

EFFECTS OF TIBOLONE ON THE DEVELOPMENT OF OSTEOPENIA INDUCED BY OVARIECTOMY IN RATS

Maria Pytlik

Department of Pharmacology, Silesian Medical University, Jagiellońska 4, PL 41-200 Sosnowiec, Poland

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Experimental osteopenia caused by ovariectomy in rats may reflect postmenopausal bone changes, which are the effect of osteogen deficiency. The aim of the present study was to investigate the effects of tibolone (0.25 mg/kg/day and 2.5 mg/kg *po*) administered for 4 weeks on the development of osteopenia caused by bilateral ovariectomy in 3-month-old female Wistar rats.

The experiments were carried out on six groups of animals: I (C) – control sham operated rats, II (OVX) – ovariectomized rats, III (OVX+T-0.25) – ovariectomized rats which were administered tibolone at a dose of 0.25 mg/kg, IV (OVX+T-2.5) – ovariectomized rats which were administered tibolone at a dose of 2.5 mg/kg, V (T-0.25) – sham operated rats which were administered tibolone at a dose of 0.25 mg/kg, VI (T-2.5) – sham operated rats which were administered tibolone at a dose of 2.5 mg/kg. The following parameters were examined in all the groups: body weight gain, bone mass, length and diameter, mineral and calcium contents in the tibia and femur, endosteal and periosteal transverse growth, endosteal and periosteal osteoid width, transverse cross-section area of the cortical diaphysis and that of the marrow cavity in the tibia, epiphyseal cartilage width, trabeculae width in the epiphysis and metaphysis of the femur. Mechanical properties of the femur were also studied. Bilateral ovariectomy induced osteopenic skeletal changes in mature female rats. Tibolone (0.25 mg/kg/day and 2.5 mg/kg/day *po*) administered to ovariectomized rats for 28 days decreased the development of osteopenic skeletal changes induced by bilateral ovariectomy.

Key words: *tibolone, osteopenia, ovariectomy, bones, rats*

INTRODUCTION

Osteoporosis is a general bone disease characterized by low bone mass and lesion of microarchitecture of trabecular bone [10, 19, 22]. 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 [10, 16, 23].

A model of experimental bilateral-ovariectomy-induced osteopenia in female rats causes changes in the skeletal system similar to those occurring in postmenopausal women [8, 11, 30, 31]. Experimental models of osteopenia have often been used by numerous researchers [8, 11, 12, 20, 21, 30–32] to examine the changes in the skeletal system as well as effects of various drugs on these changes.

Tibolone, (7 α , 17 α)-17-hydroxy-7-methyl-19-norpregn-5(10)-en-20-yn-3-one, is a synthetic steroid with estrogenic, gestagenic and androgenic properties [6, 28]. It is metabolized to three active tissue-specific metabolites: 3 α -OH tibolone, 3 β -OH tibolone and Δ -4 tibolone. Especially 3 α -OH tibolone and 3 β -OH tibolone show affinity for estrogen receptor, while Δ -4 tibolone binds to androgen and progesterone receptors [15]. Tibolone is used in the treatment of post-menopausal disturbances as hormone replacement therapy. The drug relieves climacteric symptoms, especially vasomotor ones (hot flash, drenching sweat). It also prevents bone loss, does not stimulate the endometrium and does not cause progesterone-induced menstrual bleeding [1–5, 9, 13–15, 18, 25, 26].

There are no available publications on the influence of that drug on the development of osteopenia in women; therefore, the aim of this study was to examine the effect of tibolone (0.25 mg/kg and 2.5 mg/kg *po*) administered for 4 weeks on the development of osteopenia induced by bilateral ovariectomy in 3-month-old female rats.

MATERIALS and METHODS

Forty-four mature female Wistar rats used in our investigations were obtained at 3 months of age from Central Animal Farm, Silesian Medical University. The permission for the animal tests and experiments was given by the Bioethical Board of Silesian Medical University. The animals were assigned to six groups of 6–10 animals per treatment

group as follows: I (C) – control sham operated rats, II (OVX) – ovariectomized rats, III (OVX+T-0.25) – ovariectomized rats which were administered tibolone at a dose of 0.25 mg/kg/day *po*, IV (OVX+T-2.5) – ovariectomized rats which were administered tibolone at a dose of 2.5 mg/kg/day *po*, V (T-0.25) – sham operated rats which were administered tibolone at a dose of 0.25 mg/kg/day *po*, VI (T-2.5) – sham operated rats which were administered tibolone at a dose of 2.5 mg/kg/day *po*.

