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EFFECT OF CONCURRENT ADMINISTRATION OF ALENDRONATE SODIUM AND RETINOL ON DEVELOPMENT OF CHANGES IN HISTOMORPHOMETRIC PARAMETERS OF BONES INDUCED BY OVARIECTOMY IN RATS

Maria Pytlik[#], Ilona Kaczmarczyk-Sedlak, Leszek Śliwiński, Waldemar Janiec, Igor Rymkiewicz

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

Effect of concurrent administration of alendronate sodium and retinol on development of changes in histomorphometric parameters of bones induced by ovariectomy in rats. M. PYTLIK, I. KACZMARCZYK-SEDLAK, L. ŚLIWIŃSKI, W. JANIEC, I. RYMKIEWICZ. Pol. J. Pharmacol., 2004, 56, 571–579.

Retinol is a commonly used vitamin, especially by elderly people. Alendronate sodium, an aminobisphosphonate, is a potent antiresorptive drug used in the treatment of osteoporosis in postmenopausal women. Frequently, alendronate sodium and retinol are used concurrently. There are no reports on the interaction between alendronate sodium and retinol.

The aim of the present study was to investigate the effect of concurrent administration of alendronate sodium and retinol on bone remodeling in ovariectomized rats. The histomorphometric parameters of long bones were studied.

The experiments were carried out on 3-month-old Wistar rats, divided into 7 groups: I (C) – sham operated control rats, II (OVX) – ovariectomized control rats, III (OVX + ALN) – ovariectomized rats + alendronate sodium (3 mg/kg *po*), IV (OVX + R-1) – ovariectomized rats + retinol (700 IU/kg *po*), V (OVX + R-2) – ovariectomized rats + retinol (3500 IU/kg *po*), VI (OVX + ALN + R-1) – ovariectomized rats + alendronate sodium (3 mg/kg *po*) + retinol (700 IU/kg *po*), VII (OVX + ALN + R-2) – ovariectomized rats + alendronate sodium (3 mg/kg *po*) + retinol (3500 IU/kg *po*). The drugs were administered to the rats daily by oral gavage (alendronate sodium in the morning, retinol in the afternoon) for 28 days. Body mass gain, bone mass, mineral content in the tibia, femur and L-4 vertebra, histomorphometric parameters of the right tibia (width of osteoid, periosteal and endosteal transverse growth, area of the transverse cross section of the bone marrow cavity and the cortical bone) and the right femur (width of epiphyseal and metaphyseal trabeculae, width of epiphyseal cartilage) were studied.

Bilateral ovariectomy induced osteopenic skeletal changes in mature female rats. Alendronate sodium administered at a dose of 3 mg/kg *po* daily inhibited the development of changes induced by ovariectomy in the skeletal system of rats. Retinol, especially administered at the dose of 3500 IU/kg daily, intensified the changes in the osseous system caused by estrogen deficiency in rats. Retinol administered concurrently with alendronate sodium attenuated the antiresorptive effect of alendronate sodium on the skeletal system in ovariectomized rats.

***Key words**: alendronate sodium, retinol, ovariectomy, bones, rats, osteoporosis*

[#] *correspondence*

INTRODUCTION

Estrogen deficiency, and in particular 17β-estradiol deficiency, in postmenopausal women results in the development of osteoporosis, which is characteristic of a decreased mass of the normal osseous tissue, disorders in its microarchitecture and increased susceptibility to fractures [16].

Disorders in the osseous system similar to those observed in women during a decline of the hormonal activity of ovaries, can be obtained in an experimental animal model as a result of bilateral ovariectomy. This animal model of osteopenia is used by many researchers to investigate changes in the osseous system as well as to assess the effects of drugs on these changes [8, 14, 31, 38, 39].

Currently the treatment of osteoporosis in postmenopausal women involves, apart from substitutive hormonotherapy, the use of biphosphonates, and in particular alendronate sodium which is an aminobiphosphonate with potent antiresorptive action [2].

Retinol (vitamin A), due to the fact that it conditions vital biological processes, such as vision, growth and differentiation of epithelial cells and immunological response, is a widely used vitamin, usually as a component of multivitamin preparations, and also as an additive in food products [3, 11, 36].

