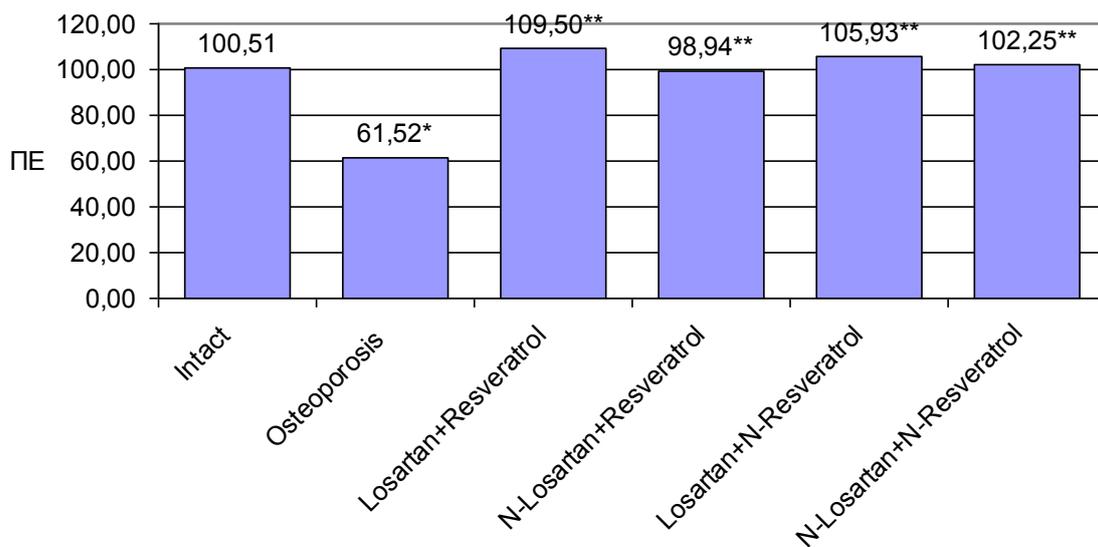


Figure 3. Results of the effect of combinations of studied medication in blood circulation in the proximal metaphysis of femur 8 weeks after bilateral ovariectomy.

Note. * - P <0.05 compared to the group of intact animals; ** - P <0.05 compared with the group of rats having osteoporosis.

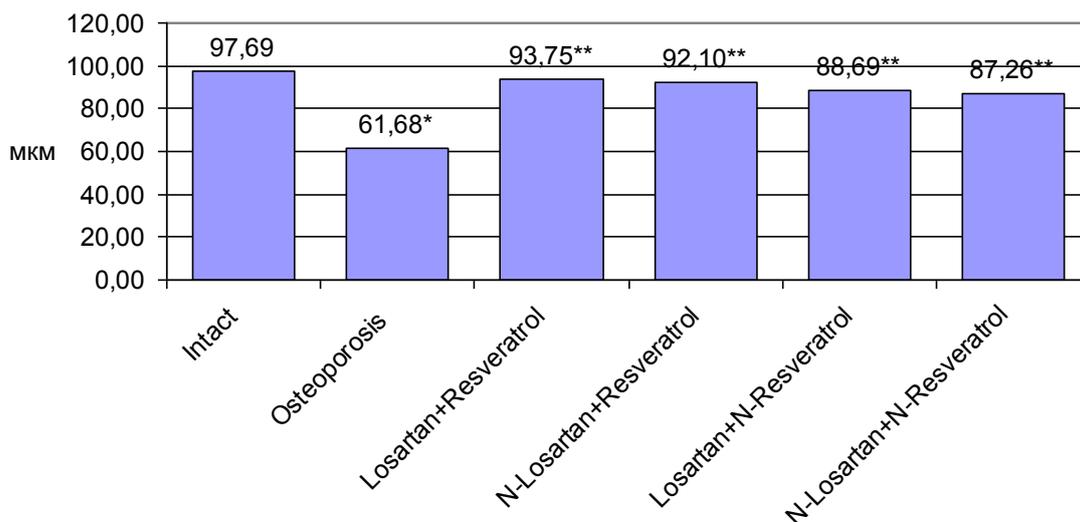


Figure 4. Results of the effect of combinations of studied medication on the average width of bone trabeculae in proximal metaphysis of femur 8 weeks after bilateral ovariectomy.

Note. * - P <0.05 compared to the group of intact animals; ** - P <0.05 compared with the group of rats having osteoporosis.

It was found that all investigated combinations of drugs led the proportion between the area of triangle above the curve of restoration of the level of microcirculation in bone, in response to Acetylcholine and Nitroprusside to those of intact animals. Thus, a combination of drugs studied significantly reduced CED, thus demonstrating endothelium protective effect to the following values: Losartan + Resveratrol - $1,1 \pm 0,1$, N-Losartan + Resveratrol - $1,2 \pm 0,1$, Losartan + N-Resveratrol - $1,4 \pm 0,2$, N-losartan + N-Resveratrol - $1,2 \pm 0,1$.