OVX, OVX+T-0.25 and OVX+T-2.5 rats were bilaterally ovariectomized under ether narcosis, using the dorsal approach [29], whereas C, T-0.25 and T-2.5 rats were subjected to a sham operation under the same conditions of anesthesia and abdominal invasion as in the case of OVX, OVX+T-0.25 and OVX+T-2.5 groups. The animals were fed on standard laboratory rodent chow (Ca 1.02%, P 0.51% and Vit D₃ 100 j.m./100 g) and distilled water *ad libitum*. Each morning all the animals were weighed immediately before administration of the tested preparations. That enabled us to determine an increase in rat's body weight and to administer a proper tibolone dose, with respect to body weight.

Each morning the rats of OVX+T-0.25 and T-0.25 groups were administered intragastrically (through a stomach tube) tibolone at a dose of 0.25 mg/kg in vehicle (0.5% carboxymethylcellulose solution) in a volume of 0.2 ml/100 g of body weight. The rats of OVX+T-2.5 and T-2.5 groups were administered intragastrically tibolone at a dose of 2.5 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 tibolone started 2 days after bilateral ovariectomy or sham operation and continued for 28 days. Twenty four hours prior to administration of tibolone and 24 h before the animals were killed, all the rats were administered *ip* 20 mg/kg of tetracycline hydrochloride in order to determine the tibial bone diameter growth. Tetracycline hydrochloride was a histomorphometric fluorescence marker [17, 24]. After 28 days of administration of tibolone all the animals were killed by spinal cord dislocation. Then, the tibias, femurs, L-4 vertebrae as well as the uterus and thymus were isolated. After isolation and freeing from muscular tissue, the bones and organs were weighed.

In the isolated bones, mass and macroscopic parameters were determined (length, diameter of the diaphysis in the mid-length).

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. The mineralized bones were dissolved in 6 M HCl and then calcium content in the bone mineral was assayed by a colorimetric method (Pointe Scientific standard kit).

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. 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 were stained using the Tripp and MacKay method [27].

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

In the unstained preparation, the distance between the tetracycline stripes was measured, on the periosteum side and on marrow cavity side (periosteal and endosteal transverse growth).

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 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 (50× magnification).

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 Messtechnik GmbH, Poznań (Poland). Examinations of femoral bone strength included the determination of the femoral strain, the maximum femoral shaft load (the force causing femoral shaft fracture) the femoral maximum strain as well as the maximum femoral neck load (the force causing

femoral neck fracture). 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 (exact to 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 rate was 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 \pm SEM. The Student's *t*-test for unpaired observations was used for estimation of statistical significance. The results for OVX, T-0.25, T-2.5 groups were compared to the ones for sham operated rats (C), whereas the results for OVX+T-0.25 and OVX + T-2.5 groups were compared with the ones for ovariectomized rats (OVX).

RESULTS

The results are presented in Tables 1–3.

Statistically significant body weight gain (by 319.91%) and statistically significant uterus mass loss (by 73.92%) as well as statistically significant thymus mass growth (by 88.00%) were observed 30 days after bilateral ovariectomy, in comparison with the results obtained in sham operated rats (C) (Tab. 1).

The examined bone mass as well as the tibial and femoral bone length and diameter in ovariectomized rats were not significantly different from those in sham operated rats (C). Mineral content in the tibia, femur and L-4 vertebra was decreased, and the ratio of mineral content to the long bone mass

was diminished, with that of L-4 vertebra being statistically significantly lower by 12.50% when compared to control rats (C). Moreover, a statistically significant decrease in calcium content in L-4 vertebra by 19.74% was observed, in comparison to the results in control group (C) (Tab. 1).