The effect of retinol on the osseous tissue remodeling process is not well recognized. The studies published so far and carried out on animals and humans have indicated that too high doses of vitamin A may result in the osseous system damage and deformation [4, 13, 17, 19, 20, 29, 37]. As the vitamin is frequently administered as a component of a therapy, which is not always justified, and especially to elderly people displaying the symptoms of decreased bone mineral density, the problem emerges of what effect retinol has on the development of osteoporosis as well as on the action of drugs used in its treatment. The aim of the present study was to examine the effects of retinol and alendronate sodium administered concurrently on the development of changes in the osseous system in rats induced by bilateral ovariectomy, based on histomorphometric parameters.

MATERIALS and METHODS

The experiments were carried out on three-month-old female Wistar rats, given water to drink and fed a standard diet *ad libitum*. The permission for the animal tests and experiments was given by the Ethics Commission, in Katowice. The animals were divided into 7 groups (n = 7): I (C) – sham-operated control rats, II (OVX) – ovariectomized control rats, III (OVX+ALN) – ovariectomized rats + alendronate sodium (3 mg/kg), IV (OVX+R-1) – ovariectomized rats + retinol (700 IU/kg), V (OVX + R-2) – ovariectomized rats + retinol (3500 IU/kg), VI (OVX + ALN + R-1) – ovariectomized rats + alendronate sodium (3 mg/kg) + retinol (700 IU/kg), VII (OVX + ALN + R-2) – ovariectomized rats + alendronate sodium (3 mg/kg) + retinol (3500 IU/kg).

Bilateral ovariectomy or sham-operation were performed under ether anesthesia. A longitudinal incision was made inferior to the rib cage on the dorsolateral body wall [35]. The ovaries were exteriorized, ligated and excised. Rats subjected to the sham surgical procedure had only the ovaries exteriorized and then replaced. The animals were weighed every day. The drugs were administered to the rat daily by oral gavage (alendronate sodium in the morning, retinol in the afternoon) for 4 weeks in the volume of 2 ml/kg. The control (C) and (OVX) rats were given destilled water in the same volume of 2 ml/kg *po* daily. Administration of drugs started 2 days after bilateral ovariectomy and continued for 28 days. Twenty four hours prior to the first administration and the last day administration of the drugs, the animals were given tetracycline hydrochloride 20 mg/kg *ip* in order to mark the calcification front. Tetracycline hydrochloride was a histomorphometric fluorescence marker [18, 28]. After 28 days of retinol administration, all the animals were sacrificed.

The right and left tibial femoral bones, L-4 vertebrae as well as the uterus, and thymus were isolated. After isolation and freeing of muscle tissue, the bones and organs were weighed (Analytical Standard AS200, OHAUS, accuracy 0.0001 g).

In order to determine the content of mineral substances in bones, the left tibia and femur and L-4 vertebra 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 lon-

gitudinal 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 using the Tripp and MacKay method (without decalcification) [32]. Staining times were subjected to the authors' own modification.

The histomorphometric measurements were made using a microscope Optiphot 2 (Nikon), connected through RGB camera (Cohu) to personal computer (program Lucia G 4.51, Laboratory imaging), with final magnifications 200 and 500 times.

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 area of the transverse cross-section of the cortical bone in the tibial diaphysis and the area of the transverse cross-section of the marrow cavity in the tibia were measured in the stained preparation, with the use of a lanameter (magnification 50 times).

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 all groups were compared with the ones for ovariectomized rats (OVX), whereas the results for (OVX + ALN + R-1) and (OVX + ALN + R-2) groups were compared with the ones for ovariectomized rats which were administered alendronate sodium (OVX + ALN).

RESULTS

Estrogen deficiency resulting from bilateral ovariectomy (Group II) caused a statistically significant increase in body weight (by 73.18%), a statistically significant decrease of uterus mass (by 72.2%) and a statistically significant increase of thymus mass (by 59.6%) when compared to the results obtained for control sham-operated rats (C). The increase in body weight only for the ovariectomized group of rats, which were administered alendronate sodium and concurrently alendronate sodium and retinol at the dose of 700 IU/kg daily, was similar to the results in the sham-operated control group and significantly smaller by 36.17% and 34.05%, respectively, when compared to the results for ovariectomized rats (Tab. 1).

The uterus mass was statistically significantly decreased in the group of ovariectomized rats which were administered retinol at the dose of 3500 IU/kg daily and alendronate sodium concurrently with retinol at both examined doses by 15.54%, 24.06% and 14.66%, respectively, in comparison with control ovariectomized rats (Tab. 1).

