HISTOLOGICAL STRUCTURE OF FEMORAL BONE TISSUE IN ADULT MALE RATS AFTER SUBCHRONIC PERORAL ADMINISTRATIONS OF CADMIUM AND SELENIUM

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ABSTRACT

The aim of this study was to examine effects of subchronic peroral administrations of cadmium and selenium on femoral bone microscopic structure in adult male rats. 1-month-old male Wistar rats were randomly divided into three groups, each containing 10 males. In the first group (EG1), rats were administered by cadmium at the dose of 30 mg of CdCl₂/L in drinking water for 90 days. In the second group (EG2), animals received a drinking water containing 5 mg of Na₂SeO₃/L for the same treatment period. The third group of rats without cadmium and selenium applications, served as a control group (CG). At the end of the experiment (90 days), all animals were killed and their right femora were collected for microscopic evaluation. We found that the qualitative histological characteristics of the compact bone tissue were different in the middle part of compact bone in medial and lateral views between experimental (EG1, EG2) and control groups (CG). In Cd- and Se-treated rats, a smaller number of primary and secondary osteons was identified. Moreover, a few
resorption lacunae were observed in rats perorally exposed to Cd (EG1). Histomorphometric evaluation showed a significant decrease ($P<0.05$) in all variables (area, perimeter, maximum and minimum diameter) of the primary osteons' vascular canals in the Cd-exposed rats (EG1) as compared to the control group. In comparison with the group CG, they were significantly increased ($P<0.05$) in the Se-exposed rats (EG2). Values of the Haversian canals and secondary osteons were significantly decreased ($P<0.05$) for all variables in rats from both experimental groups (EG1, EG2) as compared to the control (CG). The results allow for the conclusion that subchronic exposure to Cd and Se at the levels used in this study significantly influenced microscopic structure of femoral bone tissue in adult male rats.

**Keywords:** femoral bone, histomorphometry, rat, cadmium, selenium

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**INTRODUCTION**

Cadmium (Cd) is a heavy metal that is widely present in our environment as a pollutant (Moulis and Thévenod, 2010). Populations worldwide are exposed to Cd by low-level intake mainly through their food or by inhalation of tobacco smoke and exposure to Cd contaminated airborne particles (Järup and Åkesson, 2009). The kidneys, lungs, bones, liver and testes have been identified as the target organs for Cd toxicity (Siddiqui, 2010). In respect to bone tissue, the results obtained by Brzóska and Moniuszko-Jakoniuk (2005) have shown that chronic, or even low-level exposure to Cd, disturbs bone metabolism during skeletal development and maturity by affecting bone turnover. Indeed, rats exposed to Cd at only 1 μg/mL (or 1 mg/L) in their drinking water, had demineralized lumbar vertebrae and decreased mechanical strength as compared to controls (Brzóska and Moniuszko-Jakoniuk, 2004). Galicka et al. (2004) reported that receiving 50 mg/L Cd in drinking water for 6 months influenced collagen content and its solubility in the femoral bone of female rats. Cytotoxic and genotoxic effects of chronic exposure to Cd on rat bone marrow have also been demonstrated in the study by Celík et al. (2005).

Selenium (Se), an essential element in almost all biological systems, has pharmacological properties and it is considered an antioxidant (Meotti et al., 2004). Se is found in some selenoproteins such as glutathione peroxidase, and thioredoxin reductase. According to Ebert and Jakob (2007) several selenoproteins are expressed in bone and play important roles in bone metabolism. Se deficiency has been associated with the bone growth
retardation (Moreno-Reyes et al., 2001; Dobbelaere et al., 2003). On the other hand, excess of Se induces apoptosis in mature osteoclasts (Chung et al., 2006) and osteoblast-like cells (Milgram et al., 2008). Furthermore, Se is reported to be teratogenic (Greenberg, 2003).

According to our knowledge, a detailed histological analysis of compact bone tissue including histomorphometric evaluation after long-term peroral administrations of Cd and Se in rats has not been done prior to our experiment. Therefore, the aim of the present study was to analyse microstructural changes in the femoral bone tissue of rats subchronic exposed to Cd and Se in their drinking water.

