EFFECTS OF SELENIUM AND SIMULTANEOUS EXPOSURE TO SELENIUM AND DIAZINON ON FEMORAL BONE STRUCTURE IN ADULT RATS

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The present study aimed to investigate the macro- and the microscopic structure of femoral bone tissue in adult male rats after selenium (Se) and simultaneous exposure to Se and Diazinon (DZN). One month-old male, Wistar rats were randomly divided into three groups, of 10 males each. In the first group (EG1), rats were administered by Se at a dose of 5 mg of Na_2SeO_3/l in drinking water for 90 days. In the second group (EG2), animals received a drinking water containing 5 mg of Na_2SeO_3/l and 40 mg of DZN/l for the same time period. The third group of males without Se and DZN administration served as a control group (CG). Our results revealed a significant decrease in femoral length and cortical bone thickness in experimental groups (EG1, EG2) of rats compared to the control ones (CG). Rats from experimental groups (EG1, EG2) also displayed different microstructure in the middle part of the femur, where vascular canals expanded into central area of the bone while, in control rats, these occurred near endosteal surfaces only. Additionally, a smaller number of primary and secondary osteons was identified in experimental groups of these rats. A few resorption lacunae were observed in them simultaneously administered to Se and DZN. Based on these findings we can conclude that sub-chronic exposure to Se and simultaneous exposure to Se and DZN influences significantly macro- and microscopic structure of femoral bone tissue in adult male rats.

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1. Introduction

Selenium is an essential trace element involved in several key metabolic activities, such as fertility, protection against oxidative damage, regulation of immunity and thyroid function.[1,2] In China, the deficiency of selenium in endemic regions causes Kashin–Beck disease (KBD) - a chronic endemic degenerative osteoarthritis.[3-5] The nutrition range between selenium essentiality and toxicity is fairly narrow.[6] Excess of Se causes abnormal bone and cartilage development and it is reported to be teratogenic.[7] Harr et al.[8] mention that 4 to 16 ppm Se in the diet causes adverse effects in the bones of rats; the bones were soft and the epiphyseal plates were easily separable. Chung et al.[9] found that apoptosis in mature osteoclasts supplemented for 6 hours in the additional presence of 5μM or 10μM sodium selenite was mediated by the mitochondrial pathway. Also excess of Se causes a death of osteoblast-like cells,[10] leads to abnormal bone and cartilage development.

Organophosphorus (OP) compounds are one of the most common types of organic pollutants found in the environment.[11] Residual amounts of OP pesticides have been detected in the soil, water bodies, vegetables, grains and other food products.[12] Diazinon is an OP insecticide used to control a variety of insects.[13] Diazinon exerts its toxicity by binding its oxygen analog to the neuronal enzyme acetylcholinesterase (AChE), resulting in the accumulation of endogenous acetylcholine in nervous tissues and effects organs.[14] Recent evidence suggests that AChE may also have a functional role in bone.[15-17] Genever et al.[16] identified AChE expression in the bone cells - osteoblasts. This fact suggest that AChE might have a function in a noncholinergic capacity of the bone, possibly through its ability to mediate cell function as demonstrated in various other tissues.[18-20] In order to support this hypothesis, binding motifs for osteogenic factors, including Cbfa-1, 17h-estradiol and vitamin D3, have been identified in the extended promoter region of the AChE gene.[17] In addition, OP pesticides as the potent inhibitors of AChE, cause a significant reduction in bone mass and density in individuals following chronic low-level exposure.[15] Several skeletal deformities such as an undulatin notochord and fused cervical rings induced by these pesticides have also been observed in the study by Misawa.[21]

To our knowledge, there are no studies describing the detailed histological analysis of the femoral bone after Se and DZN intoxication. Therefore, the main aim of the present study was to determine macro- and microscopic changes in the femoral bone of adult male rats exposed to Se and simultaneously exposed to Se and DZN in their drinking water diets.

