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OSTEOTOXIC EFFECT OF SIMULTANEOUS ADMINISTRATION TO CADMIUM AND DIAZINON ON BONE IN ADULT MALE RATS

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Abstract
Bone is a metabolically active tissue which can be influenced by various toxicants presented in the environment. The study was aimed to investigate the osteotoxic effect of simultaneous peroral administration to heavy metal cadmium (Cd) and nonselective organophosphorous insecticide diazinon (DZN) on bone in adult male rats. A total of twenty 1-month-old male Wistar rats were randomized into two experimental groups. In the first group (A), young males were dosed with combination of 30 mg $CdCl_2$/L and 40 mg DZN/L in drinking water, for 90 days. Ten 1-month-old males without toxicant administration served as a control group (B). After treatment period, detailed histological analysis of compact bone was performed in each group. We found that rats from the group A displayed different microstructure in the middle part of the *substantia compacta* where primary vascular radial bone tissue appeared (due to radial extension of vascular canals from the endosteal surfaces). In some cases, vascular expansion was so enormous that canals were also present near the periost. On the other hand, they occurred only near the endosteal surfaces in rats from the group B. In Cd-DZN-exposed rats, a smaller number of primary and secondary osteons was also identified signalizing reduced bone mechanical properties. Our results suggest an adaptive response of bone to Cd-DZN-induced toxicity in rats in order to prevent osteonecrosis.

Key words: Bone. Osteotoxicology. Cadmium. Diazinon. Rats.

Introduction

Cadmium (Cd) is a toxic metal which still attracts the attention of researchers and the public because its level in food products often exceeds the maximum allowable limits (Toman *et al*., 2011). The diet is the major source (~ 99%) of Cd exposure in the general non-smoking population (Järup and Akesson, 2009). Concentrations of Cd were determined in various organs of experimental animals (Massányi *et al*., 2001; Kolesárová *et al*., 2008). In respect to bone, which is one of the important organs for Cd toxicity (WHO, 1992), exposure to Cd has been linked to bone loss, low bone mass, and osteoporosis and even to an increased incidence of bone fractures (Wilson *et al*., 1996; Wang *et al*., 2003). The results obtained by Brzóska and Moniuszko-Jakoniuk (2005) have shown that chronic, even low-level exposure to Cd disturbs bone metabolism during skeletal development and maturity by affecting bone turnover most probably through a direct influence on bone formation and resorption, and indirectly via disorders in Ca metabolism.

Diazinon (DZN) is a contact organophosphate pesticide which is extensively used in agriculture (Salehi *et al*., 2009). Like other organophosphates (OPs), the main toxic action of DZN is inhibition of acetylcholinesterase activity (AChE) which results in accumulation of acetylcholine

(ACh) and associated neurotoxicity (Oruc and Usta, 2007). According to Garg *et al*. (2004), a potential target of pesticide toxicity is the skeletal system. Marked impairment in the development of the backbone in ducklings due to OPs toxicity has been observed in the study by Ludle *et al*. (1979). Higher amounts of DZN caused additional defects in quail and chicken including folding of the spinal chord, shortening of the neck (Wyttenbach and Hwang, 1984), fusing and twisting of vertebrae, abnormal development of ribs and breastbone (Meneely and Wyttenbach, 1989), curled claws, reduced growth of leg and wing bones (Cho and Lee, 1990), and reduced bone calcification (Cho and Lee, 1991). In addition, OPs cause a significant reduction in bone mass and density in individuals following chronic low-level intoxication (Compston *et al*., 1999). Results by Lari *et al*. (2011) indicate that DZN exposure is associated with decrease in trabecular and cortical bone density and might be one of the causes for worldwide increasing prevalence of osteoporosis.
The aim of current study was to investigate the osteotoxic effect of peroral Cd-DZN co-administration on bone in adult male rats.

