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EFFECTS OF SIMVASTATIN ON THE DEVELOPMENT OF OSTEOPENIA CAUSED BY OVARIECTOMY IN RATS

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Simvastatin is a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-determining enzyme for cholesterol synthesis which is used in the treatment of hypercholesterolemias, particularly in type IIa and IIb hyperlipoproteinemias, frequently in postmenopausal women. Inhibition of cholesterol synthesis by simvastatin may cause disorders of bone remodelling.

The aim of the present study was to investigate the effects of simvastatin (3 mg and 6 mg/kg/day *per os*) administered for 4 weeks on the development of ovariectomy-induced osteopenia in 3-month-old female Wistar rats. The experiments were carried out on six groups of animals: I (C) – sham operated rats, II (S-3) – sham operated rats + simvastatin 3 mg/kg/day *po*, III (S-6) – sham operated rats + simvastatin 6 mg/kg/day *po*, IV (OVX) – ovariectomized rats, V (OVX + S-3) – ovariectomized rats + simvastatin 3 mg/kg/day *po*, VI (OVX + S-6) – ovariectomized rats + simvastatin 6 mg/kg/day *po*. In all the groups, we examined body weight gain, and macrometrical, histomorphometrical and mechanical parameters.

Bilateral ovariectomy induced osteopenic skeletal changes in mature female rats. In cortical bone, ovariectomy intensified resorption processes at the marrow cavity, as indicated by a decrease in endosteal transverse growth and an increase in transverse cross-section surface area of the marrow cavity in the tibia. Intensification of resorption processes was observed in cancellous bone (a statistically significant decrease in the width of trabeculae in the epiphysis and metaphysis of the femur). Structural changes in the long bones resulting from bilateral ovariectomy were manifested by deterioration of mechanical properties of the shaft and neck of the femur. The force needed to fracture the neck and shaft of the femur was significantly smaller than that in sham operated rats. Simvastatin (3 and 6 mg/kg/day *po*) slightly influenced bone remodelling in sham operated rats. Simvastatin (3 and 6 mg/kg *po* daily) administered to ovariectomized rats intensified bone formation processes and decreased bone resorption processes induced by bilateral ovariectomy, showing stronger activity at 6 mg/kg.

Key words: *simvastatin, bone, ovariectomy, rats, osteopenia*

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INTRODUCTION

Simvastatin is a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is an enzyme catalyzing a conversion of HMG-CoA into mevalonate at the initial phase of cholesterol biosynthesis. As a result of this activity, the amount of cholesterol in the liver cells decreases and the density of LDL receptors increases, leading to accelerated catabolism of LDL cholesterol. Simvastatin also attenuates the production of VLDL and triglycerides and raises HDL concentration [11]. It is used in the treatment of hypercholesterolemia and hyperlipoproteinemia type IIa and IIb, frequent in the age group with a higher risk of osteoporosis [15].

Osteoporosis is a general bone disease characterized by low bone mass and lesion of microarchitecture of trabecular bone. These changes result in the diminishing of bone endurance and, in effect, bones are susceptible to fractures. The commonest type of osteoporosis is bone loss due to estrogen deficiency after the menopause [11, 17].

A model of experimental bilateral-ovariectomy-induced osteopenia in female rats was developed to mimic the changes in the skeletal system occurring in postmenopausal women [9, 12, 34, 35]. This experimental model of osteopenia has often been used by researchers to examine both the changes in the skeletal system caused by estrogen deficiency and the effects of various drugs on these changes [9, 21–23, 34, 35].

In 1999, Mundy et al. [19] for the first time reported a beneficial influence of statin on bones in rodents. Mundy et al. [19] link this beneficial activity of statin on the skeleton with the increased expression of osseous growth factor, morphogenetic protein-2 (BMP-2) which initiates differentiation of osteoblasts. The increased bone formation following *in vivo* administration of statin was confirmed by Gutierrez et al. [10] and Wilkie et al. [33].

Some controversial results of retrospective research concerned the influence of statin on the skeleton in humans have been published recently. A beneficial influence of statin on fracture index and mineral content of bones was described by Chan et al. [3], Cummings and Bauer [6], Edwards et al. [7], Meier et al. [16], Wang et al. [31] and Chung et al. [4]. On the other hand, a lack of statin influence on bone fracture was noted by Cauley et al. [2], La Croix et al. [13], Van Sta et al. [30]. Further research is required to investigate the problem of statin influence on osteoporotic changes in the skeleton.

