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Bone mineral content of the mandible and spine in ovariectomized rats with estrogen deficiency

After menopause (natural or induced after ovariectomy) when the endogen estrogens level is decreased, physiological balance between resorption and bone formation is disturbed. As a result of this, calcium depletion from bones is accelerated. Estrogens regulate calcium absorption and elimination from the organism by direct or indirect influence on parathormone, calcitonin, active form of D vitamin and estrogen receptors present on the osteoclasts and osteoblasts (8). Actually, the receptory mechanism of the bone metabolism is not entirely known. But it was discovered that classic estrogen receptors (ERα) and lately identified estrogen receptors β (ERβ) play an important role in osteoblasts differentiation (9).

Recent examinations indicate that estrogens deficiency in woman organism during postmenopausal period or after ovariectomy causes disorders of the bone metabolism balance with a prevalence of resorption processes. But the process of bone mass depletion is coursed not linearly but dynamically (phasically). High bone turnover (active phase) occurs alternately with low bone turnover (nonactive phase) (5). Thus, actual surveys concern quantitative and qualitative estimation of the process of the bone mass depletion and the methods assessing bone mineral content are still improved.

Now, dual x-ray absorptiometry – DXA or dual x-ray absorptiometry were generally accepted as the method of detecting and treatment monitoring of the osteoporosis (6, 10). The obtained results can be presented in the following unit – BMC – bone mineral content (g/cm^3, g). DXA is a useful and safe method of measurement of the bone density, especially in pathological processes. An amount of ionizing radiation necessary to perform examination is below 3 milirem, time of examining is short (5–15 min) and depends on scanning speed of densitometer (6, 11). Because of that, this method was approved as the most adequate in the assessment of changes in the bone mineral content of the mandible and spine in female rats with estrogen deficiency during this experiment.

MATERIAL AND METHODS

Young adult female Wistar rats, weighing 250–300 g were used for the experiment. The animals were fed a standard chow and housed in cages with light-dark cycle and were allowed free access to water and feed. The experiment was carried out in accordance with guidelines of Animal Ethical Research Committee of Medical University of Lublin. After two-week adaptation to the diet and new environment, rats were divided at random into the following seven groups, of 10 animals in each: CL – control group; SH – rats sham operated; OV – rats after bilateral ovariectomy; OVO – rats after bilateral ovariectomy receiving *oleum pro injectione*; OVH_1 – rats after bilateral ovariectomy taking 17β-estradiol in a dose of 1.25 μg per animal, twice a week, during seven weeks; OVH_2 – rats after

bilateral ovariectomy taking 17β-estradiol in a dose of 12.5 μg per animal, twice a week, during seven weeks, and OVH_3 – rats after bilateral ovariectomy taking 17β-estradiol in a dose of 125 μg per animal, twice a week, during seven weeks.

Sham operated rats (SH group) were used to determine the influence of operation stress on the calcium content in the examined tissues. In OV group, the ovaries were removed under general anaesthesia. To examine the influence of the oil base of estradiol *oleum pro injectione* was supplied in OVO group. In OVH_1-OVH_3 groups *Oestradiolum benzoicum* (Jelfa – Jelenia Góra) was administered intramuscularly.

After the experiment the rats were anaesthetized by the lethal dose of Tiopenthal. Bone mineral content (BMC) of the spine and mandible was measured using DPX-A densitometer and computer programme Small Animal Software. The obtained data (g) were analyzed by calculating mean (M) and standard deviation (SD). The significance of differences between groups has been determined on the basis of confidence intervals (NIR), obtained from variance analysis (ANOVA). Differences between means are significant when means are not designated the same letter.

RESULTS

Table 1 presents bone mineral content of the mandible and spine. In the control group mean BMC of the mandible was 0.335 g and in SH group – 0.318 g, and differences between these groups were not statistically significant. BMC of the mandible was the lowest in OV group – 0.271 g and in OVO group – 0.288 g. Administration of 17β-estradiol caused an increase in BMC of the mandible from 0.303 g in OVH_1 group to 0.548 g in OVH_2 group, and 0.386 g in OVH_3 group. Statistically significant differences occurred only between OVH_2 and OVH_3 groups and CL and OV groups.

The mean result of BMC of the spine in CL group was 0.678 g and in SH group 0.667 g and differences were not statistically significant. After ovariectomy bone mineral content of the spine decreased to 0.531 g and this result was statistically significant in relation to all groups. The administration of 17β-estradiol increased the mean BMC of spine. It was 0.676 g in OVH_1 group, 0.743 g in OVH_2 group and 0.693 in OVH_3 group. These values were statistically significant in comparison with OV group.