Histomorphometric measurements showed a statistically significant decrease in osteoid width by 46.27% in the periosteal and by 16.09% in the en-

dosteal (Tab. 2). As determined by a tetracycline method, tibia growth was not significantly different in the periosteal, however, there was a statistically significant decrease by 31.14% in the endosteal, compared to the results in control group (C). Furthermore, transverse cross-section area of the marrow cavity was greater and a statistically significant decrease by 19.24% in trabeculae thickness in the epiphysis of the femur and by 21.48% in the

Table 1. Effects of tibolone administered at the doses of 0.25 mg/kg and 2.5 mg/kg *per os* daily for 28 days on body weight and macrometrical parameters in the ovariectomized and sham operated female rats

Examined parameters		Groups					
		C	OVX	OVX+T-0.25	OVX+T-2.5	T-0.25	T-2.5
Body weight (g)	Initial (g)	236.06 ± 1.20	229.48 ± 6.49	231.90 ± 5.76	235.85 ± 2.17	241.33 ± 3.28	243.27 ± 2.67
	After 28 days (g)	245.72 ± 4.05	269.96 ± 8.23	234.14 ± 3.49	220.48 ± 7.17	250.42 ± 3.58	234.57 ± 4.63
	Increase after 28 days	9.64 ± 2.79	40.48 ± 4.70 ^{ccc}	2.24 ± 1.61 ^{ooo}	-15.57 ± 6.50 ^{ooo}	9.08 ± 2.86	-8.70 ± 2.35 ^c
Mass of the examined organs (g)	Uterus	0.46 ± 0.07	0.12 ± 0.02 ^{ccc}	0.23 ± 0.02 ^{oo}	0.26 ± 0.01 ^{ooo}	0.34 ± 0.04	0.35 ± 0.17
	Thymus	0.25 ± 0.04	0.47 ± 0.03 ^{ccc}	0.27 ± 0.03 ^{oo}	0.19 ± 0.02 ^{ooo}	0.24 ± 0.02	0.26 ± 0.03
Bone mass (g)	Tibia	0.53 ± 0.02	0.51 ± 0.02	0.52 ± 0.02	0.55 ± 0.01	0.56 ± 0.02	0.55 ± 0.01
	Femur	0.76 ± 0.04	0.76 ± 0.03	0.76 ± 0.02	0.77 ± 0.02	0.80 ± 0.04	0.80 ± 0.03
	L-4 vertebra	0.25 ± 0.01	0.28 ± 0.04	0.29 ± 0.05	0.30 ± 0.02	0.29 ± 0.02 ^c	0.29 ± 0.01 ^c
Bone length (cm)	Tibia	3.61 ± 0.03	3.64 ± 0.05	3.73 ± 0.04	3.70 ± 0.02	3.70 ± 0.03	3.71 ± 0.04
	Femur	3.35 ± 0.09	3.38 ± 0.10	3.48 ± 0.02	3.46 ± 0.03	3.45 ± 0.03	3.49 ± 0.02
Bone diameter (cm)	Tibia	0.28 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.28 ± 0.01	0.28 ± 0.01
	Femur	0.34 ± 0.01	0.33 ± 0.01	0.34 ± 0.01	0.36 ± 0.01 ^o	0.34 ± 0.01	0.35 ± 0.01
Mineral contents (g)	Tibia	0.24 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.26 ± 0.01
	Femur	0.34 ± 0.01	0.32 ± 0.01	0.33 ± 0.01	0.34 ± 0.01	0.36 ± 0.01	0.36 ± 0.01
	L-4 vertebra	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.02	0.10 ± 0.01	0.10 ± 0.005 ^c	0.10 ± 0.004 ^c
Ratio of mineral content to bone mass	Tibia	0.45 ± 0.01	0.45 ± 0.01	0.46 ± 0.02	0.45 ± 0.02	0.46 ± 0.01	0.47 ± 0.01
	Femur	0.45 ± 0.01	0.42 ± 0.01	0.43 ± 0.01	0.44 ± 0.01	0.45 ± 0.01	0.45 ± 0.01
	L-4 vertebra	0.32 ± 0.01	0.28 ± 0.02 ^c	0.28 ± 0.01	0.33 ± 0.01 ^o	0.34 ± 0.01	0.34 ± 0.02
Ca contents (mg/g of minerals)	Tibia	349.72 ± 21.40	327.92 ± 26.70	351.10 ± 22.42	359.76 ± 24.20	358.29 ± 31.40	363.72 ± 20.22
	Femur	385.61 ± 18.60	367.13 ± 27.21	370.21 ± 27.80	382.12 ± 19.30	384.90 ± 32.50	394.16 ± 23.70
	L-4 vertebra	392.58 ± 29.81	315.11 ± 12.03 ^{cc}	347.35 ± 27.40	345.24 ± 21.40	391.20 ± 18.90	398.26 ± 24.80

