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Osteoprotective Effect of Enalapril, Losartan and Resveratrol in Experimental Osteoporosis

Faitelson AV*, Gudyrev OS, Dubrovin GM, Pokrovsky MV, Ivanov AV and Rajkumar DSR

Department of Traumatology and Orthopaedic, Kursk State Medical University, Kursk, Russia

Abstract

Osteoporosis (OP) - a systemic skeletal disease characterized by low bone mass per unit volume and an impairment of the micro architecture of bone tissue, leading to an increase in bone fragility and a high risk of fractures. The basic cause of development of osteoporosis is an imbalance between the two major processes of bone remodeling: bone resorption and bone formation.

Introduction

Osteoporosis (OP) - a systemic skeletal disease characterized by low bone mass per unit volume and an impairment of the micro architecture of bone tissue, leading to an increase in bone fragility and a high risk of fractures. The basic cause of development of osteoporosis is an imbalance between the two major processes of bone remodeling: bone resorption and bone formation [1,4].

An important element in the pathogenesis of osteoporosis is the reduction of the blood supply to the bone tissue [11], leading to inhibition of osteoblastic activity and also increases activity of osteoclasts. During the research we have found there is a close interdependence between the size of the microcirculation in the bone and "quality" of bone; which is expressed by the thickness of bone trabeculae and also the stability of the bone tissue to external influences [9].

It is known that the structure of the micro vessels of bone morphology differs significantly from other vascular tissues. Bone microvessels have only endothelium, which consequently mediate the humoral regulation of all exchanges between bone cells and blood. In the present time, in the available literature contains no information about anyone using the vascular endothelium of bone as a target for pharmacological action aimed at osteoporotic changes.

In previous studies we also demonstrated that the generalized hypo-estrogen induction of Osteoporosis in females Westar rats is accompanied by signs of endothelial dysfunction (ED), which leads to a deterioration of regional blood flow and may cause disturbances to the of processes of osteogenesis and osteoreparation, causing osteoporosis.

Modern pathogenetic therapy of Osteoporosis has neglected drugs with endothelial protective properties and consequently a positive influence on the blood supply of bone. This indicates the importance of studying the action of osteoprotective drugs with proven positive endothelial effects: Enalapril, Losartan, and Resveratrol, whereby underliethe purpose of this study.

Materials and Methods

Experiments were performed on 162 white females Wistar rats weighing 250 ± 50 g. For modeling systemic osteoporosis, rats are narcotized with intra-peritoneal injection of chloral hydrate solution at a dose of 300 mg/kg and performed bilateral ovariectomy surgery [6,22]. The development of generalized Osteoporosis was evaluated in eight weeks (57 days) after surgery.

The level of the microcirculation was assessed in the tissue of proximal metaphysis of femur. For this, after fixing the animal on the table for surgical procedures [8], then drilled a monocortical hole in femur, into this stabilized the sensor probefor the measurement of microcirculation of bone by applying the rod-conductor [7]. Then to obtain data of microcirculation in the bone, used equipment from company Biopac systems: polygraph MP100 model with laser Doppler flowmetry (LDF) LDF100C and sensor probe TSD144. Registering the results of LDF was performed by acknowledge software version 3.8.1., The values of microcirculation are expressed as perfusion unit (PU).

The development of hypo-estrogen induction ED was assessed after measurement of intra-osseous microcirculation level, which was performed on samples by endothelium-dependent vasodilation (EDVD) in response to a bolus intravenous injection of a solution of acetylcholine at a dose of 40 mg/kg [17] and endothelium non-dependant vasodilation (ENVD) in response to bolus of sodium nitroprusside solution at a dose of 30 mg / kg [2].To objectively assess the development of endothelial dysfunction in generalized Osteoporosis, calculated coefficient of endothelial dysfunction (CED) based on the data from LDF in the bone.To determine the area of right angled triangle abovecurverestored microcirculation after conduction of reaction EDVD&ENVD. Before this from one of the two sides of a triangle taken the absolute increase of the fall of the level of the microcirculation, for another - recovery time and stabilizing values of microcirculation. CED is calculated as the ratio of the area of the triangle above the recovery curve of microcirculation in response to the introduction of sodium nitroprusside to the area of the triangle above the recovery curve of microcirculation in response to the introduction of acetylcholine.

To confirm the development of Osteoporosis and in a comprehensive assessment of the effectiveness of study medication was performed morphological investigation of proximal femoral

***Corresponding author:** Faitelson AV, Department of Traumatology and Orthopaedic, Kursk State Medical University, Kursk, Russia, Tel: 8-919-212-95-38; E-mail: vladimirfaitelson@gmail.com

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metaphyses. The slides with the histological preparations were subjected to light microscopy (zoomx100, objective lens x10, eyepiece x10) and photographed bone trabeculae by matching objective of camera lens and the eyepiece of the microscope.

