ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LVII, N 1, 46 SECTIO D 2002

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Influence of estrogen deficiency on the copper level in rat teeth and mandible

The loss of estrogen in postmenopausal women has been implicated as one of several factors in the etiology of osteoporosis. Estrogen deficiency leads to increase in the resorption of bone caused by increased osteoclast numbers and their activity. It leades to demineralization, depletion of bone mass and increases risk of fractures. Loss of calcium is mainly responsible for this state. Last reviews present a new concept that bone health depends not just on estrogen and calcium, but on a wide range of other nutrients (4).

Minerals are the inorganic component of biologic materials. Although many of the inorganic compounds reside in the skeleton, other play key roles in cell metabolism. Bone is an active, living tissue, with diverse nutritional needs. For example copper is necessary for normal erythropoiesis as well as for iron absorption (1). This ion is also required for metalloenzymes like ceruloplasmin, ferroxidase, cytochrome c oxidase, superoxide dismutase, tyrosinase. It has been known that copper is a cofactor for the lysyl oxidase too, which strengthens connective tissue by cross-linking collagen strands (4, 9). Deficiency of copper in horses leads to osteochondrosis (2). Rats fed a copper deficient diet had reduced bone mineral content and reduced bone strength (4). In drug-induced osteoporosis after application of hydrocortisone the copper level in rat teeth was also decreased (7).

Lately, the role of copper in postmenopausal osteoporosis is widely discussed. The aim of this study was to evaluate the copper level in rat teeth and mandible after ovariectomy, when the level of estrogen is decreased.

MATERIAL AND METHODS

Young adult female rats were classified for the experiment, after two-week adaptation period to experimental conditions. They were divided into 7 groups – 10 rats in each: CL

– control group, healthy animals, SH – rats sham operated, OV – rats after bilateral ovariectomy, OVO – rats after bilateral ovariectomy given oleum pro injectione, OVH_1– rats after bilateral ovariectomy taking 17β – estradiol in the dose of 1.25 μg twice a week, for seven weeks, OVH_2 – rats after bilateral ovariectomy taking 17β – estradiol in the dose of 12.5 μg twice a week, for seven weeks, OVH_3 – rats after bilateral ovariectomy taking 17β – estradiol in the dose of 125 μg twice a week, for seven weeks.

After the experiment rats from groups CL, SH, OV, OVO, OVH_1, OVH_2, OVH_3 were anaesthetised, decapitated and the mandible and incisors were prepared. The samples of each experimental group were carefully labelled and kept separately. Finally, rat teeth and mandible were mineralized in muffle furnace at 450^0 C (dry method) and the copper level was then estimated with a Pye-Unicam atomic absorption spectrophotometer (6). The copper level per unit teeth and bone amount (μg/g tissue) were calculated. The data obtained were analysed by calculating mean (M) and standard deviation (SD). The significance of differences between the groups have been determined on the basis of confidence intervals (NIR), which were determined from variance analysis (ANOVA). Differences between means are significant, when means are not designated by the same letter.

RESULTS AND DISCUSSION

Table 1 and Figure 1 presented the level of copper in rat teeth in the examined groups. The mean teeth copper level in the control group was 8.75 μg/g and in the sham operated group – 8.70 μg/g. After ovariectomy the mean copper level showed a significantly marked decrease – 7.06 μg/g. Administration of 17β-estradiol caused increasing of the copper level in rat teeth from 7.91 μg/g in OVH_1 group to 9.40 μg/g in OVH_3 group. The observed differences were statistically significant in comparison to OV group and CL group.

Table 1. Copper level in rat teeth in examined groups (μg /g)

Experimental group	Number of rats (n)	Mean (M)	Standard deviation (SD)	Significance of differences (p)*
CL	10	8.75	0.45	c
SH	10	8.70	0.20	c
OV	10	7.06	0.70	a
OVO	10	7.89	0.80	b
OVH_1	10	7.91	0.80	b
OVH_2	10	9.39	0.17	d
OVH_3	10	9.40	0.07	d

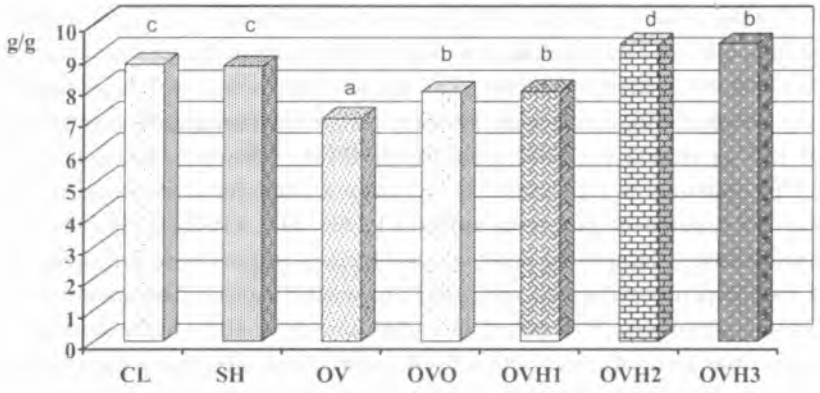

Fig. 1. The level of copper in rat teeth in experimental groups

Table 2. Copper level in rat mandible in examined groups (μg /g)

