INFLUENCE OF ESTROGEN DEFICIENCY ON THE LEVEL OF MAGNESIUM IN RAT MANDIBLE AND TEETH

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On the basis of atomic absorption spectrophotometry rat teeth and mandible magnesium levels in experimental postmenopausal osteoporosis and after administration of 17β-estradiol were measured. The results showed that estrogen deficiency after ovariectomy decreased the magnesium content in rat teeth and mandible and administration of 17β-estradiol influences in good way the mineral components of the examined tissues.

Key words: rat, magnesium, estrogen deficiency.

Magnesium (Mg) is the fourth most abundant cation in the body and plays a pivotal role as an enzyme cofactor in biosynthesis of proteins and mineral administration. Its metabolism is connected with the bone and it is indispensable to osteogenesis and mineralization of bones. Alkaline phosphatase, an enzyme involved in forming new calcium crystals, is activated by magnesium. The conversion of vitamin D to its biologically active form, 1,25-dihydroxyvitamin D3, also appears to require magnesium (3).

Disorders in which magnesium depletion is common are associated with high incidence of osteoporosis. Mg depletion in humans results in hypocalcaemia, low serum parathyroid hormone (PTH) and 1,25(OH)2 vitamin D levels, as well as PTH and vitamin D resistance which may serve as mechanisms for the development of osteoporosis. Experimental Mg deficiency in animal models has resulted in impaired bone growth, osteopaenia, and increased skeletal fragility. Magnesium deficiency is also revealed in dentition. Incisors of rats maintained on a Mg-deficient diet reveal a marked reduction in eruption rate. Histologically, these teeth show atrophy of the formative, basal tissues and degenerative changes in the odontoblasts and enamel epithelium with disturbances in calcification (11, 12). In drug induced-osteoporosis after application of a high dose of hydrocortisone magnesium level in rat maxilla, mandible and teeth was also decreased (9, 10).

Not many reports inform us about influence of estrogen deficiency for magnesium metabolism in the organism during postmenopausal osteoporosis. Kotkowiak (6) noticed that there was a lower level of total and ionized magnesium in
serum and reduced excretion of this element in the urine of fasting women with osteoporosis. Also hair magnesium levels in this examined group were lower.

The aim of this study was to examine the influence of estrogen deficiency on the magnesium content in rat mandible and teeth. Since the changes in bone and teeth following estrogen deficiency are very similar in rats and humans the ovariectomized rat is the most frequently used as a model for study the magnesium metabolism in osteoporosis (1).

**Material and Methods**

Young, adult female Wistar rats, weighing 250-300 g were used for the experiment. The animals were fed a standard diet and housed in the cages with light-dark cycle and allowed free access to water and diet. After two-week of adaptation to the diet and new environment, rats were divided at random into the following groups: CL - control group; SH - rats sham-operated; OV - rats after bilateral ovariectomy; OVO - rats after bilateral ovariectomy receiving *oleum pro iniectione*; OVH<sub>1</sub> - rats after bilateral ovariectomy taking 17β-estradiol in a dose of 1.25 µg per animal, twice a week, during seven weeks; OVH<sub>2</sub> - rats after bilateral ovariectomy taking 17β-estradiol in a dose of 12.5 µg per animal, twice a week, during seven weeks; OVH<sub>3</sub> - rats after bilateral ovariectomy taking 17β-estradiol in a dose of 125 µg per animal, twice a week, during seven weeks. Each group comprised of 10 animals.

Sham-operated rats (SH group) were used to determine the influence of operation stress on the magnesium content in examined tissues. In OV group, the ovaries were removed under general anesthesia. To examine the influence of the oil base of estradiol *oleum pro iniectione* was supplied in OVO group. In OVH<sub>1</sub>-OVH<sub>3</sub> groups *Oestradiolum benzoicum* (Jelfa – Jelenia Góra) was administered intramuscularly.

After the end of the experiment the rats were anaesthetized by the administration of the lethal dose of Tiopenthal, decapitated and the mandible and incisors were prepared. The samples for each experimental group were carefully labelled and kept separately. The use of the incisors in the experiment was due to their wide apical foramen, spacious root canal and big pulp. They are good for experimental conditions and ensure regular mineral metabolism. Rat mandible and teeth were mineralized in muffle furnace at 450°C (dry method) and the magnesium level was then estimated with a Pye-Unicam atomic absorption spectrophotometer with the following parameters: analytic wavelength - 285.2 nm, tissue width - 0.2 nm, lamp current - 5.0 mA, acetylene-flow - 1 dm³ min⁻¹, air-flow - 5 dm³ min⁻¹, burner height - 10 cm (8). The magnesium level was calculated per unit of teeth and mandible tissue amount (mg/g tissue).