Experiments

Animals

Thirty 1-month-old male Wistar rats were obtained from the accredited experimental laboratory (number SK PC 50004) of the Slovak University of Agriculture in Nitra (Slovakia). These clinically healthy rats were randomly divided into three groups of 10 each. Male rats were used as they are less susceptible than female rats. The rats were housed individually in plastic cages in an environment maintained at 20-24°C, 55±10% humidity. They had access to water and food (feed mixture M3, Bonagro, Czech Republic) ad libitum.

The first experimental group (EG1; n=10 rats) was exposed daily to 5 mg/L Se, as Na_2SeO_3, in their drinking water for a total of 90 days. Ten 1-month-old males of the group EG2 received a drinking water containing 5 mg of Na_2SeO_3/l and 40 mg of DZN/l for the same time period of treatment. The third group (*n*=10 rats), without Se and DZN supplementation, served as the control group (CG). This study was approved by the Animal Experimental Committee of the Slovak Republic.

Macroscopic analysis

At the end of the 90 days, all the rats were killed and their femurs were used for macroscopic and microscopic analyses. The femurs were weighed on an analytical scale to 2 decimal places and the length was measured with a sliding instrument.

Microscopic analysis

For histomorphometric analysis, the right femurs were sectioned at the midshaft of the diaphysis and the segments fixed in HistoChoice fixative (Amresco, USA). The segments were then dehydrated in increasing grades (40 to 100%) of ethanol and embedded in Biodur epoxy resin (Günter von Hagens, Heidelberg, Germany) according to method described by Martiniaková et al.[22] Transverse thin sections (70-80 μm) were prepared with a sawing microtome (Leitz 1600, Leica, Wetzlar, Germany) and fixed on to glass slides by Eukitt (Merck, Darmstadt, Germany) as previously described.[23]

The qualitative histological characteristics of the compact bone tissue were determined according to the internationally accepted classification systems of Enlow and Brown[24] and Ricqlés et al.[25] Diaphyseal cortical bone thickness was measured by the Motic Images Plus 2.0 ML software. Twenty random areas were selected, and average thickness was calculated for each femur.

Statistic

Statistical analysis was performed using SPSS 8.0 software. All data were expressed as mean ± standard deviation (SD). The one-way analysis of variance (ANOVA), Tukey and Games-Howell tests were used for establishing statistical significance (significance level of $P < 0.05$) between experimental and control groups.

Results and discussion

Macroscopic analysis

Sub-chronic peroral exposure to Se and simultaneous exposure to Se and DZN, in drinking water for 90 days resulted in a significant decrease in femoral length and cortical bone thickness in these rats from both experimental groups (EG1, EG2) compared to the control group (CG). Femoral weight and cortical bone thickness were significantly increased in the Se-exposed rats (EG1) in comparison with those simultaneously exposed to Se and DZN (EG2). The results are summarized in Table 1.

Table 1 Average femoral weight, femoral length and cortical bone thickness in the control (CG) and experimental (EG1, EG2) groups of rats.

Rat´s group	No.	Femoral weight (g)	Femoral length (cm)	Cortical bone Thickness,mm
CG(1)	10	1.05 ± 0.17	3.94 ± 0.09	0.572±0.054
EG1(2)	10	1.07 ± 0.11	3.74 ± 0.07	0.528±0.051
EG2(3)	10	0.93 ± 0.07	3.75 ± 0.07	0.507±0.050
T-test		2:3 P<0.05	1:2; 1:3 P<0.05	1:2; 1:3, 2:3 P<0.05

Data are expressed as means ± SD. N – number of rats

Selenium is known to accumulate in the anterior pituitary of the pituitary gland, Thorlacius-Ussing et al.[27] mentioned decreased secretion of growth hormone (GH) and somatomedin C in rats after receiving 15 mg/L Na_2SeO_3 in their drinking water, suggesting that growth retardation could be mediated by reduced GH and somatomedin C production. Ip[26] also reported a reduction in growth in rats following 12 weeks exposure to 5 mg/kg Na_2SeO_3 in the diet. Gronbaek et al.[28] showed that Se treatment of 3.3 mg/L Na_2SeO_3 in the drinking water for 35 days induced a significant reduction in circulating IGF-I of rats. These rats also disposed a significantly shorter tibia length. In the study by Misawa et al. (1982) diazinon-induced inhibition in growth of some skeletal elements, such as femur, tibia, metatarsi and digits of the leg in chick embryos were also demonstrated.