For histomorphometry used the program Image J version 1.39, pre-calibrated as follows: using the same equipment to get photographs of bone trabeculae, photographed a "scale" of 1 mm on a transparent substrate. Measured the "scale" in pixels by program Image J, the length for 1425 pixels is1 mm. Later measured the width of bone trabeculae, and expressed it in micrometers (mm).

To study the osteoprotective effect we selected drugs with endothelioprotective action which has been proven in previous studies in the laboratory of cardio-pharmocology-SRI-Ecology Medicine KSMU: enalapril maleate, losartan potassium, and resveratrol. ACE inhibitor Enalapril maleate and AT1-receptor blocker losartan potassium ("Renipril" and "Bloktran" produced by OAO "Pharmstandard-Leksredstva") was administered intra-gastric at doses of 0.5 mg/kg and 6 mg/kg, respectively, resveratrol (Greensyn, Guangzhou Ltd.) administered intraperitoneally at a dose of 2 mg/kg daily once a day for eight weeks, which fits with the data available and reveals the effective endothelioprotective action of these drugs in the experiments previously conducted in our laboratory [10].

The control group was a group of animals with experimental Osteoporosisand they were not receiving pharmacological correction. The intact group of rats consist falsely operated animals (false operation-ovariectomy without removal of ovaries).

Statistical data analysis was performed in the program Microsoft Excel. "Descriptive statistics" was used to determine the average value (M) and error of the mean (m). "Two-Sample t-test with different variances" was used for comparisons in different groups of animals and determining the reliability of the differences between them. Statistically significant differences is felt in the values of the two-sided p <005.

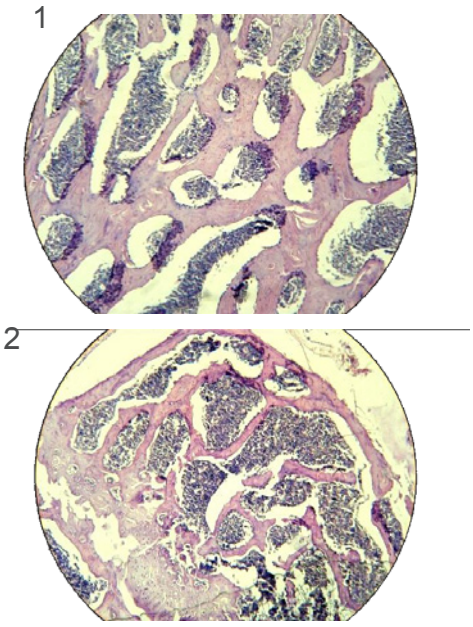
Results of the Study

The results of LDF allowed to comment the significantlylower level of the microcirculation in the bone tissue of rats eight weeks after ovariectomy (61,5 ± 3,7 PU; n = 42) when compared with intact animals (100,5 ± 4,4 PU; n = 30).

In response to systemic administration of acetylcholine and sodium nitroprusside observed a decrease in the microcirculation with subsequent normalization of blood flow. Thus, the decrease in the level of the microcirculation during sample EDVD in the group of intact animals averaged 46,7 ± 3,8% from baseline values in the group of rats with experimental OP - 38,9 ± 3,8%. The reaction ENVD level of microcirculation in the group of intact rats decreased by an average of 29,0 ± 3,5% of its original value in the control group of animals - in 27,3 ± 5,3%.

In the group of intact animals received CED = 1,3 ± 0,2, in the group of rats with experimental OP CED was statistically significantly in higher and was 2,4 ± 0,2.

Generalized osteoporotic changes in the bones of the skeleton were confirmed histologically in all rats eight weeks after ovariectomy: there was thinning of bone trabeculae and widening of intertrabecular space (Figure 1). In addition, some histological preparations were observed microfractures of bone trabeculae. O the intravital occurrence of



Note: 1 - intact rats, 2 –rat's with osteoporosis.

Figure 1: Morphological picture of section from proximalmetaphysis of femor of rats (stained by hematoxylin and eosin, zoom-100).

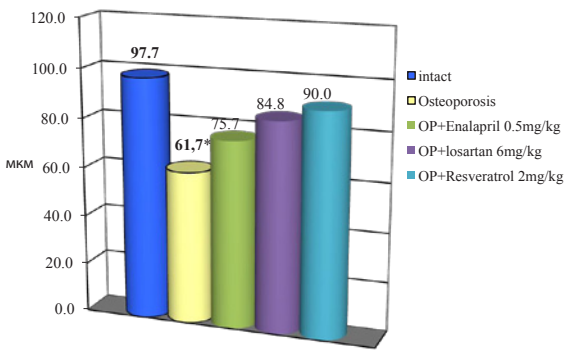
microfractures was judged by the germination of the connective tissue intrabecula the fracture site.