The mass of tibia, femur and L-4 vertebra immediately after their isolation in the ovariectomized controls was insignificantly statistically bigger than the mass of the examined bones in the sham-operated control rats. The biggest mass of the examined bones, although statistically insignificant, was observed in the alendronate sodium-administered group, whereas the smallest value was noted in the groups which were administered retinol, where after the dose of 3500 IU/kg daily a statistically significant decrease in tibial bone mass was observed (by 8.83% and femur mass by 8.49%). Concurrent administration of alendronate sodium and retinol caused a decrease in the examined bone mass which was statistically significant for the femur after the dose of 3500 IU/kg daily when compared with ovariectomized rats administered alendronate sodium (Tab. 1).

The mineral content in the examined bones in the group of control ovariectomized rats was smaller, and in case of L-4 vertebra – statistically significantly smaller (by 9.55%) in comparison with the results obtained for control sham-operated rats.

Alendronate sodium administered to ovariectomized rats statistically significantly increased the mineral content in the tibial and femoral bones and in L-4 vertebra by 7.36%, 5.84% and 13.05%, re-

Table 1. Effects of alendronate sodium (3 mg/kg *po* daily) and retinol (700 IU/kg *po* or 3500 IU/kg *po* daily) administered for 4 weeks on body mass and bone parameters in rats

Parameters		Groups						
		I – C	II – OVX	III – OVX + ALN	IV – OVX + R-1	V – OVX + R-2	VI – OVX + ALN + R-1	VI – OVX + ALN + R-2
Body mass [g]	Initial	211.85 ± 2.61	204.42 ± 2.58	214.16 ± 4.70	212.00 ± 3.13	208.28 ± 3.25	216.00 ± 4.15	206.85 ± 4.29
	Increase after 28 days	27.14 ± 1.59^{ooo}	47.00 ± 4.28	30.00 ± 8.16	41.00 ± 4.00	41.00 ± 3.41	31.00 ± 5.44^{o}	38.43 ± 4.50
Organs mass [mg]	Uterus	322.62 ± 28.7^{ooo}	89.68 ± 3.31	83.78 ± 6.57	76.21 ± 6.72	75.75 ± 4.15^{o}	68.1 ± 2.2^{oo}	76.54 ± 3.48^{o}
	Thymus	325.7 ± 19.64^{ooo}	519.84 ± 40.22	526.66 ± 28.2	582.61 ± 26.21	573.58 ± 37.84	613.95 ± 53.81	550.41 ± 40.59
Bone mass [mg]	Tibia	516.88 ± 7.56	539.37 ± 10.15	541.95 ± 2.81	511.01 ± 11.63	491.73 ± 10.21^{oo}	531.17 ± 11.99	522.79 ± 13.86
	Femur	725.84 ± 12.88	742.27 ± 12.21	753.52 ± 5.90	717.22 ± 15.36	679.22 ± 13.51^{oo}	719.43 ± 14.67	703.91 ± 17.03^{A}
	L-4 vertebra	254.29 ± 7.89	261.90 ± 9.21	280.06 ± 14.1	254.57 ± 7.39	245.19 ± 3.77	249.8 ± 12.39	254.82 ± 5.01
Bone mineral content [mg]	Tibia	226.01 ± 4.11	216.00 ± 3.28	231.91 ± 2.35^{oo}	210.81 ± 5.49	209.17 ± 6.12	219.35 ± 3.05^{AA}	219.22 ± 5.06
	Femur	311.04 ± 6.12	295.77 ± 5.22	313.05 ± 2.56^{o}	289.02 ± 8.22	283.72 ± 9.08	293.57 ± 4.59^{A}	295.3 ± 7.32
	L-4 vertebra	90.22 ± 2.24^{o}	81.61 ± 2.55	92.26 ± 2.07^{oo}	81.08 ± 2.82	80.08 ± 1.6	81.82 ± 3.1^{AA}	84.08 ± 2.1^{A}
Bone mineral content/bone mass ratio	Tibia	0.44 ± 0.01^{ooo}	0.40 ± 0.01	0.43 ± 0.01^{ooo}	0.41 ± 0.01	0.42 ± 0.02	0.41 ± 0.01	0.42 ± 0.004^{oo}
	Femur	0.43 ± 0.01^{ooo}	0.40 ± 0.01	0.42 ± 0.01^{o}	0.40 ± 0.01	0.42 ± 0.02	0.41 ± 0.01	0.42 ± 0.004^{o}
	L-4 vertebra	0.36 ± 0.01^{ooo}	0.31 ± 0.004	0.33 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.33 ± 0.01	0.33 ± 0.01