The aim of this paper is to examine simvastatin influence on the development of ovariectomy-induced osteopenia in rats.

MATERIALS and METHODS

Forty mature female Wistar rats used in our investigations were obtained at 3 months of age from the Central Animal Farm of the Silesian Medical University. The permission for the animal tests and experiments was given by the Bioethical Board of the Silesian Medical University. The animals were assigned to six groups of 6–7 animals per treatment group as follows: I (C) – control sham operated rats, II (S-3) – sham operated rats which were administered simvastatin at a dose of 3 mg/kg/day *po*, III (S-6) – sham operated rats which were administered simvastatin at a dose of 6 mg/kg/day *po*, IV (OVX) – ovariectomized rats, V (OVX + S-3) – ovariectomized rats which were administered simvastatin at a dose of 3 mg/kg/day *po*, VI (OVX + S-6) – ovariectomized rats which were administered simvastatin at a dose of 6 mg/kg/day *po*.

OVX, OVX + S-3 and OVX + S-6 rats were bilaterally ovariectomized under ether narcosis, using the dorsal approach [32], whereas C, S-3 and S-6 rats were subjected to a sham operation under the same conditions of anesthesia and abdominal invasion as in the case of OVX, OVX + S-3 and OVX + S-6 groups. The animals were fed on standard laboratory rodent chow (Ca 1.02%, P 0.51% and Vit D_3 100 j.m./100 g) and distilled water *ad libitum*. Each morning all the animals were weighed (Precision Advanced GT 2100-V OHAUS, accuracy 0.01 g.), immediately before administration of the tested preparations. Each morning the rats of S-3 and OVX + S-3 groups were administered intragastrically (through a stomach tube) simvastatin at a dose of 3 mg/kg in vehicle (0.5% carboxymethylcellulose solution) in a volume of 0.2 ml/100 g of body weight. The rats of S-6 and OVX + S-6 groups were administered intragastrically simvastatin at a dose of 6 mg/kg in vehicle (0.5% carboxymethylcellulose solution) in a volume of 0.2 ml/100 g of body weight. The control (C) and OVX rats were given vehicle.

Administration of simvastatin started 2 days after bilateral ovariectomy or sham operation and continued for 28 days. Twenty four hours prior and the last day administration of simvastatin, the animals were given tetracycline hydrochloride 20 mg/kg *ip* in order to mark the calcification front. Tetracycline hydrochloride was a histomorphometrical fluorescence marker [18, 27]. After 28 days of simvastatin administration, all the animals were sacrificed. The right and left tibial femoral bones, L-4 vertebrae as well as the uterus, thymus and liver were isolated. After isolation and freeing from muscular tissue, the bones and organs were weighed (Analytical Standard AS200, OHAUS, accuracy 0.0001 g). Macrometric parameters (length, diameter of the diaphysis in the mid-length) in the isolated bones were determined. A slide caliper (accuracy: 0.1 mm) was used for the macrometric measurements.

In order to determine the content of mineral substances in bones, the left tibia and femur and L-4 vertebrae were mineralized at the temperature of 640°C for 48 h and weighed using Analytical Plus, OHAUS, accuracy 0.00001 g.

The right femoral and tibial bones were used to prepare histological specimens. From the tibial bone, transverse cross-sections were made perpendicularly to the long axis, starting from the point where fibula grows into it. Three tibial slices were obtained by cutting. From the femoral bone, a longitudinal section of the distal epiphysis was made, in the medial part, in the median plane. The sections were ground on the tarnished glass. The first preparation from the tibia remained unstained. The rest of the preparations (2nd and 3rd tibial cross-section slices together with the longitudinal section slice of the femoral distal epiphysis) were stained undecalcified using the Tripp and MacKay method [28]. Staining times were subjected to the authors' own modification.

Histomorphometrical measurements were made using Optiphot 2 (Nikon) microscope, connected by RGB camera (Cohu) with a personal computer (program Lucia 3.1), with final magnifications 150× and 390×.

In the unstained preparation, the distance between the tetracycline stripes was measured, on the periosteum side and on the marrow cavity side (periosteal and endosteal transverse growth). Determinations of transverse growth of the tibia were done in UV light on unstained preparation whereas determinations of other histomorphometrical parameters were done in the visible light.

