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INTERMITTENT THREE-MONTH TREATMENT WITH UKRAIN IN INTACT AND OVARIECTOMIZED RATS. PART II: EFFECT ON BONE MINERAL DENSITY OF THE FEMUR

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Summary: *Ukrain, an acid alkaloid derivative of* Chelidonium majus L., *was administered intraperitoneally to ovariectomized and control sexually mature female rats at doses of 7, 14 and 28 mg/kg once daily for 10 days, followed by 10-day break. This procedure was repeated five times. At the end of the Ukrain treatment (24 h after the last dose of the drug) the right femora of the rats were harvested and the bone densitometric parameters of the whole bone and distal metaphysial and intertrochanteric subregions were assessed using the dual energy x-ray absorptiometry densitometric method. The results showed no apparent decrease in bone mineral density in groups of rats studied. A nearly significant (p = 0.08) decrease of bone mineral content was observed in ovariectomized rats treated with 14 mg/kg of Ukrain.*

Introduction

Hohenwarter *et al.* (1) and Nowicky *et al.* (2, 3) demonstrated that Ukrain, a semisynthetic drug, shows wide biological activity. This drug administered to sexually mature female rats was capable of influencing mineral metabolism and bone parameters in ovariectomized and also in intact rats (4-6). Bone mineral density (BMD; g/cm^2) depending on mineral content, is quantified by dual energy x-ray absorptiometry (DEXA) (7). Rats are suitable animals for such investigations (8, 9). DEXA is a recognized method of evaluating bone status, both in humans (10) and experimental animals, since special computer software is available to assess the small bones of animals (11). Bone mineral density (BMD) is directly related to bone mineral content (BMC, g) and assessed area (cm^2)).

Our studies used the DEXA method to assess changes in rat femoral bone density induced by ovariectomy and long-term parenteral intermittent treatment with three different doses of Ukrain.

Materials and methods

Animals. Female, sexually mature Wistar rats (340 g ± 30 g) were used for the study. The animals were housed at room temperature (20-21 °C) on a natural day-night cycle with free access to food (1.1% Ca) and water. Experimental and control groups consisted of 10 rats.

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Drugs. Ukrain was received in its purest state from the Ukrainian Anti-Cancer Institute, Vienna, Austria. Water for injection was obtained from Polfa, Poland.

Experimental procedures and treatments. The intact and ovariectomized rats received i.p. injections of Ukrain (7, 14, or 28 mg/kg at a volume of 0.5 ml/100 g) every day for 10 days, followed by 10-day break. This procedure was repeated five times. The control and sham-operated animals received the same volume of water for injection. Operative procedure was performed from the dorsal approach (12).

Bone densitometry. Bone area, including the calculations of area (cm^2), BMC (g) and BMD (g/cm^2)), were assessed with Hologic QDR 4500A DEXA bone densitometer (Subregion Hi-Res V8.20h, line spacing – 0.0311 cm, point resolution – 0.0311 cm) in a-p projections. Measurements were performed in the air (Shepherd J.A., Hologic, Waltham, personal communication). Values for entire femur were calculated automatically, metaphysial region (R1), rich in cancellous bone, was set manually eight lines long starting seven lines from the distal margin of the femoral condyles. The intertrochanteric subregion (R2), corresponding to the lower part of the basis of the femoral neck, was semiautomatically set on a 4 x 4 line size on each scan, as in previous investigations (5, 6).

Statistical analysis. After assessment for normality with Kolmogorow-Smirnow test, statistical differences between means within the groups were calculated with ANOVA. *Post hoc* comparisons of means were performed using Duncan's test (Statistica 5.0). $p = 0.05$ was regarded as significant.

Results

Results of our investigation are presented in Table I and Figures 1-6. It appeared that no significant

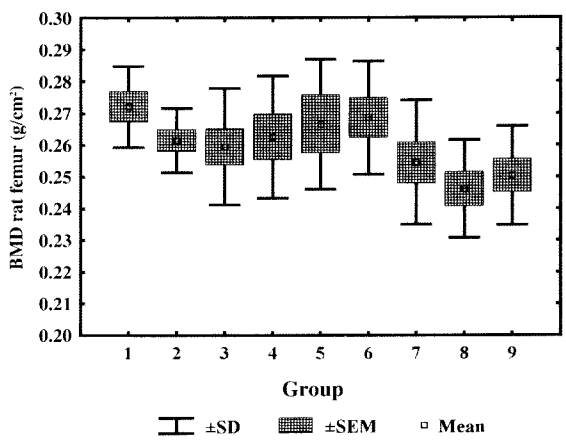

Fig. 1 Bone mineral density of the whole rat femur in the study groups: 1) control, 2) sham-operated, 3) ovariectomized, 4) intact + 28 mg/kg Ukrain, 5) intact + 14 mg/kg Ukrain, 6) intact + 7 mg/kg Ukrain, 7) ovariectomized + 28 mg/kg Ukrain, 8) ovariectomized + 14 mg/kg Ukrain, 9) ovariectomized + 7 mg/kg Ukrain.

