CHANGES OF THE CALCIUM METABOLISM IN MINERALIZED TISSUES OF RATS DURING EXPERIMENTAL POSTMENOPAUSAL OSTEOPOROSIS

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Received for publication March 08, 2004.

Abstract

The aim of this study was to assess the hypoestrogenism influence on the calcium content in rat teeth and mandible. The calcium level was measured by atomic absorptive spectrometry technique. This level depended on the kind of experimental group: control, sham-operated, after ovariectomy (OV) and rats after ovariectomy receiving 17β-oestradiol (OVH) in three different doses (1.25, 12.5, 125 µg). After the end of the experiment, the decreased calcium level in teeth and mandible of rats from OV group was observed. In OVH1-OVH3 groups the calcium level in the examined tissues increased in the comparison with OV group. These results indicate that decreased oestrogen level changed mineralization of bone and tooth tissues and can cause damage of stomatognatic system.

Key words: rats, calcium, postmenopausal osteoporosis, teeth, mandible.

One of the elements securing the homeostasis of calcium metabolism within the bone tissue of the female body is the correct level of oestrogens - especially oestriadol. A significant drop in the production of this hormone in postmenopausal phase (among other factors) results in a rapid decrease in calcium content within the bones (ca. 60 mg per day), leading to demineralization and eventually to development of postmenopausal osteoporosis. The main characteristics of this disease are: decrease in bone volume despite the balance between their organic and mineral components, damage in microarchitecture of the bone tissue and increased brittleness of long bones and vertebrae. In reference to norm, age and sex, osteoporosis shows also an abnormal increased skeletal resorption. Many elements propagating its progress lead to a negative balance of calcium within the body, which then leads to a decrease in volume and mineralization of the bone tissue. The densitometric testing of mineral density of the bones in women affected by osteoporosis decreases by more than ±2.5 (SD) under the top end of norm of the bone mass (3).

The mineral basis of a bone is a junction of calcium and phosphorus in the form of hydroxyapatite crystals and amorphous phosphates. Bone apatite shows a high reactive chemical activity during which the exchange of ions with the environment can takes place. The body of an adult man contains on average 1000-1200 g of calcium, which accumulates mainly in bones, teeth and nails. This makes 99% of an entire pool of calcium in a human body. The remaining 1% is spread within tissues and systemic fluids. Oestrogens affect calcium distribution through induction of parathormone secretion (PTH) which stimulates renal 1-alpha-hydroxylasis catalysing the conversion from 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol. This active state of vitamin D3 positively affects the balance of calcium, therefore we can talk about a particular oestrogens-depending calcium distribution within the body (4).

However, well documented and tested is the process of demineralization of long bones and vertebrae in postmenopausal osteoporosis, still so little do we know about the changes taking place within calcium metabolism of the mandible and teeth. Therefore one can conclude that the aim of this experiment was to determine changes within calcium content in the mandible and teeth of animals after ovariectomy (state of experimental postmenopausal osteoporosis) and undergoing the treatment with 17β-oestradiol.

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 allowed free access to water and feed. The experiment was carried out in accordance with guidelines of Animal Ethical Research Committee of
Medical University in 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*; OVH₁ - rats after bilateral ovariectomy taking 17β-oestradiol in a dose of 1.25 µg per animal, twice a week, during seven weeks; OVH₂ - rats after bilateral ovariectomy taking 17β-oestradiol in a dose of 12.5 µg per animal, twice a week, during seven weeks, and OVH₃ - rats after bilateral ovariectomy taking 17β-oestradiol 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 oestradiol *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 anaesthetized by the lethal dose of Tiopenthal, decapitated and incisive tooth and mandible bones were prepared. The samples for each experimental group were carefully washed in a distilled water, dried and weighed with exactitude to 0.0001g, after careful labelling the examined samples were kept separately. Rat mandible and teeth were mineralized in muffle furnace at 500°C (dry method). The mineralization process was accelerated with using a concentrated nitric acid (65% HNO₃, Suprapur, Merck), then the ashes were dissolved in 15% water solution of hydrochloric acid (30% HCl Suprapur, Merck) and transmitted to calibrated flask with capacity of 15 cm³ using deionized water. The calcium level in the examined mineralized samples was calculated per unit of tooth and mandible tissue amount (mg/g tissue) and then estimated with a Pye-Unicam atomic absorption spectrophotometer type SP192, with the following parameters: analytic wavelength - 422.7 nm, lamp current - 10.5 mA, fissure width – 0.2 nm, acetylene-flow – 1 dm³ x min⁻¹, air-flow – 5 dm³ x min⁻¹, burner height – 10 cm.