MATERIAL AND METHODS

Our experiment was conducted on thirty 1-month-old male Wistar rats (obtained from the accredited experimental laboratory of the Slovak University of Agriculture in Nitra). Clinically healthy rats were randomly divided into three groups, of 10 animals each. In the first group (EG1), young males were dosed with a daily intake of 30 mg of CdCl₂/L in drinking water for 90 days. Ten 1-month-old males of the group EG2 received a drinking water containing 5 mg of Na₂SeO₃/L for the same treatment period. The third group without Cd and Se suplementations served as a control group (CG). At the end of the experiment, all animals were killed and their right femora were used for microscopic analyses. The present experiment was approved by the Animal Experimental Committee of the Slovak Republic.

For histological analysis, each right femur was sectioned at the midshaft of its diaphysis. The obtained segments were placed in HistoChoice fixative (Amresco, USA). Specimens were then dehydrated in ascending grades of ethanol and embedded in epoxy resin Biodur (Günter von Hagens, Heidelberg, Germany) according to Martiniaková et al. (2008). Transverse thin sections (70-80 µm) were prepared with a sawing microtome (Leitz 1600, Germany) and affixed to glass slides by Eukitt (Merck, Darmstadt, Germany) as previously described (Martiniakova et al., 2010). The qualitative histological characteristics of the femoral bone tissue were determined according to the internationally accepted classification systems of Enlow and Brown (1956) and Ricqlès et al. (1991). The quantitative (histomorphometric) variables were assessed using the software Motic Images Plus 2.0 ML (Motic China Group Co., Ltd.) in anterior, posterior, medial and lateral views of thin section(s). We measured area, perimeter, and the minimum and maximum diameter of primary osteons' vascular canals, Haversian canals and secondary osteons in all views of thin section(s) in order to minimize inter-animal differences. The measured values were expressed
as mean ± standard deviation. The differences in the quantitative histological characteristics of the compact bone between Cd- and Se-exposed rats and those of the control groups were determined using the unpaired T-test. The criterion significance level was set at $P<0.05$.

RESULTS AND DISCUSSION

The femurs of rats from the control group (CG) had the following microstructure in common: an internal layer surrounding the medullary cavity consisted of a zone of non-vascular bone tissue in all views (anterior, posterior, medial and lateral) of the thin section(s). This type of bone tissue was composed of concentric lamellae with osteocytes. In addition, some areas of primary vascular radial bone tissue were observed in lateral, anterior and posterior views. This tissue created vascular canals (branching and non-branching) radiating from the marrow cavity. Moreover, some primary and secondary osteons were especially found in anterior and posterior views near the endosteal surfaces. In the middle parts of the compact bone, a few primary and secondary osteons were identified. Finally, the periosteal border was composed of non-vascular bone tissue, mainly in anterior and posterior views.

On the other hand, femoral bone microstructure of rats from the experimental groups (EG1, EG2) was different in the middle part of the compact bone (in medial and lateral views) in comparison with the control group. In these views, primary vascular radial bone tissue occurred because vascular canals expanded from endosteal border into central area of the bone and supplanted primary and secondary osteons. Therefore, a smaller number of the osteons was also observed in the Cd- and Se- exposed rats. Moreover, a few resorption lacunae were identified near endosteal surfaces in Cd exposed rats (EG1) (mainly in antero-medial and postero-medial views), which could indicate the early stage of osteoporosis.

For the quantitative histological characteristics, 1080 vascular canals of primary osteons, 916 Haversian canals and 916 secondary osteons were measured. The results are summarized in Table 1, 2 and 3. We found an opposite effect of Cd and Se administration on the size of the primary osteons’ vascular canals in adult male rats. All measured variables (area, perimeter, maximal and minimal diameter) of the primary osteons’ vascular canals were significantly decreased ($P<0.05$) in the Cd-exposed rats (EG1) as compared to the control group (CG). On the other hand, they were significantly increased ($P<0.05$) in rats exposed to Se (EG2) in comparison with the control (CG). In both experimental groups (EG1, EG2), all variables of the Haversian canals and secondary osteons had significantly lower values ($P<0.05$) as compared to the control group (CG).
Table 1 Characteristics of the primary osteons' vascular canals of compact bone tissue in control (CG) and experimental groups (EG1, EG2)