Results are presented as means ± SEM (n = 6–10). Student's *t*-test for unpaired observations was used for estimation of statistical significance. ^c – significantly different from the control group (C), ^c p < 0.05; ^{cc} p < 0.01; ^{ccc} p < 0.001; ^o – significantly different from the ovariectomized group (OVX), ^o p < 0.05; ^{oo} p < 0.01; ^{ooo} p < 0.001

Table 2. Effects of tibolone administered at the doses of 0.25 mg/kg and 2.5 mg/kg *per os* daily for 28 days on histomorphometrical parameters in the ovariectomized and sham operated female rats

Examined parameters		Groups					
		C	OVX	OVX+T-0.25	OVX+T-2.5	T-0.25	T-2.5
Width of tibial osteoid (μm)	periosteal	15.43 \pm 1.29	22.57 \pm 1.07 ^{ccc}	13.83 \pm 0.75 ^{ooo}	12.94 \pm 0.40 ^{ooo}	12.46 \pm 0.32	13.36 \pm 0.38
	endosteal	8.45 \pm 0.20	9.81 \pm 0.36 ^c	9.83 \pm 0.44	9.55 \pm 0.26	9.14 \pm 0.39	9.25 \pm 0.25 ^c
Transverse growth of cortical bone in tibia	periosteal	46.41 \pm 3.90	42.39 \pm 3.16	55.30 \pm 4.90 ^o	58.27 \pm 4.50 ^{oo}	51.24 \pm 2.33	56.51 \pm 3.25 ^{cc}
	endosteal	26.53 \pm 1.19	18.27 \pm 0.47 ^{ccc}	25.78 \pm 1.71 ^{oo}	29.17 \pm 3.29 ^{ooo}	25.24 \pm 0.46	26.51 \pm 2.35
Transverse cross-section area of the tibial shaft cortex (mm^2)		3.65 \pm 0.12	3.51 \pm 0.11	3.55 \pm 0.13	3.89 \pm 0.07 ^o	3.76 \pm 0.15	3.84 \pm 0.15
Transverse cross-section area of the tibial bone marrow cavity (mm^2)		0.81 \pm 0.05	0.89 \pm 0.06	0.87 \pm 0.03	0.83 \pm 0.07	0.80 \pm 0.02	0.74 \pm 0.02
Width of trabeculae in femoral bone (μm)	in femoral epiphysis	71.78 \pm 1.82	57.97 \pm 1.13 ^{ccc}	66.88 \pm 1.94 ^{oo}	70.24 \pm 1.24 ^{ooo}	75.91 \pm 1.30	75.98 \pm 0.95
	in femoral metaphysis	49.40 \pm 1.33	38.79 \pm 1.03 ^{ccc}	46.28 \pm 1.14 ^{ooo}	46.79 \pm 1.07 ^{ooo}	49.08 \pm 1.71	48.54 \pm 1.12
Width of epiphyseal cartilage of femoral bone (μm)		62.85 \pm 2.76	76.40 \pm 1.47 ^{cc}	68.17 \pm 4.68	74.69 \pm 4.59	64.39 \pm 3.06	61.40 \pm 5.15

Results are presented as means \pm SEM (n = 6–10). Student's *t*-test for unpaired observations was used for estimation of statistical significance. ^c – significantly different from the control group (C), ^c p < 0.05; ^{cc} p < 0.01; ^{ccc} p < 0.001; ^o – significantly different from the ovariectomized group (OVX), ^o p < 0.05; ^{oo} p < 0.01; ^{ooo} p < 0.001

metaphysis was observed, compared to the results in control group (C). Epiphyseal cartilage width was significantly widened by 21.56%, compared to the results in control group (C) (Tab. 2). The deterioration of mechanical properties of the femur was observed in ovariectomized rats (OVX). The femoral shaft and neck were fractured by a force statistically smaller by 33.88% and 9.83%, respectively, compared to the results in control group (C).

