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STUDY OF THE MICROCIRCULATION LEVEL IN BONE WITH OSTEOPOROSIS AND OSTEOPOROTIC FRACTURES DURING THERAPY WITH RECOMBINANT ERYTHROPOIETIN, ROSUVASTATIN AND THEIR COMBINATIONS

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Abstract. The experiment was carried out in female white Wistar rats. The effects of recombinant erythropoietin, rosuvastatin and their combination were investigated based on the blood supply to the bone after a modelled experimental osteoporosis. It was found that the studied drugs prevent decrease of bone microcirculation in cases of osteoporosis and in callus tissue in experimental osteoporotic fractures, positively influencing the course of reparative regeneration of bone tissue.
Keyword: osteoporosis, osteoporotic fracture, microcirculation, endothelial dysfunction, recombinant erythropoietin, rosuvastatin

Introduction

Osteoporosis is a systemic disease of skeleton, characterized by decrease in density of bone mass and microarchitecture defects in bone tissue that leads to high bone fragility and fracture risks. Osteoporosis development is based on imbalance of two processes of bone remodelling: resorption and regeneration [1, 2].

The number of operative treatment of bone fractures using different implants is growing every year, including the use of endoprosthesis for large joints. According to recent investigations, each fifth operation of primary endoprosthesis replacement includes one re-endoprosthesis manipulation. This is generally connected with the development of aseptic instability because of osteoporotic abnormalities in bone tissue [3].

The blood supply in the bone plays a significant role in the processes of bone remodelling and reparative regeneration of bone tissue. Bone microvessels have only endothelium and don't have muscular or connective layers. This means that the endothelium mediates all humoral regulation between osteoblasts, osteoclasts and blood [4, 5, 6].

The reason for a poor blood circulation of bone tissue can be endothelial dysfunction, that reduces microcirculation and leads to osteopenia and gradually to osteoporosis [7, 8].

In case of endotheliotropic therapy we can observe the improvement of microcirculation and the changes of bone tissue structure [9, 10].

It is widely known that recombinant erythropoietin and rosuvastatin have endotheliotropic effect, however their influence on bone tissue has not been properly investigated, what leads to further studies of these medicines.

Objectives. To study the level of microcirculation in bone tissue in case of experimental osteoporosis and osteoporotic fractures using treatment with recombinant erythropoietin and rosuvastatin or their combination.

Methods and materials. For the experiment, 240 female white Wistar rats (200 – 250 gr) were used. All the manipulations were done under anaesthesia (by intraperitoneal introduction of a solution of chloral hydrate at a dose of 300 mg/kg). The animals were divided into twelve groups – 20 rats each.

I – intact - false bilateral ovariectomy was performed (laparotomy incision without removal of the ovaries was done followed with layer-by-layer stitching of the wound).

II – control - bilateral ovariectomy was performed (laparotomy incision with removal of the

ovaries was done followed with layer-by-layer stitching of the wound).

III – Treatment with recombinant erythropoietin was given eight weeks after the ovariectomy and the development of osteoporosis (50 IU/kg subcutaneously once a week from the ninth to the twelfth week).

IV – Treatment with rosuvastatin was given eight weeks after ovariectomy (0,86 mg/kg daily intraperitoneally from the tenth to the twelfth week).

V – Treatment with recombinant erythropoietin and rosuvastatin was given eight weeks after bilateral ovariectomy using the same method as in groups III and IV.

VI – Bivalos (strontium ranelate), a drug for comparison, was administered eight weeks after bilateral ovariectomy (171 mg/kg daily intraperitoneally from the tenth to the twelfth week).

VII – Eight weeks after a false bilateral ovariectomy a proximal femoral metaphysis fracture was performed;

VIII – Eight weeks after bilateral ovariectomy a proximal femoral metaphysis fracture (osteoporotic fracture) was performed.

IX – Eight weeks after bilateral ovariectomy a proximal femoral metaphysis fracture was performed. After that recombinant erythropoietin treatment was given. (50 IU/kg subcutaneously, once a week from the tenth to the twelfth week).

X – Eight weeks after bilateral ovariectomy a proximal femoral metaphysis fracture was performed. After that rosuvastatin treatment was given (0,86 mg/kg daily intraperitoneally from the ninth to the twelfth week).