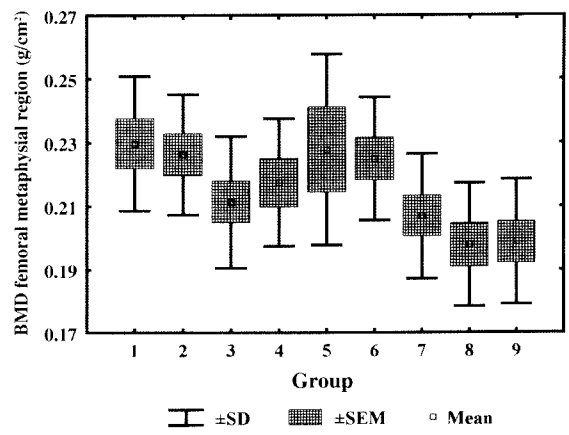

Fig. 2 Bone mineral density of the femoral methaphyseal region in the study groups. For group definitions see Figure 1.

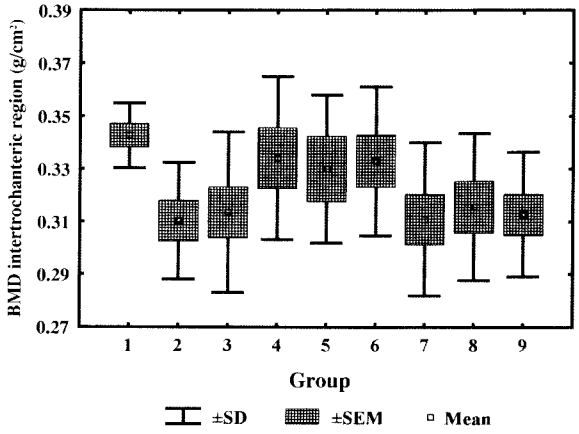

Fig. 3 Bone mineral density of the intertrochanteric region in the study groups. For group definitions see Figure 1.

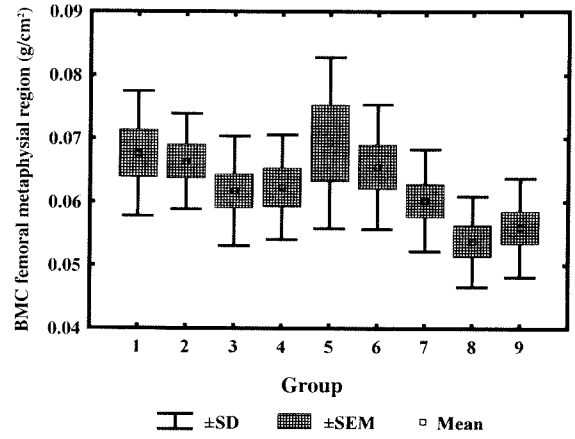

Fig. 5 Bone mineral content of the methaphyseal region of the femur in the investigated groups. For group definitions see Figure 1.

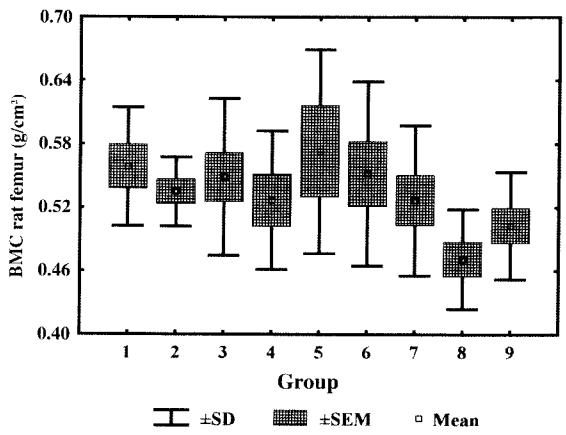

Fig. 4 Bone mineral content of the whole femur in the study groups. For group definitions see Figure 1.

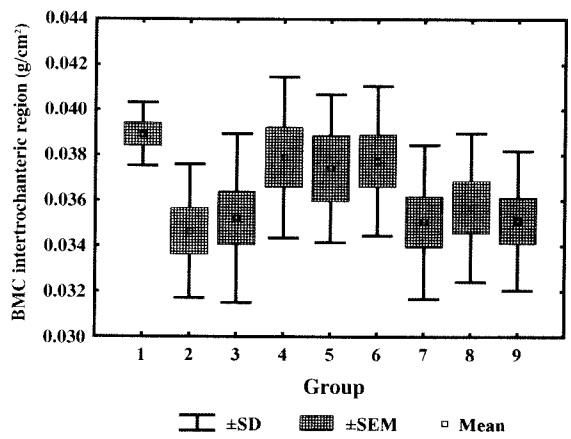

Fig. 6 Bone mineral content of the intertrochanteric region in the investigated groups. For group definitions see Figure 1.

decrease in bone mineral density occurred in the investigated groups of rats. A nearly significant (p = 0.08) decrease in bone mineral content was observed in ovariectomized rats treated with 14 mg/kg of Ukrain. This group also showed as light but significant decrease in the investigated areas (p = 0.02).