Results are presented as means ± SEM (n = 7). I (C) – sham-operated control rats, II (OVX) – ovariectomized control rats, III (OVX + ALN) – ovariectomized rats + alendronate sodium (3 mg/kg *po*), IV (OVX + R-1) – ovariectomized rats + retinol (700 IU/kg *po* daily), V (OVX + R-2) – ovariectomized rats + retinol (3500 IU/kg *po*), VI (OVX + ALN + R-1) – ovariectomized rats + alendronate sodium (3 mg/kg *po*) + retinol (700 IU/kg *po*), VII (OVX + ALN +R-2) – ovariectomized rats + alendronate sodium (3 mg/kg *po*) + retinol (3500 IU/kg *po*). Students *t*-test for unpaired observations was used for estimation of statistical significance. ^{o} – significantly different *vs.* the ovariectomized rats (OVX), ^{o} $p < 0.05$, ^{oo} $p < 0.01$, ^{ooo} $p < 0.001$. ^{A} – significantly different *vs.* ovariectomized rats which were administered alendronate sodium (OVX + ALN), ^{A} $p < 0.05$, ^{AA} $p < 0.01$

spectively, when compared to the ovariectomized controls. Retinol administered to ovariectomized rats statistically insignificantly decreased the mineral content in the examined bones, and when administered in combination with alendronate sodium at both doses significantly decreased the mineral content compared to the results obtained for ovariectomized rats administered alendronate sodium (Tab. 1).

The ratio of mineral content to the mass of tibia, femur and L-4 vertebra in the ovariectomized control group was statistically significantly smaller by 9.1%, 6.98% and 13.89%, respectively, in comparison with the results obtained for sham-operated controls.

Alendronate sodium administered to ovariectomized rats statistically significantly increased the calculated ratio in the tibia by 7.50%, and in the fe-

Table 2. Effects of alendronate sodium (3 mg/kg *po* daily) and retinol (700 IU/kg *po* or 3500 IU/kg *po* daily) administered for 4 weeks on the histomorphometric parameters of bones in rats

Parameters		Groups						
		I – C	II – OVX	III – OVX + ALN	IV – OVX + R-1	V – OVX + R-2	VI – OVX + ALN + R-1	VI – OVX + ALN+R-2
Width of osteoid in the tibia [μm]	Periosteal	17.42 ± 0.21^{ooo}	20.76 ± 0.25	15.02 ± 0.48^{oo}	22.65 ± 0.84	23.95 ± 0.76^{oo}	16.82 ± 0.57^{oo}	17.16 ± 0.89^{o}
	Endosteal	8.16 ± 0.37^{oo}	9.86 ± 0.33	8.57 ± 0,46^{o}	9.52 ± 0.74	11.87 ± 0.97^{o}	10.18 ± 0.37^{A}	11.18 ± 0.74 ^{A}
Transverse growth of the tibia [μm]	Periosteal	45.69 ± 5.39	48.36 ± 5.04	55.36 ± 3.8	37.01 ± 2.49^{oo}	31.85 ± 2.76^{o}	44.84 ± 2.02^{A}	41.84 ± 4.93 ^{A}
	Endosteal	19.76 ±1.59^{o}	14.76 ± 0.89	19.84 ± 1.01^{oo}	13.56 ± 0.81	12.05 ± 1.34	17.11 ± 0.77	17.51 ± 1.12
Transverse cross-section area of the cortical bone in the tibial diaphysis [mm^2]		3.00 ± 0.03	2.99 ± 0.06	3.14 ± 0.09	2.99 ± 0.08	2.90 ± 0.08	2.91 ± 0.09	2.98 ± 0.1
Transverse cross-section area of the tibial marrow cavity [mm^2]		0.83 ± 0.03	0.98 ± 0.09	0.93 ± 0.05	0.98 ± 0.08	0.99 ± 0.06	0.94 ± 0.08	0.95 ± 0.06
Transverse cross-section area of the tibial marrow cavity/tibial diaphysis ratio		0.28 ± 0.01	0.33 ± 0.01	0.29 ± 0.02	0.32 ± 0.01	0.34 ± 0.01	0.32 ± 0.03	0.32 ± 0.02
Width of trabeculae in the femur [μm]	Epiphysis	78.16 ± 3.49^{ooo}	54.42 ± 1.14	71.15 ± 1.66^{ooo}	56.41 ± 3.56	55.09 ± 4.49	63.6 ± 2.35$^{o\,AA}$	55.17 ± 4.32^{AAA}
	Metaphysis	45.16 ± 1.9^{oo}	35.16 ± 1.4	47.68 ± 1.22^{ooo}	41.78 ± 2.77	36.62 ± 0.91	43.14 ± 0.86$^{ooo\,A}$	40.51 ± 0.94$^{o\,AA}$
Width of epiphyseal cartilage in the femur [μm]		94.39 ± 6.78	106.71 ± 6.24	101.03 ± 6.1	104.28 ± 4.92	100.89 ± 3	103.04 ± 4.25	104.31 ± 2.2