An objective measure of assessing the development of OP eight weeks after bilateral ovariectomy was significant decreased in the average width of bone trabeculae in the study localization. Thus, the average width of bone trabeculae in the proximal femur metaphysis in rats with experimental OP (61,7 ± 1,2 mm) was lower than in intact animals (97,7 ± 1 mm) to 36.8%.

When analyzing the activity of the studied drugs it was found that enalapril 0.5 mg / kg, 6 mg losartan / kg resveratrol, and 2 mg / kg effectively prevented the decrease in regional blood flow in the femur bone, preserving the values of the microcirculation at the level of intact rats, the results of LDF in groups of rats treated with enalapril (93,3 ± 4,4 PU; n = 35), losartan (100,0 ± 2,3 PU; n = 35) and resveratrol (91,0 ± 12,8 PU, n = 20) did not differ from those in intact animals (p> 0,1) and did not differ among themselves (p> 0,1), as well as significantly different from that of the control group (p <0,05).

It was found that enalapril, losartan and resveratrol resulted in proportions between the areas of triangles on the curves of recovery level of microcirculation in bone in response to the nitroprusside and acetylcholine to those in intact animals. Thus the studied drugs significantly reduced the values of CED to 1,6 ± 0,1, 1,5 ± 0,2 and 1,3 ± 0,2, respectively.

In the light microscopy sections of bones in rats treated, discovered maintainance of the structure of bone and a large width of bone trabeculae than in rats with OP who were not receiving treatment. Thus revealed that enalapril, losartan and resveratrol prevented the decrease in the average width of bone trabeculae to the level of animals with experimental Osteoporosis, but the average width of the trabeculae did not reach the value of intact rats (Figure 2). Enalapril, losartan, and resveratrol had a statistically significant impact on the average width



Note: * - p <0,05 when compared with group of intact animals;

** - P <0,05when compared with the group of rats with osteoporosis.

Figure 2: Effect of Enalapril, losartan and resveratrol in the average width of bone trabeculaein the proximal metaphysis of the femur 8 weeks after bilateral ovariectomy.

of bone trabeculae: Enalapril increased the width of trabeculae in an average of 22.7%, losartan - 37.4%, resveratrol - at 45.9% as compared to rats suffering from OP.

Discussion

In order to maintain homeostasis of bone, it's extremely important the development of functionally meaningful microcirculation and the general condition of regional microcirculation in bone tissue. The deterioration of the blood supply of bone tissue can lead to the development of such pathologies of musculoskeletal system, as osteonecrosis [13], osteomyelitis [18] or osteoporosis [12, 20].

Endothelium of mature vascular network plays a central regulatory role, providing a link with the other layers of the vessel wall and adequately responding to their needs, the release of mediators [3]. Thus, in our opinion the vascular endothelium of bone tissue, as an integral part of the bone, in many respects determines the state of regional microcirculation, and thus is responsible for the maintenance of homeostasis in the bone.

This situation is confirmed by several authors, who claim, 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 remodeling process [14,19,21,25] and the repair of damage [15,23,24] of bone tissue. So, it was demonstrated that inhibition of VEGF leads to an increase in the width of the femoral and tibial growth zones [26], the decrease in the intensity of angiogenesis in the growth zones, loss of blood vessels in the metaphyseal area, as well as reducing the formation of trabecular bone structure [14,25], and the intensification of resorption of cancellous bone [16].

In the current time there is active study of drugs with endothelioprotective properties, as well as formulation of possible pathogenetic mechanisms of their effects on the vascular endothelium.

In particular, one possible way of the protective effect of ACE inhibitors on endothelial dysfunction is associated with restoration of metabolism of bradykinin: blockade of tissue ACE not only leads to a decrease in the synthesis of AT-2, but also slow down the degradation of bradykinine. Also under the action of these drugs may decrease basal and insulin-induced secretion of endothelin-1 and increased production of NO.

Endothelioprotective influence of angiotensin receptor antagonists occurs, presumably due to blockade of AT1 receptors, leading to reduced production of superoxide radicals, reduce the binding of NO and its accumulation. Since the stimulation of AT1 receptors promotes the formation of superoxide inactivating NO, and stimulation of AT2 receptors leads to vasodilation and increased natriuresis by activation of bradykinine, NO and cGMP, the effect of AT-2 (increased synthesis or inactivation of NO) depends on with which the receptors it interacts mainly. It is therefore evident that in the context of the blockade of AT1-receptor creates favorable conditions for the intensified functioning of free AT2 receptor, which leads to the accumulation of NO.