In the stained preparation of the transverse cross-section of the tibia, the width of the endosteal and periosteal osteoid was determined. In the longitudinal preparation from the femur, the width of epiphyseal cartilage and the width of trabeculae in the epiphysis and metaphysis were measured. The width of trabeculae in the epiphysis and metaphysis was measured as an arithmetic mean of the measurements of the all trabeculae within one field of view of a microscope fixed 600 μm above the cartilage (the trabeculae in the epiphysis) and 600 μm below the epiphyseal cartilage (the trabeculae in the metaphysis).

The transverse cross-section area of the cortical diaphysis and that of the marrow cavity in the tibia were measured in the stained preparation, with the use of a lanameter with magnification 50×.

Mechanical properties of the femoral shaft and neck strengths were measured using a set designed at the Department of Pharmacology, Faculty of Pharmacy, Silesian Medical University in Sosnowiec in the cooperation with Hottinger Baldwin Messtechnic GmbH, Poznań (Poland). Examinations of femoral bone strength included the determination of the femoral strain, the maximum femoral shaft load as well as the maximum femoral neck load. Briefly, the femoral shaft strength was determined by mechanical testing performed for each isolated left femur. The linearly increasing force was applied to the length midpoint vertically to the femoral shaft axis. The femur was supported at its epiphysis and at its head. A tensiometric sensor (manufactured by HBM) was used to measure the load and a WBL-4A inductive sensor (accuracy 0.05 mm; manufactured by HBM) was used to measure the bone strain.

Amplified signals from the sensors were recorded by means of XY recorder (type KP-6801A) as a function of the examined bone strain relative to the applied load. The load increased at a rate of 100 N/min.

To establish the femoral neck strength, the right femurs were isolated and tested mechanically as follows. The femoral head was cut from the bone 17 mm below the bone head. The bone head was embedded in a plastic plate using epoxy resin, to a point just underneath the lesser trochanter. The bone was embedded in a special hole whose diameter was adjusted to the bone diameter. Then, the linearly increasing force (100 N/min) was applied to the femoral head, parallel to the femoral long axis, using a curved cup to avoid local damage to the femoral head.

The results were given as arithmetic mean values ± SEM. Student's *t*-test for unpaired observations was used for estimation of statistical significance. The results for OVX, S-3, S-6 groups were compared to the ones for sham operated rats (C), whereas the results for OVX + S-3 and OVX + S-6 groups were compared with the ones for ovariectomized rats (OVX).

RESULTS

Thirty days after bilateral ovariectomy in rats, statistically significant body weight gain (by 337.44%) and statistically significant uterus mass loss (by 72.09%) as well as statistically significant thymus mass growth (by 120%) were observed, in comparison with the results obtained in sham operated rats (C) (Tab. 1). The examined bone mass was smaller than that of sham operated rats (C) and, in the case of the tibia, its mass was significantly smaller by 10.8%. Macrometrical parameters of the tibia and femur did not vary statistically significantly from the results in the sham operated rats (C). Mineral content in the tibia, femur and L-4 vertebra markedly decreased by 13.00%, 11.93% and 11.75%, respectively. Moreover, the ratio of mineral content to the mass of the femoral bone and L-4 vertebra was statistically significantly lower by 7.15% and 8.34%, respectively, when compared to the control group (C) (Tab. 1).

Histomorphometric measurements showed a statistically significant increase in the osteoid width by 30.92% in the periosteal and by 39.94% in the endosteal when compared to the results in (C) group (Tab. 2). As determined by a tetracycline method, the tibial diameter growth was statistically significantly lower by 17.62% in the periosteal and by 8.11% in the endosteal, when compared to the results in (C) group. Moreover, there was a statistically significant decrease by 9.97% in transverse cross-section surface area of the tibia and an increase by 7.69% in that of the marrow cavity in comparison with the results in (C) group. Furthermore, histomorphometric measurements showed a statistically significant decrease by 29.49% in the trabecula thickness in the epiphysis and by 31.06% in the metaphysis, compared to the results in control group (C). Additionally, the epiphyseal cartilage width was significantly widened by 23.62%, compared to the results in control group (C) (Tab. 2).