Table 1. Bone mineral content of the mandible BMC-M and spine BMC-S (g)

Group	No. of rats (n)	BMC-M (M± SD)	Significance of differences $(P_1)^*$	BMC-S (M± SD)	Significance of differences $(P_2)^*$
CL	10	0.335 ± 0.07	c	0.678 ± 0.10	bc
SH	10	0.318 ± 0.04	bc	0.667 ± 0.03	bc
OV	10	0.271 ± 0.02	a	0.531 ±0.13	a
OVO	10	0.288 ± 0.03	ab	0.639 ± 0.07	b
OVH_1	10	0.303 ± 0.01	abc	0.676 ± 0.15	bc
OVH_2	10	0.548 ± 0.03	e	0.743 ± 0.05	c
OVH_3	10	0.386 ± 0.02	d	0.693 ± 0.07	bc

DISCUSSION

The carried out examination of bone mineral content of the mandible and spine of female rats allows very careful assessing of changes of mineralization in the examined bone tissues. During the experiment, there had taken place a statistical decrease of BMC of the mandible and spine in rats after ovariectomy. This state of induced hypoestrogenism caused significant disorders of bone mineral metabolism. Also, the studies of Giardino indicate that in rats two weeks after ovariectomy there was observed osteopenia (4). Deficiency of estrogen, also in postmenopausal women causes activation of osteoclasts to the bone resorption by parathormone and 1.25 dihydroxycholecalciferol, although the level of these hormones is decreased (1). The other mechanism is to inhibit osteoblasts to the bone formation. Manifesting of bone mass depletion and some disorders in the structure of bones concerns organic and inorganic parts as well. Architectonic changes are visible in the spongosa bone where trabeculae are thinned and they lose continuity. In the long bones there are dilatatored bone marrow lacunas and thinned cortical lamina. These changes cause osteopenia and in the next step, postmenopausal osteoporosis (3, 11). This process we can inhibit and sometimes regress these bone changes by using hormonal substitute therapy. The most effective and safe up to this time was 17β-estradiol (1). In 1940 Albright paid attention to the fact that estrogen therapy can have a protective effect in postmenopausal osteoporosis prevention (5).

In the carried out experiment using of three doses of 17β-estradiol significantly increased bone mineral content of the mandible and spine in rats after ovariectomy. It was observed that this increasing of BMC of the examined bones was comparable with the level of BMC in the control group. It was also found that the minimal (1.25 μg) and medium (12.5 μg) dose of 17β-estradiol causes biological effects but administration of the higher dose (125 μg) did not influence the response of rats' organism and increasing of BMC of the examined bones. It may be an effect of saturation of receptors' capacity.

Also the studies of Christiansen showed that hormonal therapy after menopause rises bone mineral content in women bones. The frequency of femur, forearm and spine fractures is also decreased (2). Huges et al. noticed that estrogens induced *in vivo* osteoclasts apoptosis and estradiol adding to the bone marrow culture *in vitro* twice times increased osteoclasts death. Such effect is observed in mice after ovaries removing (7). Also the histomorphometrical and biochemical examination of the rats revealed that estrogens inhibit bone resorption and in consequence cause new bone formation (12).

CONCLUSIONS

1. The carried out examination showed that estrogen deficiency after ovariectomy has an important influence on bone mass depletion expressing the low BMC of the mandible and spine.

2. 17β-estradiol application effectively rise bone mineral content of the examined bones.

3. For earlier diagnosis of osteoporotic changes a quantitative estimation of real bone mass depletion is necessary.

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SUMMARY

The aim of this study was to assess the bone mineral content of the mandible and spine in rats with estrogen deficiency after ovariectomy. Female rats were divided into the following groups: CL – control, SH – sham-operated, OV – after ovariectomy, OVH – after ovariectomy receiving 17β-estradiol in three different doses (1.25, 12.5, 125 μg) during seven weeks. After the experiment densitometric examinations of the mandible and spine were made using DEXA method and there was measured bone mineral content (BMC). The results of the examination indicate that estrogen deficiency after ovariectomy leads to decreasing of BMC index of the examined bones, and densitometric examinations allow to carefully evaluate changes in the mineral part of the examined bones.

Zawartość składników mineralnych w kościach żuchwy i kręgosłupa samic szczura z niedoborem estrogenów

Celem pracy była ocena zawartości składników mineralnych (BMC) w żuchwie i kręgosłupie u samic szczurów z niedoborem estrogenów po usunięciu jajników. Zbadano również wpływ 17β-estradiolu zastosowanego w trzech różnych dawkach na mineralną masę badanej tkanki kostnej. Stwierdzono, ze w wyniku usunięcia jajników dochodzi do istotnego obniżenia BMC żuchwy i kręgosłupa, a badania densytometryczne pozwalają dokładnie prześledzić ilościowe zmiany w części mineralnej kości.