The thickness of cortical bone is an important parameter in the evaluation of cortical bone quality and strength. The value of cortical bone thickness in rats from the control group (CG) was different in comparison with values reported by Comelekoglu et al.[29] and Chovancová et al.[30] However; this discrepancy may be influenced by the different age of the rats in these studies. We could not compare the value of cortical bone thickness in rats from both experimental groups with published data, since it was not reported in previous studies.

Se either increases or decreases toxicity of various xenobiotics, including pesticides, depending on its amounts introduced into an organism.[31] According to many studies, Se is considered to be a relatively protective factor or antagonistic element against harmful impact of various toxicants.[32-35] In contrary, Se administration into the organism in higher amounts is able to induce oxidative stress and toxicity.[36] Moreover, Se is probably able to affect the atom of sulphur in the molecule of diazinon and amplificates the diazinon toxicity.[37] Therefore we suppose

that significantly decreased femoral weight and cortical bone thickness could be mediated by higher oxidative stress and amplification of the DZN toxicity by the Se in rats from group EG2 compared to the EG1.

Microscopic analysis

Endosteal border of the femurs from the control rats (CG) was formed by non-vascular bone tissue in all views of the thin sections. The bone tissue contained cellular lamellae and osteocytes. Areas of primary vascular radial bone tissue were identified in anterior, posterior and lateral views. Vascular canals (branching or non-branching) radiating from the marrow cavity were also presented. Moreover, some primary and secondary osteons were especially found in the anterior and posterior views near the endosteal surfaces. In the middle part of the compact bone, a few primary and secondary osteons were identified. The periosteal border was again composed of non-vascular bone tissue, mainly in anterior and posterior views (Fig.1).

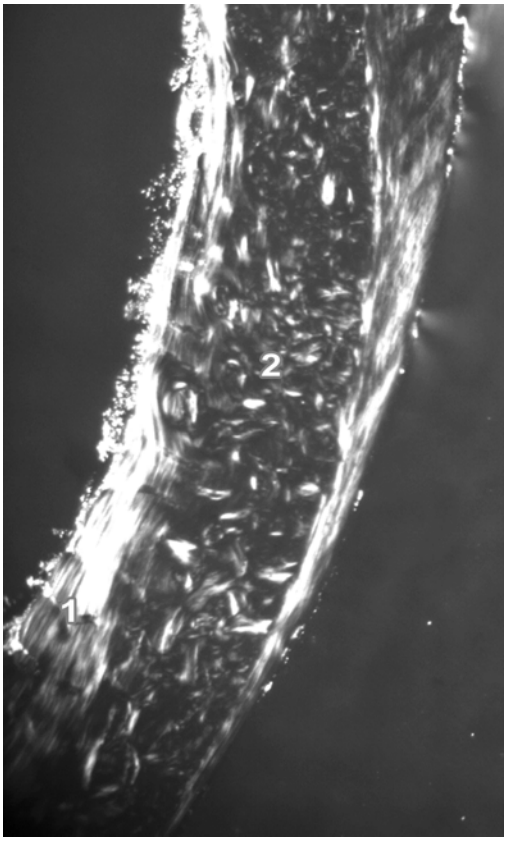

Figure 1. Microscopic structure of compact bone tissue in rat from the control group (1 - vascular canals radiating from marrow cavity, 2 - primary and secondary osteons).

The results of the qualitative histological analysis from the control rats corresponded with previous works.[38-41] We identified non-vascular and 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.[40,42] Therefore, some secondary osteons can be observed in the long bones (near the endosteal border). In our study, the newly formed remodeling units within compact bone originated from the endocortical surface and extended deep into the underlying compact bone. The same findings have also been documented in the study of Reim et al.[41] in 13 month-old male rats.