In ovariectomized rats mechanical properties of the femur ameliorated. The force required to break the femoral neck was smaller by 14.49% and the one to break the femoral shaft by 40.08%, when compared to the results in control rats (C) (Tab. 2).

Both at a dose of 3 mg/kg and 6 mg/kg *po,* simvastatin administered to sham operated rats did not statistically significantly change macrometric parameters of the long bones, masses of the examined organs and bone masses or mineral content and the ratio of mineral content to the bone mass in comparison with the results in the control (C) group (Tab. 2). No statistically significant differences were observed in the mechanical properties and in the evaluated histomorphometric parameters, except for a statistically significant increase by 11.09% in the epiphyseal cartilage width in (S-3) rats.

Both at a dose of 3 mg/kg and 6 mg/kg *po,* simvastatin administered to ovariectomized rats did not statistically significantly change the gain of body weight, uterus mass, liver mass as well as thymus mass. Macrometric parameters of the long bones were also not changed, when correlated to the results in (OVX) group.

Mineral content in the examined bones and bone mass in ovariectomized rats, which were administered simvastatin at both doses of 3 and 6 mg/kg *po*, were greater in comparison with the results in (OVX) group.

A statistically significant increase in the tibia mass and mineral content was observed. The tibia mass was greater by 11.49% in (OVX + S-3) group and by 10.88% in (OVX + S-6) group, whereas mineral content was greater by 13.63% and by 13.37%, respectively, when compared to (OVX) group (Tab. 1).

In ovariectomized rats which were administered simvastatin at both doses of 3 and 6 mg/kg *po,* histomorphometric measurements showed a statistically significant decrease in the endosteal osteoid width, by 39.75% and 29.28%, respectively, and there was also observed a statistically significant decrease in the periosteal osteoid width by 14.08% in (OVX + S-6) group, in comparison with the results obtained in (OVX) rats (Tab. 2).

The tibia mass gain in (OVX) rats, which were administered simvastatin at both doses of 3 and 6 mg/kg *po*, was greater in the periosteal and endosteal by 26.59% and 31.85%, respectively (Tab. 2).

Table 1. Effects of simvastatin administered at doses 3 and 6 mg/kg *per os* daily for 28 days on body mass and macrometrical parameters in the ovariectomized and sham operated female rats

Examined parameters		Groups					
		C	S-3	S-6	OVX	OVX + S-3	OVX + S-6
Body mass [g]	Initial [g]	227.67 ± 3.63	229.11 ± 4.89	221.85 ± 7.14	222.43 ± 4.01	223.00 ± 5.13	218.42 ± 4.39
	After 28 days [g]	237.50 ± 3.21	240.28 ± 3.26	230.05 ± 3.25	265.43 ± 4.63	262.17 ± 4.64	258.56 ± 4.85
	Increase after 28 days	9.83 ± 3.14	11.17 ± 2.98	8.20 ± 2.13	43.00 ± 4.84^{ccc}	39.17 ± 3.82	40.14 ± 4.28
Mass of examined organs [g]	Uterus	0.43 ± 0.04	0.45 ± 0.06	0.45 ± 0.09	0.12 ± 0.03^{ccc}	0.12 ± 0.02	0.17 ± 0.06
	Thymus	0.20 ± 0.03	0.27 ± 0.03	0.30 ± 0.04	0.44 ± 0.03^{ccc}	0.48 ± 0.08	0.47 ± 0.01
	Liver	7.98 ± 0.32	8.59 ± 0.31	8.55 ± 0.34	8.70 ± 0.29	9.30 ± 0.37	9.03 ± 0.32
Bone length [mm]	Tibia	36.76 ± 0.42	37.08 ± 0.27	36.26 ± 0.31	36.00 ± 0.41	36.33 ± 0.24	36.57 ± 0.24
	Femur	34.33 ± 0.44	34.80 ± 0.43	34.49 ± 0.40	33.98 ± 0.31	33.48 ± 0.29	34.24 ± 0.23
Bone diameter [mm]	Tibia	2.68 ± 0.03	2.70 ± 0.06	2.66 ± 0.04	2.61 ± 0.03	2.71 ± 0.04	2.68 ± 0.03
	Femur	3.43 ± 0.07	3.43 ± 0.07	3.44 ± 0.07	3.36 ± 0.09	3.44 ± 0.027	3.36 ± 0.07
Bone mass [mg]	Tibia	503.70 ± 10.70	522.61 ± 14.11	496.41 ± 12.20	449.31 ± 17.51^{c}	500.93 ± 11.40°	498.19 ± 6.91°
	Femur	739.00 ± 22.11	768.11 ± 21.24	736.98 ± 31.52	684.91 ± 21.23	710.72 ± 21.52	712.44 ± 15.53
	L-4 vertebra	245.71 ± 8.63	254.00 ± 11.32	258.10 ± 15.71	239.90 ± 3.57	248.11 ± 9.43	249.30 ± 9.18
Bone mineral content [mg]	Tibia	228.98 ± 5.23	240.82 ± 7.37	223.44 ± 6.12	199.23 ± 6.81^{cc}	226.38 ± 7.46°	225.87 ± 4.71°
	Femur	309.92 ± 5.91	328.64 ± 12.7	309.85 ± 11.9	272.95 ± 3.43^{ccc}	282.74 ± 3.44	284.32 ± 8.61
	L-4 vertebra	88.48 ± 1.78	88.28 ± 3.57	88.5 ± 3.45	78.08 ± 1.46^{ccc}	81.87 ± 2.63	82.27 ± 3.35
Bone mineral content/bone mass ratio	Tibia	0.45 ± 0.01	0.46 ± 0.01	0.45 ± 0.01	0.44 ± 0.01	0.45 0.01	0.45 ± 0.01
	Femur	0.42 ± 0.01	0.43 ± 0.01	0.42 ± 0.01	0.39 ± 0.01^{c}	0.40 ± 0.01	0.40 ± 0.01
	L-4 vertebra	0.36 ± 0.01	0.35 ± 0.01	0.34 ± 0.01	0.33 ± 0.01^{c}	0.33 ± 0.01	0.33 ± 0.01