In sham operated rats, tibolone administered at a dose of 0.25 mg/kg *po* did not change body weight gain, however, at a dose of 2.5 mg/kg *po* tibolone markedly decreased body weight in comparison with control rats (C). Tibolone, both at a dose of 0.25 mg/kg and 2.5 mg/kg *po*, did not markedly change uterus and thymus masses in sham operated rats, compared to control rats (C), however, tibolone insignificantly increased the tibia and femur masses. In addition, a statistically insignificant increase in mineral content, a larger ratio of mineral content to body mass and elevated calcium content was observed in the tibia and femur. Furthermore, a statistically insignificant in-

crease in the examined bones length and diameter was observed in comparison with sham operated (C) rats. However, marked differences were observed in L-4 vertebra mass and in mineral content in L-4 vertebra after tibolone administration, both at a dose of 0.25 mg/kg and 2.5 mg/kg *po*. L-4 vertebra mass increased by 17.4% and 17.81% in T-0.25 and T-2.5 groups, respectively, whilst mineral content in L-4 vertebra increased by 23.81% and 21.43% in the abovementioned groups of rats, respectively, compared to sham operated (C) rats (Tab. 1).

Histomorphometric measurements showed an insignificant decrease in periosteal osteoid width in sham operated rats which were administered tibolone, and a statistically significant increase in periosteal tibia growth by 21.76% in comparison with the results obtained in control rats (C). Other parameters assessed in the histomorphometric measurements did not vary in a statistically significant way (Tab. 2).

In sham operated rats, which were administered tibolone, the mechanical properties of the femoral

Table 3. Effects of tibolone administered at the doses of 0.25 mg/kg and 2.5 mg/kg *per os* daily for 28 days on mechanical parameters in the ovariectomized and sham operated rats

Examined parameters		Groups					
		C	OVX	OVX+T-0.25	OVX+T-2.5	T-0.25	T-2.5
Femoral neck strength	Max load (N)	107.92 ± 1.66	97.32 ± 5.07 ^c	107.57 ± 3.64	109.75 ± 3.26	108.00 ± 2.56	110.48 ± 3.28
Femoral shaft strength	Max load (N)	94.14 ± 3.68	77.07 ± 2.17 ^{cc}	94.33 ± 3.25 ^{oo}	109.67 ± 3.36 ^{ooo}	102.37 ± 1.61	107.68 ± 4.14
	Strain (mm)	0.42 ± 0.02	0.39 ± 0.01	0.41 ± 0.01	0.40 ± 0.02	0.41 ± 0.01	0.37 ± 0.02
	Force causing fracture (N)	86.59 ± 3.80	57.26 ± 3.45 ^{cc}	84.53 ± 4.07 ^{oo}	94.43 ± 2.84 ^{ooo}	98.45 ± 3.28	102.08 ± 5.34
	Max strain (mm)	0.47 ± 0.01	0.48 ± 0.01	0.46 ± 0.01	0.45 ± 0.01	0.46 ± 0.01	0.44 ± 0.01

Results are presented as means ± SEM (n = 6–10). Student's *t*-test for unpaired observations was used for estimation of statistical significance. ^c – significantly different from the control group (C), ^c p < 0.05; ^{cc} p < 0.01; ^{ccc} p < 0.001; ^o – significantly different from the ovariectomized group (OVX), ^o p < 0.05; ^{oo} p < 0.01; ^{ooo} p < 0.001

bone ameliorated; however, the assessed parameters were not significantly different in comparison with the results in control rats (C) (Tab. 3).

In ovariectomized rats, treated with tibolone at a dose 0.25 mg/kg and 2.5 mg/kg *po*, a marked reduction in body weight gain was evidenced in comparison with the results obtained in ovariectomized (OVX) rats (Tab. 1). Moreover, in OVX+T-2.5 rats a reduction in body weight gain relative to the initial body weight was evidenced. In comparison with ovariectomized (OVX) rats, tibolone administered to ovariectomized rats both at a dose of 0.25 mg/kg and 2.5 mg/kg *po*, induced a statistically significant increase in uterus mass by 91.66% and by 166.66%, respectively. However, thymus mass was markedly decreased by 42.56% in OVX+T-0.25 rats and by 59.58% in OVX+T-2.5 rats in comparison with ovariectomized (OVX) rats (Tab. 1).

