EXPERIMENTAL INVESTIGATIONS ON BONE DENSITY OF THE MANDIBLE AND SPINE IN OVARIECTOMIZED RATS

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Received for publication December 18, 2002.

The aim of this study was to estimate a bone density of the mandible and spine in experimental model of postmenopausal osteoporosis. Female Wistar rats were the investigative material. Rats were qualified for 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 end of the experiment densitometric examinations of the mandible and spine were made using DEXA method and measured bone mineral density (BMD). The results of the examination indicate that estrogen deficiency after ovariectomy leads to a decrease in BMD index of the examined bones, whereas the administering of 17β-estradiol influences the bone density and depends on hormone dose.

Key words: rats, bone mineral density, postmenopausal osteoporosis, experimentation.

Postmenopausal osteoporosis concerns women after surgical removing of ovaries or after natural menopause. As the result of this, more intensive bone metabolism is observed. Resorption of bone tissue prevalences for bone formation. According to Riggs (7), appearance of bone mass depletion is also caused by temporary limiting of osteoblasts activity. Therefore an estrogen deficiency can be pathophysiological cause of bone mass defect in women after menopause (5).

Bone density is an essential, possible to assay indicator of osteopenic and osteoporotic changes in the skeletal system. For early, quantitative, real assessment of bone mass depletion, densitometric examinations are used. This form of radiological examination is developing very quickly, especially last years. Short time of examination, the better resolving power of picture, the lower radiation dose, the higher precision, the higher possibility of adaptation to examine various bones of skeleton and the lower costs service - there is everything what decides, that densitometry is an universal and useful method in diagnosing osteoporosis. The most often used unit of the bone density is bone mineral density (g/cm², g/cm) index (BMD) (8).

Postmenopausal osteoporosis manifests changes of bone density, which are observed mainly in long bones and vertebrae. To assess the changes in bone tissues of masticatory system and to compare them with spine lesions, experimental
investigations on bone mineral density in Wistar rats after ovariectomy were undertaken.

**Material and Methods**

Young, adult female Wistar rats, weighing 250-300 g were used. The animals were fed a standard chow and housed in cages with light-dark cycle and allowed free access to water and feed. 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; OVH₁ - rats after bilateral ovariectomy taking 17β-estradiol in a dose of 1.25 μg per animal, twice a week, during seven weeks; OVH₂ - rats after bilateral ovariectomy taking 17β-estradiol in a dose of 12.5 μg per animal, twice a week, during seven weeks; and OVH₃ - 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 examined parameters. 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 OVH₁-OVH₃ groups Oestradiolum benzoicum (Jelfa – Jelenia Góra) was administered intramuscularly.

After the end of the experiment the rats were sacrificed by the lethal dose of Tiopenthal and decapitated. Bone mineral density (BMD) of the spine and mandible was measured using DPX-A densitometer and computer programme Small Animal Software. The obtained data (g/cm²) 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, obtained from variance analysis (ANOVA). Correlations between bone mineral density of the mandible and spine were determined by the r-Pearson test.

**Results**

Table 1 presents bone mineral density of the mandible and spine. In the control group mean BMD of the mandible was 0.0792 g/cm² and in SH group - 0.0730 g/cm², and differences between these groups were not statistically significant. BMD of the mandible was the lowest in OV group - 0.0627 g/cm². The administration of 17β-estradiol increased the mean BMD of the mandible. It was 0.0782 g/cm² in OVH₁ group, 0.1236 g/cm² in OVH₂ group and 0.0887 g/cm² in OVH₃ group. Differences between these groups and OV group were statistically significant. The mean result of BMD of the spine in CL group was 0.2848 g/cm² and in SH group 0.2797 g/cm² and differences were not statistically significant. After ovariectomy bone mineral density of the spine decreased to 0.2711 g/cm² and this result was statistically significant in relation to all groups. Administration of 17β-estradiol cause an increase in BMD of the spine from 0.2812 g/cm² in OVH₁ group to 0.2909 g/cm² in OVH₂ group, and 0.2842 g/cm² in OVH₃ group. Differences between mean results of OVH₁ - OVH₃ groups and control group were not statistically significant.
Table 1
Bone mineral density of the mandible BMD-M and spine BMD-S (g/cm²)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats (n)</th>
<th>BMD-M (M± SD)</th>
<th>Significance of differences (P₁)</th>
<th>BMD-S (M± SD)</th>
<th>Significance of differences (P₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>10</td>
<td>0.0792 ±0.0148</td>
<td>bc</td>
<td>0.2848 ± 0.0086</td>
<td>bc</td>
</tr>
<tr>
<td>SH</td>
<td>10</td>
<td>0.0730 ±0.0181</td>
<td>ab</td>
<td>0.2797 ± 0.0118</td>
<td>b</td>
</tr>
<tr>
<td>OV</td>
<td>10</td>
<td>0.0627 ±0.0109</td>
<td>a</td>
<td>0.2711 ±0.0074</td>
<td>a</td>
</tr>
<tr>
<td>OVO</td>
<td>10</td>
<td>0.0653 ±0.0104</td>
<td>a</td>
<td>0.2792 ± 0.0116</td>
<td>b</td>
</tr>
<tr>
<td>OVH₁</td>
<td>10</td>
<td>0.0782 ±0.0147</td>
<td>bc</td>
<td>0.2812 ± 0.0073</td>
<td>b</td>
</tr>
<tr>
<td>OVH₂</td>
<td>10</td>
<td>0.1236 ±0.0083</td>
<td>d</td>
<td>0.2909 ± 0.0032</td>
<td>c</td>
</tr>
<tr>
<td>OVH₃</td>
<td>10</td>
<td>0.0887 ±0.0102</td>
<td>c</td>
<td>0.2842 ± 0.0076</td>
<td>bc</td>
</tr>
</tbody>
</table>