Results are presented as means ± SEM (n = 7). I (C) – sham-operated control rats, II (OVX) – ovariectomized control rats, III (OVX + ALN) – ovariectomized rats + alendronate sodium (3 mg/kg *po*), IV (OVX + R-1) – ovariectomized rats + retinol (700 IU/kg *po* daily), V (OVX + R-2) – ovariectomized rats + retinol (3500 IU/kg *po*), VI (OVX + ALN + R-1) – ovariectomized rats + alendronate sodium (3 mg/kg *po*) + retinol (700 IU/kg *po*), VII (OVX + ALN + R-2) – ovariectomized rats + alendronate sodium (3 mg/kg *po*) + retinol (3500 IU/kg *po*). Students *t*-test for unpaired observations was used for estimation of statistical significance. ^{o} – significantly different *vs*. the ovariectomized rats (OVX), ^{o} $p < 0.05$, ^{oo} $p < 0.01$, ^{ooo} $p < 0.001$. ^{A} – significantly different *vs.* ovariectomized rats which were administerd alendronate sodium (OVX + ALN), ^{A} $p < 0.05$, ^{AA} $p < 0.01$, ^{AAA} $p < 0.001$

mur by 5.0%. Concurrent administration of alendronate sodium and retinol at the dose of 3500 IU/kg *po* caused a statistically significant increase in this ratio when compared to the results obtained for the ovariectomized controls (Tab. 1).

Histomorphometric examinations of the compact cortical bone demonstrated a statistically significant increase in the osteoid width by 19.17% on the side of periosteum and by 20.83% on the side of endosteum for the ovariectomized rats compared to the results obtained for control sham-operated rats.

The group of ovariectomized rats which were administered alendronate sodium displayed a statistically significant decrease in osteoid width on the side of periosteum by 27.65%, and on the side of endosteum by 13.08% compared to the results obtained for ovariectomized controls.

Retinol at the dose of 3500 IU/kg daily administered to ovariectomized rats statistically significantly increased the osteoid width on the side of periosteum by 15.37% and on the side of endosteum by 20.39%. Retinol administered concurrently with alendronate sodium at both doses statistically significantly decreased the osteoid width on the side of periosteum by 18.98% and 17.35%, respectively, in comparison with the ovariectomized control group, and on the side of endosteum it significantly increased by 18.79% and 30.46%, respectively, compared to ovariectomized group receiving alendronate sodium (Tab. 2).

The tibial transverse growth marked using the tetracycline method was statistically insignificantly bigger for the ovariectomized rats on the side of periosteum, whereas on the side of endosteum it was statistically significantly smaller by 25.31% compared to the results obtained for sham-operated control rats.

Alendronate sodium administered to ovariectomized rats increased the bone transverse growth, where a statistically significant increase was observed on the side of endosteum (by 34.42%) compared to the results obtained for ovariectomized controls.

Retinol administered at both doses to ovariectomized rats decreased in a statistically significant way the growth on the side of periosteum by 18.79% and 30.46%, respectively, when compared to the results obtained in ovariectomized controls, and when administered concurrently with alendronate sodium – by 19.00% and 24.42%, respectively, compared to the ovariectomized group given alendronate sodium (Tab. 2).