XI – Eight weeks after bilateral ovariectomy a proximal femoral metaphysis fracture was performed. After that a recombinant erythropoietin and rosuvastatin therapy was given, as described for groups IX and X.

XII – Eight weeks after bilateral ovariectomy a proximal femoral metaphysis fracture was performed. After that Bivalos (strontium ranelate) was administered (171 mg/kg daily intraperitoneally from the tenth to the twelfth week).

In fracture modelling closed technique was used (proposal for technical improvement № 1975-11 from 15.11.2011. «A closed technique for a metaphysis fracture model for small laboratory animal»). A cutting blade with silicon tubes on the cutting edges was used, external force was applied on proximal femoral metaphysis until the characteristic signs of fracture were present (specific pathologic movements, fractured fragment crepitation, limb axis change) the load was applied perpendicular to the limb axis. The fracture was stabilised and fixed by screw clamp and twist drill needle from the distal to proximal part of the femur, intramedullary through the bone canaliculus and was firmly fixed in cortical layer of proximal part of the femur. The control was done by the absence of pathological movements in the fractured area. The extending part of the needle was shortly cut. The wound was sutured by one stitch through all layers.

Twelve weeks (on the eighty-fifth day) after the beginning of the experiment the measurement of microcirculation level in bone tissue was performed. The measurement was done in proximal femoral metaphysis by laser Doppler flowmeter (LDF). In the cortical layer of bone tissue a hole of 2-3mm deep (in the group of animals without fracture) was made. For animals with a fracture, the hole was made in callus tissue formed after the femoral fracture. A needle sensor was used for the microcirculation measurement. In the trial rats from groups I, II, III, IV, V and VI some probes were taken without changing the position of the sensor: for endothelium dependent vasodilation (in response to a single intravenous dose of acetylcholine solution at a dose of 40 μg/kg), and for endothelium independent vasodilation (in response to a single intravenous dose of sodium nitroprusside solution at a dose of 30 μg/kg).

To confirm the role of endothelial dysfunction in the development of regional microcirculation disorders the endothelial dysfunction coefficient based on the laser Doppler flowmeter data was calculated. Endothelial dysfunction coefficient was defined as the ratio of the area of triangle above the curve of microcirculation restoration in the response to nitroprusside administration to the area of triangle above the curve of microcirculation restoration in the response to acetylcholine administration. The parameters of microcirculation were taken by laser Doppler flowmeter Biopac systems MP_{150} and sensor TSD_{144}. The data of laser Doppler flowmeter was processed and recorded by software AcqKnowlwdge version 3.9 – 4.2, microcirculation was measured in perfusion units (PU).

The descriptive statistics and statistical analysis of initial research data were processed in Microsoft Excel. The group data was used to calculate the average value (M) and error of the mean (m). Assessment of the statistical significance of differences between groups was based on «two-sample t-test with different variances». The differences were considered statistically significant for values of $p < 0,05$.

Results. The experiment showed that twelve weeks after the operation intact animals had the microcirculation level in proximal femoral metaphysis (99,91±3,41 PU), statistically more significant than in the control group with

osteoporosis (58,75±3,76 PU). Animals with osteoporosis had a higher endothelial dysfunction coefficient (2,57±0,23) compared to intact animals (1,28±0,18). This larger values prove that there are signs of endothelial vessel dysfunction. The endothelial dysfunction of bone vessels and low microcirculation parameters lead to an imbalance of bone remodelling processes that causes and stimulates osteoporotic changes.

The experimental osteoporosis therapy using recombinant erythropoietin and rosuvastatin and their combinations has shown an improvement in microcirculation of proximal femoral metaphysis of laboratory animals (80,27±3,05 PU, 81,88±3,39 PU and 86,30±2,75 PU respectively), compared to the use of the reference product Bivalos (67,48±2,98 PU, p=0,077). In case of using recombinant erythropoietin and rosuvastatin and their combinations endothelial dysfunction coefficient was statistically smaller compared to the control group (1,70±0,21, 1,72±0,18 and 1,69±0,23 respectively). The product for comparison Bivalos, according to endothelial dysfunction coefficient statistics, did not have any endothelial protective activity (EDC = 2,44±0,19).