Discussion

Postmenopausal osteoporosis in humans results from insufficient production of female sexual hormones and the stage of this metabolic disease is usually assessed with bone densitometry (10). Ovariectomized female rats are often used as a stan-

Table I *Densitometric parameters in investigated groups of intact and ovariectomized rats under prolonged administration of different doses of Ukrain*

Groups		1	2	3	4	5	6	7	8	9
Area (cm^2)	R	2.0499 (0.1302)	2.0448 (0.0777)	2.1066 (0.1587)	2.0004 (0.1217)	2.1375 (0.2045)	2.0468 (0.2219)	2.0626 (0.1608)	1.9090 (0.0816) $p = 0.02$ *vs.* 3 group	2.0042 (0.1111)
	R1	0.2934 (0.0216)	0.2927 (0.0130)	0.2909 (0.0136)	0.2857 (0.0155)	0.3022 (0.0220)	0.2906 0.0273	0.2900 (0.0157)	0.2709 (0.0107) $p = 0.04$ *vs.* 3 group	0.2806 (0.0147)
	R2	0.1136 (0.0000)	0.1116 (0.0020)	0.1122 (0.0020)	0.1134 (0.0003)	0.1134 (0.0004)	0.1133 (0.0007)	0.1127 (0.0009)	0.1131 (0.0013)	0.1122 (0.0017)
BMC (g)	R	0.5585 (0.0559)	0.5348 (0.0328)	0.5484 (0.0739)	0.5267 (0.0655)	0.5729 (0.0965)	0.5517 (0.0869)	0.5265 (0.0712)	0.4708 (0.0472) $p = 0.08$ *vs.* 3 group	0.5025 (0.0508)
	R1	0.0676 (0.0098)	0.0663 (0.0075)	0.0617 (0.0086)	0.0623 (0.0083)	0.0692 (0.0135)	0.0655 (0.0098)	0.0602 (0.0080)	0.0537 (0.0072)	0.0559 (0.0078)
	R2	0.0389 (0.0014)	0.0346 (0.0029)	0.0352 (0.0037)	0.0379 (0.0035)	0.0374 (0.0032)	0.0377 (0.0033)	0.0350 (0.0034)	0.0357 (0.0033)	0.0351 (0.0031)
BMD (g/cm^2)	R	0.2720 (0.0127)	0.2615 (0.0100)	0.2595 (0.0182)	0.2626 (0.0192)	0.2666 (0.0204)	0.2686 (0.0177)	0.2545 (0.0195)	0.2462 (0.0155)	0.2504 (0.0156)
	R1	0.2297 (0.0210)	0.2262 (0.0189)	0.2113 (0.0207)	0.2174 (0.0201)	0.2277 (0.0301)	0.2248 (0.0193)	0.2068 (0.0197)	0.1978 (0.0195)	0.1986 (0.0197)
	R2	0.3426 (0.0123)	0.3102 (0.0222)	0.3135 (0.0305)	0.3341 (0.0307)	0.3300 (0.0280)	0.3329 (0.0281)	0.3108 (0.0291)	0.3156 (0.0279)	0.3126 (0.0237)

Standard deviation in brackets. R = total femur. R1 = distal metaphysis. R2 = intertrochanteric.
Groups: 1) control, 2) sham-operated, 3) ovariectomized, 4) intact + 28 mg/kg Ukrain, 5) intact + 14 mg/kg Ukrain, 6) intact +7mg/kg Ukrain, 7) ovariectomized + 28 mg/kg Ukrain, 8) ovariectomized + 14 mg/kg Ukrain, 9) ovariectomized + 7 mg/kg Ukrain

dard model of experimental osteopenia (4, 5, 13-16). It is known that anticancer drugs are capable of producing osteopenia (10). Ukrain exerts its multifactorial effects on several tissues (1-3), including bones (4), possibly by indirectly affecting calcium metabolism since it influences sexual hormone levels in ovariectomized rats (17).

In this study, Ukrain was given intermittently for 10 consecutive days and a break of 10 days preceded the next series of intraperitoneal injections of Ukrain. Previous preliminary results indicated that long-term daily administration of high doses of Ukrain prevented loss of humeral strength induced by ovariectomy (17). Studies with a regimen in which Ukrain was given intermittently on 5 alternative days in a series showed decrease in bone mineral density in ovariectomized rats with low and intermediate doses, whereas high dose did not affect mineral density (5).

The same regimen applied to intact rats did not affect bone mineral density of their femora (6).

Bone mineral density and content assessed with DEXA are closely related to bone quality and its resistance to load (18). Results of this study show no adverse effect of Ukrain on bone mineral density on the drug regimen tested.

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