Results are presented as means ± SEM (n = 6–8). Student's *t*-test for unpaired observations was used for estimation of statistical significance. ^{c} – significantly different from the sham operated group (C), ^{c} $p < 0.05$, ^{cc} $p < 0.01$, ^{ccc} $p < 0.001$. $^{\circ}$ – significantly different from the ovariectomized group (OVX), $^{\circ}$ $p < 0.05$

Table 2. Effects of simvastatin administered at doses 3 and 6 mg/kg *per os* daily for 28 days on histomorphometrical and mechanical parameters in the ovariectomized and sham operated female rats

Examined parameters		Groups					
		C	S-3	S-6	OVX	OVX + S-3	OVX + S-6
Width of tibial osteoid [μm]	Periosteal	18.82 ± 0.92	20.36 ± 1.07	18.65 ± 1.41	24.64 ± 0.99^{cc}	21.83 ± 0.76	21.17 ± 1.00°
	Endosteal	6.76 ± 0.31	6.78 ± 0.24	6.13 ± 0.18	9.46 ± 0.44^{ccc}	5.70 ± 0.09$^{\circ\circ\circ}$	6.69 ± 0.59$^{\circ\circ}$
Transverse growth of the tibia [μm]	Periosteal	67.36 ± 3.27	65.08 ± 2.93	67.36 ± 3.66	55.49 ± 1.42^{cc}	60.58 ± 5.33	61.79 ± 4.40
	Endosteal	19.85 ± 0.85	20.67 ± 0.87	20.98 ± 0.59	18.24 ± 0.37	23.09 ± 1.36°	24.05 ± 0.51$^{\circ\circ\circ}$
Transverse cross-section area of the tibial diaphysis [mm^2]		3.51 ± 0.08	3.77 ± 0.18	3.49 ± 0.01	3.16 ± 0.09^{c}	3.53 ± 0.17	3.49 ± 0.13
Transverse cross-section area of the tibial marrow cavity [mm^2]		0.78 ± 0.04	0.77 ± 0.05	0.75 ± 0.02	0.84 ± 0.02	0.78 ± 0.02	0.75 ± 0.02
Width of trabeculae in the femur [μm]	In femoral epiphysis	82.82 ± 4.53	73.04 ± 3.99	77.38 ± 5.01	58.40 ± 2.48^{ccc}	77.21 ± 2.19$^{\circ\circ\circ}$	78.00 ± 1.84$^{\circ\circ\circ}$
	In femoral metaphysis	29.81 ± 2.39	25.11 ± 0.88	24.64 ± 0.95	20.55 ± 1.11^{cc}	28.17 ± 1.04$^{\circ\circ}$	29.06 ± 3.09$^{\circ\circ}$
Width of epiphysis cartilage in the femur [μm]		76.84 ± 1.54	85.36 ± 2.66^{c}	81.26 ± 1.36	94.99 ± 5.99^{c}	90.05 ± 1.74	85.34 ± 1.30
Femoral neck strength	Max load [N]	75.16 ± 5.76	75.32 ± 5.8	71.58 ± 4.38	64.27 ± 4.92	65.95 ± 5.86	87.57 ± 4.42$^{\circ\circ}$
Femoral shaft strength	Max load [N]	72.27 ± 8.98	68.26 ± 5.48	71.84 ± 7.25	43.31 ± 1.57^{cc}	45.93 ± 3.55	51.97 ± 5.23
	Strain [mm]	0.34 ± 0.04	0.32 ± 0.02	0.31 ± 0.16	0.28 ± 0.04	0.24 ± 0.01	0.27 ± 0.17