In animals with models of experimental osteoporosis of proximal femoral metaphysis reparative regeneration processes in cases of osteoporosis had statistically smaller parameters of microcirculation in callus tissue (66,59±3,61 PU), compared to animals with fractures, but without osteoporosis (89,30±4,75 PU). The fracture union in animals with osteoporotic femoral fractures occurred in 55% of cases, while in cases without osteoporosis in 75%.

The osteoporotic fracture therapy using recombinant erythropoietin, rosuvastatin and their combination has shown higher parameters of microcirculation in callus tissue of proximal femoral metaphysis fractures of laboratory animals (96,31±3,16 PU, 94,34±2,54 PU and 101,05±2,75 PU respectively). These parameters were statistically more significant than in other experimental and control groups. In cases of usage of comparative product (Bivalos) was observed a tendency of microcirculation improvement in fracture callus tissue in proximal femoral metaphysis but this numbers were not statistically significant (70,39±2,39 PU, p=0,386). Experimental fracture union using all mentioned medical products occurred in 100% of the cases.

Discussion. Endothelial dysfunction is characterized by imbalance between vasoconstrictive and vasodilator ability of the vessels. The cause is based on the imbalance between vasodilative and vasoconstrictive factors such as nitrogen oxides (NO) and prostacyclin, endothelin-1 and angiotensin II [11]. Vascular endothelium of bone tissue is an important part of the bone, it has a central regulatory role [12]. Many clinical trials and experimental investigations showed that recombinant erythropoietin is a multifunctional trophic factor, it has different sites of expression, specific tissue regulation and some other transmissions. Erythropoietin functional receptors were found not only on red blood cells membranes of bone marrow, but also on the myeloid cells, lymphocytes, megalokaryocytes and also on endothelial, mesangial and smooth muscle cells and neurons [13]. Also recombinant erythropoietin promotes proliferation of endotheliocytes, liver embryonic stem cells and unstriated muscles [14, 15, 16]. The perspective of recombinant erythropoietin usage in cardiology is explained by its pleiotropic effects. In this way recombinant erythropoietin stimulates epithelial apoptosis, cardiac hypertrophy reduction, and increase in physical activity tolerance in patients with chronic cardiac failure [17, 18]. The researches on the heart of experimental rats showed that the treatment based on recombinant erythropoietin leads to coronary perfusion improvement, decreasing in left ventricular end diastolic pressure and improvement of its systolic function [19]. This effect is connected with endothelial NO-synthase and protein kinase B activation, that mediates phosphorylation, leading to long-term NO-dependent vasodilation [20]. The positive influence of statins on endothelial function was investigated. Through protein kinase B activation and influencing phosphorylation process in endothelial cells, eNO (endothelial synthase NO) causes high level of NO production [21], that leads to NO-dependent vasodilation. All stated above proves that recombinant erythropoietin and rosuvastatin have a positive influence on vascular endothelium and improvement of regional microcirculation of bone tissue.

Conclusion:

1. Twelve weeks after the bilateral ovariectomy the laboratory animals had the signs of vascular endothelial dysfunction, including bone tissue microvasculature, with the decrease in regional microcirculation parameters, that can negatively influence the balance on bone regeneration and resorption leading to osteoporosis.

2. The recombinant erythropoietin therapy (50 IU\kg subcutaneously, once a week from the tenth to the twelfth week) and rosuvastatin treatment (0,86 mg/kg daily intraperitoneally from the ninth to the twelfth week) or their combination twelve weeks after bilateral ovariectomy showed the signs of their endothelial protective functions. This leads to the decrease in EDC and to the increase of the microcirculation in the bone tissue of proximal

femoral metaphysis. The product used for comparison, Bivalos, has not shown any endothelial protective activity.

3. The fracture adherence of proximal femoral metaphysis in twelve weeks after its modelling in laboratory animals was accompanied by decrease of microcirculation parameters in callus tissue, consequently leading to poor consolidation parameters of experimental fractures.

4. The administration of recombinant erythropoietin and rosuvastin and of their combination increased microcirculation parameters in callus tissue in rats with proximal femoral metaphysis fracture and provided favorable conditions for reparative regeneration, that increases the parameters of experimental osteoporotic fracture adherence. Bivalos also had a positive influence on the osteoporotic fracture adherence.

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