No significant differences were observed in the measurements of transverse cross-section of the diaphysis and tibial marrow cavity, although an increase in marrow cavity transverse cross-section occurred in ovariectomized controls and in the groups which were administered retinol. Also, the marrow cavity transverse cross-section/tibial diaphysis transverse cross-section ratio was the biggest in the ovariecomized controls and in the group receiving retinol at the dose of 3500 IU/kg, although it was not statistically significant (Tab. 2).

Histomorphometric examinations of trabecular bones included the measurement of trabeculae width in the epiphysis and in the metaphysis of the femur. The ovariectomized controls displayed a statistically significant decrease in the trabecular width in the epiphysis by 30.37%, and in the metaphysis by 22.14% when compared to the results obtained for sham-operated rats. Alendronate sodium administered to ovariectomized rats caused a statistically significant increase in trabeculae width by 30.74% in the epiphysis and by 35.61% in the metaphysis of the femur, compared to ovariectomized controls.

Alendronate sodium administered concurrently with retinol at both doses decreased significantly the width of trabeculae in the epiphysis by 10.61% and 22.46%, respectively, and in the metaphysis by 9.52% and 15.04%, respectively, when compared to the ovariectomized group receiving alendronate sodium, however, the obtained results were higher than for ovariectomized controls (Tab. 2).

No significant differences were observed with regard to the measurement of epiphysial cartilage of the femur (Tab. 2).

DISCUSSION

Estrogen deficiency, and in particular, 17β-estradiol deficiency in postmenopausal women induces a decreased osteoblast activity while at the same time the activity of osteoclasts increases, which leads to a disturbed bone remodeling process and the development of osteoporosis [16].

Disorders in the osseous system similar to those observed in postmenopausal women may be obtained in an experimental model of sexually mature female rats as a result of bilaterial ovariectomy. Such animal model of osteopenia is used by many researchers to assess changes in the osseous system induced by estrogen deficiency and to examine the effects of drugs on the changes [8, 38, 39].

Estrogen deficiency 30 days after performing bilateral ovariectomy caused a statistically significant increase in the body weight of the examined female rats as well as a statistically significant increase in thymus mass and a decrease in the uterus mass. The observed changes confirm proper execution of bilateral ovariectomy and are in accordance with our earlier observations [6, 22–24], as well as with observations made by others [14].

Estrogen deficiency occuring 30 days after bilateral ovariectomy in sexually mature female rats caused also changes in the osseous system associated with bone loss, however, without spontaneous fractures. The intensification of bone loss was confirmed by our results which demonstrated a reduced ratio of mineral content to the examined bone mass and the results of histomorphometric measurements.

Histomorphometric measurements performed in our study indicated that the process of osseous tissue remodeling was disturbed, both in the cortical and trabecular bones. In the cortical bone on the side of periosteum, the bone formation process was observed to be intensified (an increased osteoid width and transverse growth of the bone), whereas on the side of marrow cavity, the process of resorption was intensified (increased marrow cavity transverse cross-section area and increased ratio of

marrow cavity transverse cross section area to tibial bone transverse cross section area). Resorption process was also observed to be intensified in the examined trabecular bone (a statistically significant decrease in trabecular width in the epiphysis and metaphysis of the femur). The obtained results confirm the development of osseous tissue disorders of osteoporosis nature and they are in accordance with the data found in literature [14, 31, 39].

In order to investigate the effect of alendronate sodium on the development of changes in the osseous system induced by ovariectomy, the drug was administered at the dose of 3 mg/kg *po* once a day in the morning for the period of 28 days. The dose was selected based on data found in literature [7].

Alendronate sodium (3 mg/kg) administered to ovariectomized rats inhibited bone loss induced by estrogen deficiency, which is acknowledged by a significantly increased ratio of mineral content to bone mass of the examined bones. Additionally, alendronate sodium intensified the process of bone formation and mineralization in the cortical bone (increased transverse growth of the tibia measured using tetracycline method, a statistically significant decrease in osteoid width and a statistically significant increase in mineral content in the examined bones) and it inhibited slightly the process of resorption on the side of marrow cavity (a decreased transverse cross-section area of the marrow cavity as well as a decreased ratio of transverse cross-section area of the marrow cavity to transverse cross-section area of the tibia). In the trabecular bone, on the other hand, alendronate sodium inhibited the process of resorption or intensified the process of bone formation induced by estrogen deficiency, which is proved by a statistically significant increase in the trabecular bone width in the epiphysis and metaphysis of the femur. The results obtained with regard to protective effects of alendronate sodium on the development of changes in the osseous system of rats induced by ovariectomy are in compliance with literature data [10, 27] and they result from its antiresorptive effect on the osseous tissue.