In ovariectomized rats treated with tibolone both at a dose 0.25 mg/kg and 2.5 mg/kg *po*, there was evidenced a marked increase in the tibia, femur and L-4 vertebra masses, and increased mineral content in the examined bones, a larger ratio of mineral content to bone mass, elevated calcium content as well as the increased tibial and femoral length and diameter in comparison with results obtained in ovariectomized (OVX) rats (Tab. 1).

Histomorphometric measurements demonstrated a statistically significant decrease in periosteal width by 38.73% and 42.67% and a statistically significant increase in periosteal tibia growth by

30.45% and 37.46% as well as in the endosteal surface by 41.10% and 59.66% in OVX+T-0.25 and OVX+T-2.5 rats, respectively, in comparison with the results obtained in ovariectomized (OVX) rats. Compared to ovariectomized (OVX) rats, in the epiphysis and metaphysis of the spongy femur, there was the statistically significant thickening of trabeculae by 15.37% in OVX+T-0.25 rats and by 21.17% in OVX+T-2.5 rats, and by 19.31% in OVX+T-0.25 rats and by 20.62% in OVX+T-2.5 rats (Tab. 2).

The assessment of mechanical properties of the femoral shaft and neck indicated that the maximum load required to fracture the femoral neck was greater by 10.53% in OVX+T-0.25 rats and by 12.77% in OVX+T-2.5 rats, while the maximum load required to fracture the femoral shaft was markedly greater by 22.39% in OVX+T-0.25 rats and by 42.30% in OVX+T-2.5 rats. The force imposed at the time of fracture of the femoral shaft was also markedly greater by 47.62% in OVX+T-0.25 rats and by 64.91% in OVX+T-2.5 rats, in comparison with the results obtained in ovariectomized (OVX) rats (Tab. 3).

DISCUSSION

Osseous tissue redevelopment depends on resorption processes and concomitant ossification processes, and appropriate balance of these two processes guarantees that the skeleton mass is pre-

served and the renewal of the mineralized matrix takes place. From the birth to the maturity, bone formation process dominates in redevelopment process, which results in bone growth. However, with ageing resorption processes start to dominate and gradual bone loss begins [10].

In postmenopausal women, bone loss is accelerated as a result of the diminishing of ovaries' hormonal activity. Estrogen deficiency, especially 17β -estradiol, brings about a reduction of osteoblast activity. At the same time, osteoclast activity is intensified as a result of the lack of its suppression by osteoblast in a paracrine way as well as due to the lack of the suppression of stimulating activity of parathormon on osteoclast by estrogen. Calcitonin secretion is also reduced [10]. These disturbances entail intensification of bone loss in postmenopausal women, which, in turn, evokes osteoporosis characterized by bone mass loss, lension of its microarchitecture and greater susceptibility to fractures [10, 16, 19, 22, 23].

A model of experimental bilateral-ovariectomy-induced osteopenia in female rats allows to observe changes in the skeletal system similar to those occurring in postmenopausal women [8, 11, 30, 31]. Experimental models of osteopenia have often been used by numerous researchers to examine the changes in the skeletal system as well as the effects of various drugs on these changes [7, 8, 11, 12, 20, 21, 30–32].

In order to elucidate tibolone effect on the development of changes in the skeleton in ovariectomized female rats, the tibia, femur and L-4 vertebra were used. The long bone shafts are composed of compact bone, whereas in 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 the redevelopment. Trabecular bone redevelopment is more intensive than that of cortical bone. Trabecular bone redevelopment takes place throughout the bone, whereas cortical bone redevelopment takes place only at the endosteal surface and Haversian canals [10].

Development of changes in the skeleton of rats was assessed 30 days after bilateral ovariectomy, because the changes in osseous tissue at that stage are characterized by the greatest dynamic. Furthermore, prolonging the time of the experiment to 6–8 weeks did not intensify the changes, as also indicated by other researchers [21].