The obtained data were analysed by calculating mean (M) and standard deviation (SD). The significance of differences between groups have been determined on the basis of confidence intervals (NIR), obtained from variance analysis (ANOVA). Differences between means were significant when means were not designated the same letter. Correlations between calcium level of the mandible and teeth were determined by the r-Pearson test.

**Results**

**Calcium content in teeth (Fig. 1).** The calcium content in the incisors of the control rats was 368.64 mg/g, in the SH group – 358.77 mg/g. The level of Ca in rats’ teeth after ovariectomy was significantly lower than that in control animals – 325.94 mg/g. 17β-oestradiol administration caused the increase in calcium content in teeth in all OVH groups, and these values were statistically significant in the comparison with OV group.

**Calcium content in mandible (Fig. 2).** The mean content of calcium in the mandible of the control rats was 450.26 mg/g. In the animals after ovariectomy calcium level was decreased to 308.15 mg/g. This result was statistically significant in the comparison with the CL and SH groups. In cases of 17β-oestradiol treatment of ovariectomized rats, the calcium content in the mandible was significantly increased only in OVH₂ and OVH₃ groups. Administration of *oleum pro injectione* did not statistically influence the calcification of the mandible.

**Correlation between calcium content in teeth and mandible (Fig. 3).** In the examined population statistically significant positive correlation was observed: correlation coefficient r – 0.93; significance of differences P<0.002.

![Fig. 1. Calcium content in rat teeth.](image-url)
Fig. 2. Calcium content in rat mandible.

Regression equation
\[ \text{Ca}_{\text{mandible}} = -923.4 + 3.7506 \times \text{Ca}_{\text{teeth}} \]
Correlation coefficient: \[ r=0.92894 \quad p<0.002 \]

Fig. 3. Correlation between calcium content in teeth and mandible.

**Discussion**

Calcium homeostasis is a complicated mechanism. It is thought that presence of calcium in human body creates optimal conditions for mineralization and growth of the bones. Calcium helps the bones to reach peak bone mass and later prevents the body from any excessive osseous loss triggered by the processes of ageing (8). Calcium pool in bone tissue subjected to renewal (ca. 4 g) can be described as two categories. First one being the calcium contained within the superficial coat of hydroxyapatites and within fluids surrounding its surface. In this area calcium ions are quickly exchanged. The second one is the calcium spread within the crystals of the mineral substance of the bone tissue. This particular cluster of ions gets renewed very slowly (4).

It is understood that those replaceable ional components (calcium being one of them) leave the bone or dental tissues when their concentration in blood decreases and they are being deposited in those tissues when their level within intercellular fluids increases (1, 2). After the performed animal tests our analysis concludes that estrogenic deficit (following the removal of ovaries in rat females) leads to a significant loss of calcium ions both within the mandible and the incisors. The deficit in female sexual hormones can lead to an increased activity of parathormone on the bone tissue (7). In bones it increases the resorption stimulated by the activities of osteoclasts and osteocytes. The removal of ovaries as well as the menopause and other cases of oestrogenic deficit interrelate with decalcification and loss of bone tissue within the female body. Oestrogens such as 17ß-oestradiol can prevent this.
The oestrogenic treatment of experimental osteoporosis after the removal of ovaries in our tests prevented demineralization of the mandible and teeth and in some cases it increased the mass of the bone tissue and its calcium content (in comparison with the control group and according to the dose of the applied 17β-oestradiol).

According to the results of tests performed by Wronski on rats with their ovaries removed, after applying the oestrogens they had their resorption process inhibited and later showed a positive balance of their bone tissue (10).

Women in their menopause receiving oestrogenic treatment show that the balance of calcium and the metabolism of minerals tend to return to the premenopausal count (5). Therefore supplementing the calcium solely through a diet proves to be insufficient, as it does not produce satisfactory results in osseous analysis.

The initial menopausal symptom is a deficit in oestrogens, which cannot be compensated by pure supplementation of calcium. Thus one can conclude that only a hormonal therapy supplemented with dietary calcium and vitamin D intakes can induce a positive effect on the bone tissue.

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

**References**