Light microscopy of slides of femur in rats treated with combinations of the studied drugs, found preserving bone structure and bone greater trabeculae width than in rats with OP, untreated. We found that the combination of study drugs effectively prevent reduction of the average width of trabecular bone to the level of animals with experimental OP (Fig. 4).

DISCUSSION

To maintain homeostasis of bone is extremely important to develop a full-fledged functional microvasculature and overall regional microcirculation in bone tissue. The deterioration of blood supply to the bone may lead to the development of pathologies of the musculoskeletal system as osteonecrosis [12], osteomyelitis [17] and osteoporosis [11, 18]. The endothelium in the mature vasculature plays a central regulatory role, providing a link with the other layers of the vessel wall and adequately responding to their needs by mediator release [3]. Thus, we believe the vascular endothelium of bone tissue, being an integral part of the bone, largely determines the state of regional microcirculation, and thus responsible for the maintenance of homeostasis in the bone.

This provision is confirmed by a number of authors who say, for example, VEGF (a key regulator of the cascade of events leading to the formation and development of the vascular system) plays a significant role in the process of remodeling [13] and the repair of damage [14] bone. Thus, it was demonstrated that inhibition of VEGF results in increased width of femoral and tibial growth plates zones, reduce the intensity of angiogenesis in growth zones, loss of blood vessels in the metaphyseal area, as well as reducing the formation of trabecular bone structure [13] and intensification of resorption of trabecular bone tissue [15].

It is currently time there is an active study on drugs with endotelioprotective properties and formulate the possible pathogenetic mechanisms of their effects on the vascular endothelium.

Endothelium protective angiotensin receptor antagonist effect is realized, apparently by blocking the AT1 receptor, leading to reduced production of superoxide radicals, reduce the degree of binding of NO and its accumulation. Since stimulation of the AT1 receptor promotes the formation of superoxide inactivating NO, and stimulation of AT2 receptor leads to vasodilation and increased natriuresis by activation of bradykinin, NO and cGMP, the effect of AT-2 (increased synthesis or inactivation of NO) depends on the with which it preferentially reacts receptors. Therefore it is obvious that in the context of the blockade of AT1 receptors are created favorable conditions for the functioning of free intensified AT2 receptor, which leads to the accumulation of NO.

Resveratrol is endothelium-dependent relaxation of blood vessels by enhancing nitric oxide production and the subsequent increase in the level of cGMP. However, these effects were attenuated by the introduction of competitive inhibitors of NO-synthase N^G -monomethyl-L-arginine and N^G -nitro-L-arginine. It is known that a short-term effect on the endothelial cells of resveratrol in low concentrations ($1-10 \mu M$) increases the amount of nitric oxide produced, due to a short-term increase in eNOS activity and reduced superoxide production in the endothelium. Resveratrol stimulates chronologically eNOS expression and VEGF. In contrast inhibition of NO production inhibitors eNOS substantially reduces mitogenic and angiogenic effects stimulated by VEGF.

Using nanoparticulate forms of Losartan and Resveratrol in this study led to a tenfold reduction of doses studied drugs maintaining a comparable therapeutic effect. This nanoparticulate form of losartan and Resveratrol showed osteoprotective effective action both as monotherapy and in combination.

Thus, nanoparticulate forms of AT1 receptor blocker Losartan, and a representative of phytoalexin group Resveratrol, providing endothelium protective effect on the endothelium of the microvasculature of bone, effectively prevent the decrease in regional blood supply to the bone in experimental osteoporosis and

have osteoprotective action to the positive impact of these drugs on the process of bone remodeling and osteoreparation.

CONCLUSIONS

- Eight weeks after bilateral ovariectomy in female Wistar rats developing endothelial dysfunction of micro vessels of bone, as evidenced by the increase in the rate of endothelial dysfunction, calculated on the results of laser Doppler flowmetry in the bone tissue. Significantly worse regional blood flow in the bone tissue that leads to the development of generalized osteoporosis, accompanied by thinning of trabeculae and their occurrence in microfractures.
- Losartan, Resveratrol and nanoparticulate forms of these drugs in monotherapy, unlike Bivalos, in the model of bilateral ovariectomy has endothelium protective effects, that is exerted in reducing the coefficient of endothelial dysfunction. All studied drugs effectively prevent reduction in the microcirculation of the thigh bone and also to prevent reduction in the width of bone trabeculae and the occurrence of microfractures shows osteoprotective action.
- Various combinations of Losartan, Resveratrol, as well as their nanoparticulate forms after bilateral ovariectomy, having expressed endothelium protective, effectively reduce the rate of endothelial dysfunction; combinations of drugs are effective in preventing the decline in blood flow in the hip bone by holding it at the values often superior than performance in intact animals, and also have a pronounced osteoprotective effect, reliably preventing the thinning of the trabeculae.

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