A representative of the phytoalexins resveratrol causes endothelium-dependent relaxation of blood vessels by increasing nitric oxide production and subsequent increase in cGMP levels. However, these effects are 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 short-term effects of resveratrol on endothelial cells in low concentrations (1-10 μM) increases the amount of produced nitric oxide, which is explained by increased activity of eNOS and decreased production of superoxide in the endothelium. Resveratrol stimulates in chronological order the expression of eNOS and VEGF. In contrast inhibition of production NO inhibition eNOS identifies significantly reduces mitogenic and angiogenic effects, stimulate VEGF.

Defined contribution at hypo-estrogen induced endothelial dysfunction makes the estrogenic properties of resveratrol. Resveratrol binds to estrogen receptors, thereby activating the process of matrix synthesis of estrogen-sensitive gene reporters. In rats with ovariectomy, resveratrol acts as an agonist of the estrogen receptor. In addition, resveratrol increases the degree of vascular relaxation, depends on the endothelin (in response to acetylcholine) and like estradiol prevents weakening of the bone.

Based on the above we formulated the hypothesis that the quality of the processes of remodeling and reparative regeneration of bone tissue is directly dependent on the quality of the functioning of the vascular endothelium, which determines the level of regional microcirculation in bone tissue. This concept, we have taken in the course of our investigations.

Complex pathological changes, lesions found eight weeks after bilateral ovariectomy in female Wistar rats, confirms our proposed theory of the development of osteoporosis: endothelial dysfunction in microvascular bone, impairing blood flow to the bone, participates in the dissociation processes of bone remodeling and as a consequence, the development of generalized of osteoporosis.

To confirm the proposed theory enalapril, losartan, and resveratrol, increases the vascular endothelium-dependent relaxation of bone tissue, normalized index of CED, leading them to the values of intact animals. The studied drugs increased the level of the microcirculation in the proximal femur metaphysis to values not significantly different from those in intact rats. Preparations have also provided a statistically significant impact on the average width of bone trabeculae as compared to rats suffering from osteoporosis.

Thus, the ACE inhibitor enalapril, AT1 blocker losartan receptors, as well as a representative group of phytoalexins resveratrol, providing endothelial protective effects on the endothelium of microcirculation of bone, effectively prevents reduction of regional blood flow in bone in experimental osteoporosis and have osteoprotective action,

consisting of the positive impact of these drugs on the processes of bone remodeling and osteo-reparation.

From this it follows that the spectrum of pleiotropic action of drugs with proven endothelio-protectivity activity can be expanded by adding osteoprotective element, which, however, requires further investigation.

Findings

1. Eight weeks after bilateralovariectomyin female Wistar rats develops endothelial dysfunction of vessels of microcirculation in bone tissue, which is proved by the increase of coefficient of endothelial dysfunction, as calculated according to the results of laser Doppler flowmetry in the bone tissue to 2,4 ± 0,2 in comparison with 1,3 ± 0,2 in intact animals. Significantly worsened regional blood flow in bone (61,5 ± 3,7 PU when compared to 100,5 ± 4,4 PU in intact rats), which leads to the development of generalized osteoporosis, accompanied by thinning of bone trabeculaeto an average of 36.8% and the emergence of theirmicrofractures.

2. Enalapril at a dose of 0.5 mg/kg on a model of bilateral ovariectomy has a pronounced endotelioprotective effect, which appears to reduce the rate of endothelial dysfunction to 1,6 ± 0,1, prevents the reduction of microcirculation in femur bone, keeping the values not to differ from intact rats (93,3 ± 4,5 PU), as well as by increasing the width of bone trabeculae in an average of 22.7% compared with a group of rats with osteoporosis and preventing the microfractures, thus it has osteoprotective action.

3. Losartan at a dose of 6 mg/kg after bilateral ovariectomy, possessing a pronounced endothelio-protective effect, reduces the coefficient of endothelial dysfunction to 1,5 ± 0,2; drug effectively prevents the decline in blood flow to the femur bone, holding it at the level of intact animals (100.0 ± 2,3 PU), and has a pronounced osteoprotective effect, increasing the width of bone trabeculae in an average of 37.4%.

4. Resveratrol in a dose of 2 mg / kg on the chosen model of Pathology has a pronounced endothelioprotective effect, which appears to reduce the coefficient of endothelial dysfunction to 1,3 ± 0,2, prevents the reduction of microcirculation in femur bone, holding it at the level of intact rats (91.0 ± 12,8 PU), as well as by increasing the width of bone trabeculae in average by 45.9% compared with a group of rats with osteoporosis and preventing the microfractures and has osteoprotective action.

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