<table>
<thead>
<tr>
<th>Rat’s group</th>
<th>N</th>
<th>Area (μm²)</th>
<th>Perimeter (μm)</th>
<th>Max. diameter (μm)</th>
<th>Min. diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>314</td>
<td>390.0 ± 95.6</td>
<td>71.89 ± 9.24</td>
<td>12.71 ± 2.06</td>
<td>9.92 ± 1.49</td>
</tr>
<tr>
<td>EG1</td>
<td>405</td>
<td>341.4 ± 65.8</td>
<td>66.74 ± 7.27</td>
<td>11.77 ± 1.81</td>
<td>9.29 ± 1.13</td>
</tr>
<tr>
<td>EG2</td>
<td>361</td>
<td>432.0 ± 51.6</td>
<td>74.19 ± 4.43</td>
<td>12.07 ± 0.99</td>
<td>10.94 ± 1.04</td>
</tr>
</tbody>
</table>

T-test: 1:2; 1:3 P<0.05 1:2; 1:3 P<0.05 1:2; P<0.05 1:2; 1:3 P<0.05

Legend: Data are expressed as means ± SD. N – number of measurements.

Table 2 Characteristics of the Haversian canals of compact bone tissue in control (CG) and experimental groups (EG1, EG2)

<table>
<thead>
<tr>
<th>Rat’s group</th>
<th>N</th>
<th>Area (μm²)</th>
<th>Perimeter (μm)</th>
<th>Max. diameter (μm)</th>
<th>Min. diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>367</td>
<td>445.6 ± 86.7</td>
<td>75.79 ± 8.11</td>
<td>13.18 ± 1.96</td>
<td>10.74 ± 1.22</td>
</tr>
<tr>
<td>EG1</td>
<td>208</td>
<td>384.9 ± 73.2</td>
<td>70.90 ± 6.94</td>
<td>12.52 ± 1.67</td>
<td>9.84 ± 1.31</td>
</tr>
<tr>
<td>EG2</td>
<td>341</td>
<td>430.7 ± 65.5</td>
<td>74.03 ± 5.75</td>
<td>12.52 ± 1.26</td>
<td>10.93 ± 1.14</td>
</tr>
</tbody>
</table>

T-test: 1:2; 1:3 P<0.05 1:2; 1:3 P<0.05 1:2; 1:3 P<0.05 1:2; 1:3 P<0.05

Legend: Data are expressed as means ± SD. N – number of measurements.

Table 3 Characteristics of the secondary osteons of compact bone tissue in control (CG) and experimental groups (EG1, EG2)

<table>
<thead>
<tr>
<th>Rat’s group</th>
<th>N</th>
<th>Area (μm²)</th>
<th>Perimeter (μm)</th>
<th>Max. diameter (μm)</th>
<th>Min. diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>367</td>
<td>5456 ± 1708</td>
<td>269.7 ± 41.9</td>
<td>49.7 ± 8.8</td>
<td>34.5 ± 6.5</td>
</tr>
<tr>
<td>EG1</td>
<td>208</td>
<td>4584 ± 1327</td>
<td>240.6 ± 35.3</td>
<td>41.5 ± 6.9</td>
<td>34.6 ± 5.3</td>
</tr>
<tr>
<td>EG2</td>
<td>341</td>
<td>4168 ± 1164</td>
<td>232.9 ± 32.6</td>
<td>41.5 ± 6.9</td>
<td>31.6 ± 5.3</td>
</tr>
</tbody>
</table>

T-test: 1:2; 1:3 P<0.05 1:2; 1:3 P<0.05 1:2; 1:3 P<0.05 1:2; 1:3 P<0.05

Legend: Data are expressed as means ± SD. N – number of measurements.

Our results from the qualitative histological analysis of the femora in rats from the control group (CG) correspond with those reported by other researches (Enlow and Brown, 1958; Martiniaková et al., 2005; Reim et al., 2008; Martiniaková et al., 2009). We identified non-vascular, primary vascular radial and, irregular Haversian bone tissues. However, there was no evidence of true Haversian intracortical bone remodeling. It is generally known that aged rats and mice lack true Haversian cortical bone remodeling but not cancellous bone remodeling activity (Erben, 1996; Reim et al., 2008; Martiniaková et al., 2009). Therefore, some secondary osteons can be observed in their long bones (near endosteal border). The same results have also been documented in the study of Reim et al. (2008) for 13 month-old male rats.