The rats from the both experimental groups (EG1, EG2) displayed a similar microarchitecture, except for the middle part of the femur in medial and lateral views. Vascular canals were shown to have expanded into the central area of the bone in these views. In some cases, the expansion was enormous and the canals occurred also near periosteal surfaces. A smaller number of primary and secondary osteons was also identified in these rats. Moreover, a few resorption lacunae were found near endosteal surfaces in rats simultaneously exposed to Se and DZN, which could indicate the early stage of osteoporosis (Fig.2).

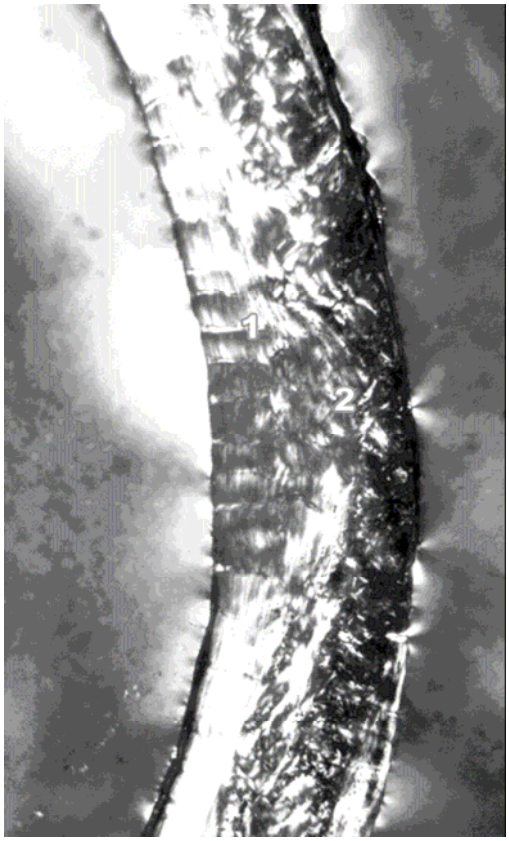

Figure 2. Microscopic structure of compact bone tissue in rat from the experimental group (1 - enormous vascular canals radiating from marrow cavity, 2 - smaller number of primary and secondary osteons).

Prolonged intake of a high dose of Se and Se in combination with DZN induced changes in the middle part of compact bone in medial and lateral views where primary vascular radial bone tissue was present. We propose that the formation of this type of bone, in the central area of the femur, could be explained as an adaptive response to Se and DZN toxicity, to protect bone tissue against the cell death. It is generally known that Se at high doses induces apoptosis in mature osteoclasts[9] and cell death in osteoblast-like cells.[10] In the study by Turan et al.[43] osteocyte loss was identified because of the destruction of the bone tissue and its replacement with large uncalcified volume of new bone matrix in rabbits fed excess Se (10 mg Na_2SeO_3/kg of diet for a period of 12 weeks). The authors also reported decreased biomechanical strength of the femur in Se-exposed animals. Diazinon causes inhibition of AChE which is expressed by osteoblasts and is present along cement lines and, to a lesser extent, in osteoid. Therefore, AChE could

have a role in the regulation of cell-matrix interactions and in the coupling of bone resorption to bone formation.[15] Therefore, higher bone resorption manifested by the presence of resorption lacunae in our study could be mediated by a low amount of AChE. In addition, Rangoonwala et al.[44] observed a progressive hypocalcemia in rats treated by sublethal dose of diazinon (daily intramuscular administration of 150 mg/kg b. w. and 225 mg/kg b.w. for 14 days). In is generally known that hypocalcemia inhibits calcitonin release. In the absence of the calcitonin, osteoclast activity is unregulated and bone resorption is accelerated[45] as it was documented also in our study.

In conclusion, sub-chronic exposure to Se and simultaneous exposure to Se and DZN in drinking water for 90 days significantly influenced macroscopic structure and also the microarchitecture of the femoral bone in adult male rats.

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