* Differences between means are significant when means are not marked with the same letter

Table 2 and Fig. 1 present the correlation between the bone mineral density of the mandible and spine. As can be seen from the Table and Figure a statistically significant positive correlation was observed.

Table 2
Correlation r-Pearson between the examined traits

<table>
<thead>
<tr>
<th>Examined traits</th>
<th>Number of rats</th>
<th>Correlation coefficient (r)</th>
<th>Significance of differences (P) *</th>
<th>Regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD-M vs BMD-S</td>
<td>70</td>
<td>0.4537</td>
<td>0.00008</td>
<td>BMD-S=0.192*BMD-M+0.265</td>
</tr>
</tbody>
</table>

* Assigned correlation coefficient is significant at P< 0.05
BMD-S = 0.192 * BMD-M + 0.265
Correlation coefficient: \( r = 0.4537 \)

Fig. 1. Correlation between bone mineral density of mandible BMD-M and spine BMD-S.

**Discussion**

Densitometric examinations carried out on the mandible and spine of rats after ovariectomy demonstrated a decrease in bone mineral density of the examined tissues in comparison with control group. This result was statistically significant. Estrogen deficiency after ovariectomy caused increasing bone tissue metabolism. Prevalence of resorption processes by osteoclasts on the restoration of bone by osteoblasts led to depletion of bone mass. In the performed experiment these changes were observed both in the spine and mandible. But no changes in bone mineral density of the mandible between SH, OV and OVO groups were observed. It means that operation stress and oil base of estadiol (*oleum pro injectione*) did not statistically influence the BMD index of the mandible.

In other studies ovariectomy resulted in an increase in failure load and stiffness of the mandible in adult rats (4). This increase in structural properties of the mandible may be a consequence of increased number of chewing cycles, which enhanced the strength and stiffness of the mandible in the ovariectomized rats. These rats in this experimental model ate by 10% more feed than did sham control. But these changes have not been confirmed by a decrease in bone mineral density of the mandible. Only a decrease in bone area fraction was observed (4). In other experimental studies carried out on rats after ovariectomy, it was showed that only extraction of molar tooth in the mandible and only ovariectomy did not cause changes in stiffness of the mandible. But when these two factors occurred together, stiffness of
the mandible was decreasing - indicating the interaction between ovariectomy and mandible molar extraction (3, 9).

Also results of bone mineral density of the spine obtained during this experiment found confirming in researches of other authors. Ammann et al. (1) observed the decreasing of BMD index of the spine and femur in rats with estrogen deficiency. Examinations carried out by Yeh (11) on rats six months after ovariectomy showed a decrease in bone mass of tibia metaphysis and carpus. These results were statistically significant. Differences were more marked in metaphysis, which contained mainly spongy bone.

Thirty years ago, it was proved that estrogen replacement therapy prevents postmenopausal bone loss. But estrogen influence on bone metabolism is not yet clear. In experimental studies, it was demonstrated that estrogens inhibit the stimulation activity of parathormone on prostaglandin E2 secretion by osteoblasts in long bones of rats (6). Putting away estrogens in experimental animals caused immediately an increase in synthesis of interleukin-6, which stimulates osteoclasts synthesis and as the result of this acceleration of bone resorption. This process is inhibited by 17β-estradiol and it is in accordance with own examinations. In the carried out experiment administration of 17β-estradiol significantly influences the increasing of BMD index of the mandible and spine in rats after ovariectomy in comparison with animals receiving placebo. These changes depended on used dose of hormone. The highest increment of the examined bone density was after dose of 12.5 μg given to rats twice a week. Application of 125μg of 17β-estradiol did not cause statistically significant increase in bone density, but kept this index on the constant level. It can be the result of decreasing of remodelling spaces caused by estrogens and transient deficiency of bone density in places, where the resorption was, but bone formation was not yet initiated. This phase - plateau can indicate the stabilization of a new level of remodelling balance (8). Another study carried out on rats, demonstrated that administration of 17β-estradiol together with exercises influenced on bone density of the tibia (11). Also movement speed of teeth during experimental orthodontic treatment after application of 17β-estradiol was decreased as the consequence of increased stiffness of masticatory bone system (10).

Problem of the discovery of postmenopausal osteoporotic changes in the maxilla and mandible is still open. During diagnostic examination, bone mineral density of the spine, femoral neck, calcaneal bone and wrist are done. But if more regions of skeleton are examined, the diagnosis of osteoporosis or osteopenia is more effective. Therefore, the observation of depletion of bone mineral density of the mandible should be monitoring, where the rate of osteoporotic changes can be different from those in the other regions of body.

Acknowledgments: This study was supported by KBN grant No 3 PO5E 078 23.

References