In the Cd- and Se- exposed rats (EG1, EG2), prolonged intake of moderate level of Cd and Se induced changes in the middle part of the compact bone in medial and lateral views,
where primary and secondary osteons were replaced by primary vascular radial bone tissue. Therefore, a smaller number of the osteons was observed in these rats. Disappearance of the Haversian canal system, which was replaced by a large quantity of degenerated, necrotic and restorative tissue, was demonstrated in the study by Li et al. (1997) in ovariectomized rats after a long-term Cd administration. In the study by Turan et al. (1997) osteocyte loss was identified because of the destruction of bone tissue and its replacement with large uncalkified volume of new bone matrix in rabbits fed diet supplemented with excess Se (10 mg Na₂SeO₃/kg of diet for a period of 12 weeks). The authors also observed decreased biomechanical strength of the femur in these animals. In our study, the formation of primary vascular radial tissue in the middle part of the central area of bone could be explained as an adaptive response of the bone tissue to Cd and Se toxicity in order to protect the tissue against cell death and subsequent necrosis. It is generally known that a high dose of Se induces apoptosis in mature osteoclasts (Chung et al., 2006) and causes a death of osteoblast-like cells (Milgram et al., 2008). Also, previous animal studies have reported that Cd stimulates the activity and differentiation of osteoclasts but inhibits the activity and differentiation of osteoblasts (Coonse et al., 2007; Chen et al., 2009). These effects result in the uncoupling of the normal balance between bone formation and bone resorption and leads to decreased bone mineral density and increased fracture incidence (Bhattacharyya, 2009), i.e. the typical manifestation that characterises osteoporosis. In our study, a few resorption lacunae were identified near endosteal surfaces in the Cd-exposed rats (EG1), which could indicate an early stage of osteoporosis.

Data obtained from the histomorphometric analysis showed a significant decrease ($P<0.05$) in all variables (area, perimeter, maximum and minimum diameter) of the primary osteons’ vascular canals in the Cd-exposed rats (EG1) as compared to control (CG). On the other hand, results obtained from the histomorphometric evaluation of these structures in Se-exposed rats (EG2) revealed the significant increased values of them. Moreover, Haversian canals were significantly decreased ($P<0.05$) in both experimental groups of rats as compared to the control. The vascular canal constriction identified in the femur of these Cd- and Se-exposed rats could be associated with changes in bone vascularization. In general, the vascular system is a critical target for metal toxicity and the actions of metals on the vascular system may play important roles in mediating the pathophysiological effects of metals in specific target organs (Prozialeck et al., 2008). Blood vessels readily adapt structurally in response to sustained functional changes, particularly those related to chronic pressure alterations or changes in the nutritional demands of the tissue (Folkow, 1983; Pries et al.,
Taking into account these considerations we propose that the observed changes in blood supply of the bone are related to a adaptive response to minimize the toxic effect of subchronic Cd or Se exposure on the bone tissue.

According to our results, subchronic peroral Cd and Se intoxication led to a decreased size of the secondary osteons. In bone, Cd may be incorporated into hydroxyapatite (HA) crystals instead of Ca (Blumenthal et al., 1995; Brzóska and Moniuszko-Jakoniuk, 2005). Blumenthal et al. (1995), showed that the incorporation of Cd into HA crystal introduced little strain in the lattice but resulted in a decreasing C-axis spacing and a corresponding decrease in crystal size along the C-axis. Also, the results of Boyar (2004) indicate that the excess of Se increased the amount of carbonate content in both femur and tibia of Wistar rats intraperitoneally injected by Na₂SeO₃/kg everyday, for 4 weeks. In general, the incorporation of the carbonate ions into the crystal structure of HA results in the changes of physical and chemical properties of HA (Liu et al., 2011). HA crystals, as a major mineral component of bones, are aligned with their long axis parallel to the axis of the collagen fibers (Boskey, 2005), creating concentric lamellae of secondary osteons. On the basis of this knowledge and the results of Blumenthal et al. (1995); Boyar (2004); Boskey (2005); Brzóska and Moniuszko-Jakoniuk (2005) and Liu et al. (2011), we speculate that a decrease in HA crystals could partially contribute to the decreased size of the secondary osteons in the Cd- and Se-exposed rats.

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