Results are presented as means ± SEM (n = 6–8). Student's *t*-test for unpaired observations was used for estimation of statistical significance. ^{c} – significantly different from the sham operated group (C), ^{c} $p < 0.05$, ^{cc} $p < 0.01$, ^{ccc} $p < 0.001$. $^{\circ}$ – significantly different from the ovariectomized group (OVX), $^{\circ}$ $p < 0.05$, $^{\circ\circ}$ $p < 0.01$, $^{\circ\circ\circ}$ $p < 0.001$

Furthermore, histomorphometric measurements showed a statistically significant increase in the trabecula width in the femoral epiphysis by 32.21% in (OVX + S-3) group and by 33.56% in (OVX + S-6) group as well as in the femoral metaphysis by 37.08% and 41.41%, respectively, in comparison with the results obtained in (OVX) rats (Tab. 2).

Simvastatin administered at both doses to (OVX) rats ameliorated mechanical properties of the femoral shaft and neck. The force required to break the femoral neck was statistically significantly greater by 36.25% in (OVX + S-6) rats, when compared to the results in (OVX) rats (Tab. 2).

DISCUSSION

In order to elucidate simvastatin effect on the development of bilateral-ovariectomy-induced changes in the skeleton of female rats, the tibia, femur and L-4 vertebra were used. Long bone shafts are composed of compact bone, whereas in the epiphysis, metaphysis and L-4 vertebra trabecular bone dominates. Diversity of anatomical and histological structure of compact and trabecular bones determines the intensity and scope of remodelling.

Trabecular bone remodelling is more intensive than that of cortical bone. Trabecular bone remodelling takes place throughout the bone, whereas cortical bone remodelling takes place only at the endosteal surface and Haversian canals [11].

Changes in the skeleton of rats were assessed 30 days after bilateral ovariectomy, because at that stage they are characterized by the greatest dynamics, as implied by the previous studies [22].

Thirty days after bilateral ovariectomy in rats, characteristic features of osteoporosis developed, however, no spontaneous fractures were observed. The obtained results of histomorphometric measurements indicated disturbances in osseous tissue remodelling, both in the cortical and trabecular bones. In the cortical bone, ovariectomy disturbed bone formation process as implied by a decrease in periosteal tibial diameter growth determined by a tetracycline method as well as a decrease in transverse cross-section surface area of the tibial shaft cortex at the point where the fibula grows into the tibia. Furthermore, ovariectomy inhibited periosteal osteoid mineralization, as manifested by a statistically significant increase in the osteoid width and reduced mineral content in the tibia.

In cortical bone, ovariectomy intensified resorption processes at the marrow cavity, as indicated by a decrease in endosteal transverse growth and an increase in transverse cross-section surface area of the tibial marrow cavity in the tibia.

Intensification of resorption processes was observed in cancellous bone (a statistically significant decrease in the width of trabeculae in the epiphysis and metaphysis of the femur). Statistically significant thickening of the epiphyseal cartilage in the femur was observed in ovariectomized rats, which may result from disturbances in cartilage ossification due to estrogen deficiency [11].

The results obtained in the present study investigating the effects of bilateral ovariectomy in rats on histomorphometric parameters of the long bones, account for development of osteoporotic changes in the skeleton, and they are in agreement with the results presented in other available publications [12, 21–23, 34, 35].