Molecular mechanism of antiresorptive action by aminophosphonates (including alendronate sodium) is connected with inhibition of farnezylodiphosphate (FPP) and geranylogeranylodiphosphate (GGPP) synthases necessary to prenilate Rab, Rac, Ras and Rho proteins of GTP family which are responsible for adhesion, movement, morphological changes and apoptosis of osteoclasts [5, 15, 25, 30, 34].

The effect of retinol on the development of changes in the osseous system of female rats with estrogen deficiency induced by ovariectomy was studied at two doses: 700 IU/kg (a preventive dose) and 3500 IU/kg (a therapeutic dose in humans) taking into account the coefficient (× 10) based on the assumption that metabolic processes in rats are ten times faster than in humans [1, 21].

Retinol, especially at the dose of 3500 IU/kg daily intensified the bone loss induced by estrogen deficiency. In the cortical bone on the side of periosteum it disturbed the bone formation process (a statistically significant reduction in transverse growth and statistically significant decrease in bone mass) and it inhibited the process of mineralization (a statistically significant increase of osteoid width and in mineral content). Simultaneously, it slightly intensified the process of resorption in the cortical bone on the side of endosteum (an increased transverse cross-section area of the marrow cavity and ratio: transverse cross-section area of the marrow cavity/tibial transverse cross-section area). The above results are confirmed by Hough and Avioli studies [13] which indicated that administering high doses of retinol reduced bone mineral density leading to bone deformation and spontaneous fractures. Also research carried out on humans proves that an increased supply of retinol above 1.5 mg daily results in an accelerated development of osteoporosis. [4, 17, 19, 29, 37].

An adverse effect of retinol on the osseous tissue is associated by some researchers with its antagonistic action against vitamin D, which may result from a similar structure and location of the nuclear RXR receptor of retinoic acid and VDR receptor of vitamin D [9, 12, 26, 33]. The results of reduced organic matrix mineralization process and increased osteoid width obtained in this study confirm the concept stating that the mechanism of retinol action on the osseous system in ovariectomized female rats may result from antagonizing vitamin D.

The last stage of research, and at the same time the main aim of the study, was to assess effect of concurrent retinol and alendronate sodium administration on the development of changes in the osseous system in rats induced by bilateral ovariectomy.

Retinol administered concurrently with alendronate sodium in female rats with estrogen deficiency inhibited the protective effect of alendronate

sodium on the development of changes in the osseous system, which was proved by decreased bone formation process in the cortical bone (a significant decrease in tibial transverse growth) and osteoid mineralization (a statistically significant increase in osteoid width as well as a decrease in mineral content) in comparison with the results obtained for ovariectomized rats which were given alendronate sodium. Also a concurrent administration of alendronate sodium and retinol at both doses to ovariectomized rats decreased the antiresorptive effect of alendronate sodium on the osseous tissue of trabecular structure (a significant decrease in trabecular width in the epiphysis and metaphysis of the femur when compared to ovariectomized rats which were administered alendronate sodium).

It seems that a significant weakening of the alendronate sodium protective action on the development of changes induced by ovariectomy after its concurrent administration with retinol does not result from antagonistic action of retinol on alendronate sodium effect on the osseous tissue, but results from a retinol-intensified bone loss induced by estrogen deficiency in rats.

In conclusion, it may be stated that administering retinol, in particular at the dose of 3500 IU/kg, intensifies bone loss in rats induced by bilateral ovariectomy.

Alendronate sodium (3 mg/kg *po*) inhibits the development of changes in the osseous system in rats induced by estrogen deficiency.

Retinol administered concurrently with alendronate sodium decreases protective action of alendronate sodium on the development of changes in the osseous system in rats induced by bilateral ovariectomy.

The results obtained in animals indicate that unjustified vitamin A administration should be limited, in particular at large doses in elderly people, especially in treating postmenopausal osteoporosis in women.

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