The obtained data were analyzed by calculating mean (M) and standard deviation (SD). The significance of differences between groups have been determined on the basis of confidence intervals (NIR) which were determined from variance analysis (ANOVA).
Results

Table 1 presents the level of magnesium in rats’ teeth in the examined groups. The level of Mg in rat teeth in the control group was 16.78 mg/g. After ovariectomy (OV group) Mg concentration was lower (13.56 mg/g) and differences between these groups were statistically significant. The administration of oleum pro injectione did not significantly influence the magnesium level in comparison with OV group. On the other hand, 17β - estradiol increased the level of the examined ion. It was 14.21 mg/g in OVH₁ group, 15.03 in OVH₂ group and 14.78 mg/g in OVH₃ group. There were significant differences in Mg levels between OVH₁- OVH₃ and OV groups.

Table 1
The level of magnesium (mg/g) in rats’ teeth

<table>
<thead>
<tr>
<th>Examined group</th>
<th>Number of rats (n)</th>
<th>Mean (M)</th>
<th>Standard deviation (SD)</th>
<th>Significance of differences (P*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>10</td>
<td>16.78</td>
<td>0.73</td>
<td>e</td>
</tr>
<tr>
<td>SH</td>
<td>10</td>
<td>16.15</td>
<td>0.26</td>
<td>d</td>
</tr>
<tr>
<td>OV</td>
<td>10</td>
<td>13.56</td>
<td>0.51</td>
<td>a</td>
</tr>
<tr>
<td>OVO</td>
<td>10</td>
<td>14.04</td>
<td>0.52</td>
<td>ab</td>
</tr>
<tr>
<td>OVH₁</td>
<td>10</td>
<td>14.21</td>
<td>1.03</td>
<td>b</td>
</tr>
<tr>
<td>OVH₂</td>
<td>10</td>
<td>15.03</td>
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<td>c</td>
</tr>
<tr>
<td>OVH₃</td>
<td>10</td>
<td>14.78</td>
<td>0.20</td>
<td>c</td>
</tr>
</tbody>
</table>

*Differences between means are significant when means are not designated the same letter.

Table 2 presents magnesium concentration in rats’ mandible in the examined groups. In the control group the level of Mg was 6.29 mg/g. After ovariectomy the mean magnesium concentration showed a significantly marked decrease (5.16 mg/g). Operation stress did not significantly influence the Mg level in comparison with the control group. Administration of 17β-estradiol increased the content of the examined element from 5.47 mg/g in OVH₁ to 6.13 mg/g in OVH₃ group. In comparison with OV group statistical significance was observed.
Table 2
The level of magnesium (mg/g) in rats’ mandible

<table>
<thead>
<tr>
<th>Examined group</th>
<th>Number of rats (n)</th>
<th>Mean (M)</th>
<th>Standard deviation (SD)</th>
<th>Significance of differences (P*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
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<td>6.29</td>
<td>0.27</td>
<td>c</td>
</tr>
<tr>
<td>SH</td>
<td>10</td>
<td>6.14</td>
<td>0.22</td>
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<tr>
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<td>0.90</td>
<td>a</td>
</tr>
<tr>
<td>OVO</td>
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<td>0.16</td>
<td>ab</td>
</tr>
<tr>
<td>OVH₁</td>
<td>10</td>
<td>5.47</td>
<td>0.13</td>
<td>b</td>
</tr>
<tr>
<td>OVH₂</td>
<td>10</td>
<td>6.13</td>
<td>0.10</td>
<td>c</td>
</tr>
<tr>
<td>OVH₃</td>
<td>10</td>
<td>5.59</td>
<td>0.72</td>
<td>b</td>
</tr>
</tbody>
</table>

*Differences between means are significant when means are not designated the same letter.

Discussion

Osteoporosis is a disease characterized by a low bone mass, microarchitectural deterioration of bone tissue leading to enhanced bone fragility, and a consequent increase in fracture risk. In humans estrogen deficiency following menopause is associated with a more or less intense osteopenia. In some subjects, such a decrease in bone mass induces osteoporosis (7). The last review presents a new concept that bone health depends not just on estrogen and calcium, but on a wide range of other nutrients and minerals (4). Mineralized tissues of bone and teeth play an important role in mineral metabolism of organism. When the level of the ions in plasma decreases these ions are mobilized from reserves in bone and teeth (13).

Histomorphometric analyses showed a significant drop in trabecular bone volume in Mg deficient animals by 16 weeks. A surprising new observation was an increase in osteoclast bone resorption with Mg depletion in rats (11).

The experiment showed that after ovariectomy the level of magnesium in rat mandible was decreasing. Deficiency of estrogens caused magnesium depletion in bone of jaws. Also in rat teeth the loss of the examined ion took place. According to Durlach, magnesium deficiency is revealed in dentition, at first (3). Also incisors of Mg-deficient rats showed some disorders: a marked inhibition of eruption and decrease in mitoses of apical tissue (5). These findings suggest that the deficit of this element induced inhibition of growth and structure changes in bone tissue and teeth. On the other hand, accumulation and mobilization of minerals from bones and teeth are controlled by a number of regulatory mechanisms including physio-chemical, endocrine and nutrient. In the group of women with osteoporosis significantly lower ionized magnesium level was found in comparison with the control group (2). This is a new aspect of an important role of minerals in postmenopausal osteoporosis. It is especially important to prevent the depletion of ions from bone and teeth.
References