Structural changes in the long bones resulting from bilateral ovariectomy are manifested by deterioration of mechanical properties of the shaft and neck of the femur. The force needed to fracture the neck and shaft of the femur was significantly smaller than that in sham operated rats.

Experiment bilateral ovariectomy also induced body weight gain, uterus mass loss and thymus mass gain, and all these effects were statistically significant. The observed changes may have resulted from estrogen deficiency, and the obtained results are in agreement with those of other researchers [12, 35].

In order to elucidate simvastatin effect on the development of osteoporotic changes in the skeleton, sham operated and ovariectomized rats were administered simvastatin at doses of 3 and 6 mg/kg *po* for 28 days. A 3 mg/kg dose was considered optimal dose (20 mg/day) whereas 6 mg/kg dose corresponded to maximum dose (40 mg/day) calculated per one kilogram of the body weight in humans taking into account the (× 10) factor based on the fact that metabolic processes in rodents are 10 times faster than in humans [1, 20].

Simvastatin administered at both doses to sham operated rats did not significantly alter bone formation processes in cortical and trabecular bones, compared to the results in control (C) rats.

Simvastatin administered (at doses of 3 and 6 mg/kg *po*) to ovariectomized rats prevented the development of osteoporotic changes in the skeleton induced by bilateral ovariectomy.

In (OVX) rats, simvastatin (at both doses) increased bone formation processes and mineralization of cortical bone, as indicated by a decrease in the periosteal osteoid width, an increase in periosteal bone growth, an increase in transverse cross-section surface area of the cortical shaft in the tibia, and a statistically significant increase in mineral content in the tibia. In cortical bones of ovariectomized rats, which were given simvastatin, an inhibition of resorption processes in the marrow cavity was observed, as manifested by a statistically significant increase in tibia diameter growth in the endosteal and a statistically significant increase in the transverse cross-section surface area of the marrow cavity in the tibia, measured at the point where the fibula grows into the tibia. Simvastatin at both doses administered to rats intensified bone formation processes and/or inhibited resorption processes in trabecular bones, as indicated by a statistically significant increase in the trabecula width at the epiphysis and metaphysis of the femur. Furthermore, simvastatin improved mechanical properties of the femur in ovariectomized rats. We also observed an increase in maximum load required to fracture the femoral neck and shaft, and in the case of the femoral neck in ovariectomized rats which were given simvastatin at the dose of 6 mg/kg, the increase was statistically significant. The obtained results are in contrast to those of other authors [19].

Mundy et al. [19] link bone formation increased by statins to the increased expression of a bone growth factor, morphogenetic protein-2 (BMP-2), which initiates osteoblast differentiation. Antiresorption activity of simvastatin can result from inhibition of cholesterol synthesis. Bisphosphonates, especially aminobisphosphonates show antiresorption activity, the mechanism of which is based on impeding enzymes (synthases) of mevalonic pathway responsible for izoprenoid synthesis, namely farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP) [5, 8, 25, 29]. Both FPP and GGPP are indispensible for prenylation of small GTP-binding proteins, such as Rho, Rac, Ras, Rab, responsible for the formation, functioning and shape of osteoclast cell membrane [14, 24, 26]. As a result of the impeded prenylation of the mentioned above signalling proteins of GTP-az family, aminobisphosphonates induce apoptosis of osteoclasts and decrease bone resorption [14, 24, 26].

Moreover, the inhibition of HMG-CoA reductase by simvastatin decreases mevalonian availability and, consequently, reduces contents of other products of mevalonic pathway, namely izoprenoids, FPP, GGPP, which are indispensible for prenylation of small GTP-binding proteins. By attenuating prenylation of signalling proteins of GTP-az family, such as aminobisphosphonates, simvastatin may induce apoptosis of osteoclasts and hinder bone resorption processes.

In conclusion, the presented data demonstrated that in mature female rats, bilateral ovariectomy induced osteopenic skeletal changes characterized by disturbances in bone formation processes and intensification of bone resorption processes. Furthermore, our results clearly show that simvastatin (3 and 6 mg/kg *po* daily) administered to ovariectomized rats intensified bone formation processes and decreased bone resorption processes induced by bilateral ovariectomy, showing stronger activity at 6 mg/kg.

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