Thirty days after bilateral ovariectomy, characteristic features of osteoporosis became apparent, however, no spontaneous fractures were observed. The obtained results of histomorphometric measurements indicated disturbances in osseous tissue redevelopment, both in the cortical and trabecular bones.

In the cortical bone, ovariectomy disturbed bone formation process (a decrease in periosteal tibial diameter growth, as determined by a tetracycline method; a decrease in cross-section surface area of the tibial shaft cortex at the point where the fibula grows into the tibia) as well as it inhibited osteoid mineralization, as indicated by a statistically significant increase in periosteal osteoid width, decreased mineral content and calcium content in the tibia. In the cortical bone, ovariectomy intensified resorption processes at the marrow cavity, as indicated by a decrease in endosteal tibia growth and an increase in cross-section surface area of the tibial marrow cavity at the point where the fibula grows into the tibia. Intensification of resorption processes was observed in cancellous bone (epiphysis and metaphysis of the femur) 30 days after ovariectomy, as indicated by a statistically significant decrease in trabecula thickness.

The thickening of the epiphyseal cartilage in the femur was observed in ovariectomized rats, what may result from disturbances in cartilage ossification due to estrogen deficiency [10].

The effects of bilateral ovariectomy in rats on histomorphometric parameters of the long bones, demonstrated in the present study, account for development of osteoporotic changes in osseous tissue, and they are in agreement with the results reported in other available publications [11, 20, 21, 30, 31]. The structural changes in the long bones resulting from bilateral ovariectomy manifested themselves as deterioration of mechanical properties of the shaft and neck of the femur. The load needed to fracture the neck and shaft of the femur was significantly smaller than in control rats.

Within 30 days after the operation, bilateral ovariectomy also induced statistically significant body weight gain, statistically significant uterus mass loss and statistically significant thymus mass gain. The observed changes resulted from estrogen deficiency and the obtained results are in agreement with those of other researchers [11, 20, 21, 30, 31].

In order to elucidate tibolone effect on the development of bilateral-ovariectomy-induced osteopenia in rats, the sham operated and ovariectomized animals were administered tibolone at a dose of 0.25 mg/kg and at a dose of 2.5 mg/kg *po* for 28 days. Tibolone administered (at both doses) to sham operated rats insignificantly intensified bone formation processes and mineralization in compact and cancellous bones, as indicated by macrometric and histomorphometric parameters as well as by the evaluation of mechanical properties of the femur.

Tibolone administered (at the doses of 0.25 mg/kg and 2.5 mg/kg *po*) to ovariectomized rats prevented the development of osteoporotic changes in the skeleton induced by bilateral ovariectomy. Tibolone at both doses increased bone formation processes and mineralization of the cortical bone, as indicated by histomorphometric measurements (i.e., a statistically significant decrease in periosteal osteoid width, a statistically significant increase in endosteal and periosteal tibia growth, an increase in transverse cross-section area of the tibial shaft, and a decrease in transverse cross-section area of the bone marrow in the tibia). Intensification of bone formation processes and/or inhibition of resorption processes in trabecular bones was observed, as indicated by a statistically significant increase in trabeculae width at the epiphysis and metaphysis of the femur, in comparison with the ovariectomized rats.

In ovariectomized rats, tibolone also improved mechanical properties of femoral shaft and neck, and the effect depended on the administered doses. The prevention of the development of osteoporotic changes in ovariectomized rats' skeletons resulted from estrogenic activity of tibolone metabolites (3 α and 3 β -OH). Moreover, estrogenic activity of the drug was indicated by statistically significant uterus mass gain and statistically significant thymus mass loss. The obtained results are in agreement with those reported by other authors [12, 28, 32]. In summary, the present study indicated that tibolone administered (at the doses of 0.25 mg/kg and 2.5 mg/kg *po* daily) for 28 days to ovariectomized rats prevented the development of ovariectomy-induced osteoporotic changes in the skeleton.

CONCLUSIONS

1. Bilateral ovariectomy induced osteopenic skeletal changes in mature female rats.

2. Tibolone (0.25 mg/kg and 2.5 mg/kg *po* daily) administered to ovariectomized rats for 28 days decreased the development of osteopenic skeletal changes induced by bilateral ovariectomy.

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