Experimental group	Number of rats (n)	Mean (M)	Standard deviation (SD)	Significance of differences (p)*
CL	10	6.97	0.32	c
SH	10	6.88	0.58	c
OV	10	5.45	0.25	a
OVO	10	5.63	0.59	ab
OVH_1	10	5.67	0.36	ab
OVH_2	10	5.68	0.25	ab
OVH_3	10	5.98	0.03	b

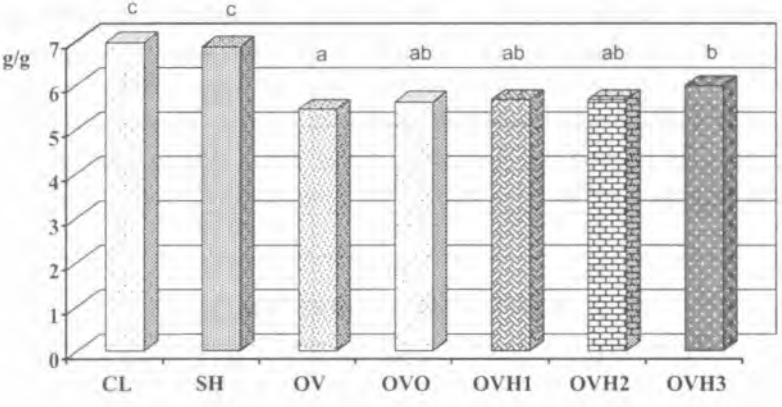

Fig. 2. The level of copper in rat mandible in experimental groups

Table 2 and Figure 2 presented the level of copper in the rat mandible in experimental groups. Comparison of the mandible examination and teeth examination was similar. In OV group, where rats have an estrogen deficiency the copper level in the mandible was 5.45 μg/g – differences statistically significant with the control group – 6.97 μg/g. After administration of 17β-estradiol the copper level was increased in OVH_1 group – 5.67 μg/g, OVH_2 – 5.68 μg/g, OVH_3 – 5.98 μg/g.

Mineralized tissues of bone an teeth play and important role in mineral metabolism of the organism. When the level of ions in plasma decreases these ions are mobilized from reserves in bone and teeth. In the experiment rat incisors were used because these teeth have wide apical foramen, spacious root canal and big pulp. They are good in experimental conditions and ensure regular mineral metabolism. In the teeth of older people the level of copper inereased with age, but in the teeth with caries the level of copper was lower (3, 8). There were also differences in copper content according to gender: women have less copper in teeth than men (10).

In my examination in rats after ovariectomy there were statistically significant differences in the level of copper in teeth and mandible. These findings suggest that when the teeth and bone copper level is affected by the estrogen deficiency the concentration of copper was decreased. Last reports indicated that deficiency of copper could lead to the development of osteoporosis (5) and copper supplementation inhibited bone resorption *in vivo* (4). This is a new aspect in postmenopausal osteoporosis – an important role of microelements. For better understanding of this aspect (theoretically and practically), experimental and clinical examination should be continued. It is especially important to prevent the depletion of minerals from bone in postmenopausal osteoporosis.

CONCLUSIONS

1. In teeth and mandible of the rat with estrogen deficiency the level of copper was decreased.
2. Administration of 17β-estradiol leads to an increase in the copper level in the examined tissues and prevents the depletion of this ion from teeth and mandible.

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2001.11.20

SUMMARY

On the basis of atomic absorption spectrophotometry rat teeth and mandible copper levels in experimental postmenopausal osteoporosis and after administration of 17β-estradiol were measured. The results showed that estrogen deficiency leads to decrease in copper content in rat teeth and mandible, and giving of 17β-estradiol positively influence the content of mineral components in the examined tissues.

Wpływ niedoboru estrogenów na poziom miedzi w zębach i żuchwie szczura

Na podstawie badań z użyciem atomowej spektrometrii absorpcyjnej określono poziom miedzi w zębach i żuchwie szczurów w przebiegu doświadczalnej osteoporozy pomenopauzalnej oraz po zastosowaniu 17β-estradiolu. Stwierdzono, że niedobór estrogenów prowadzi do obniżenia zawartości miedzi w zębach i żuchwie szczurów, a podawanie 17β-estradiolu wpływa w sposób korzystny na skład mineralny badanych tkanek.