Materials and methods
Our experiment was conducted on twenty 1-month-old male Wistar rats obtained from the accredited experimental laboratory (number SK PC 50004) of the Slovak University of Agriculture in Nitra. Clinically healthy rats were randomly divided into two groups, of 10 animals each. In the first group (A), young males were dosed with a daily intake of 30 mg $CdCl_2$/L in combination with 40 mg DZN/L in drinking water for 90 days. The second group without Cd and DZN supplementation served as a control (group B). The xenobiotics used in our experiment were chosen on the basis of their possible occurrence in the human and animal food (Toman *et al*., 2011). Indeed, correlation coefficients found between Cd and DZN in men (0.70) and women (0.69) indicate high probability of exposure to both compounds (Toman *et al*., 2012). The doses of Cd and DZN were high enough to reach a toxicity level but also safe enough to prevent animal mortality. All procedures were approved by the Animal Experimental Committee of the Slovak Republic. At the end of the experiment, all animals were killed and their right femora were used for microscopic 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*. (2007). Transverse thin sections (70-80 µm) were prepared with a sawing microtome (Leitz 1600, Leica, Wetzlar, Germany) and affixed to glass slides by Eukitt (Merck, Darmstadt, Germany) as previously described (Martiniaková *et al*., 2008). The qualitative histological characteristics of the compact bone were determined according to the internationally accepted classification systems of Enlow and Brown (1956) and Ricqlés *et al*. (1991), who classified bone into three main categories: primary vascular tissue, non-vascular tissue and Haversian bone tissue. Various patterns of vascularization can occur in primary vascular bone: longitudinal, radial, reticular, plexiform, laminar, lepidosteoid, acellular, fibriform and protohaversian. There are three subcategories indentified in Haversian bone tissue: irregular, endosteal and dense.

Results
Femoral diaphyses of rats from the control group had the following microstructure in common. The internal layer surrounding the medullary cavity (i.e. endosteal border) was formed by non-vascular bone tissue in all views of the thin sections. The bone tissue contained cellular lamellae and osteocytes. Primary and/or secondary osteons were not present. Additionally, there were also identified some areas of primary vascular radial bone tissue in anterior, posterior and lateral views. This type of bone tissue was created by branching or non-

branching vascular canals radiating from the bone marrow cavity. Some primary and secondary osteons were also found especially 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. However, dense Haversian bone tissue characterized by dense concentration of secondary osteons was not observed. Finally, the periosteal border of analysed bones was again composed of non-vascular bone tissue, mainly in the anterior and posterior views (Fig. 1). The rats simultaneously exposed to Cd and DZN displayed a similar microstructure to rats from the control group, except for the middle part of the compact bone where primary vascular radial bone tissue was observed. Vascular canals got shown to have expanded into the central area of the bones from endosteal surface. The canal expansion was in some cases so enormous that the canals also occurred near periosteal surfaces. As a result of this process, a smaller number of primary and secondary osteons was identified in the Cd-DZN-intoxicated rats (Fig. 2).

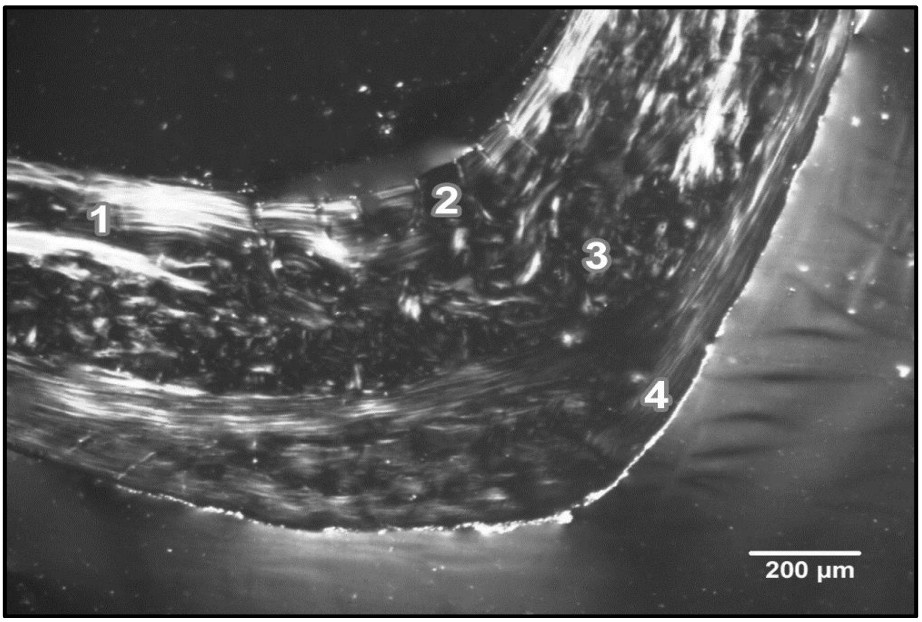

Fig. 1 Microscopic structure of compact bone in rat from the control group:
1 non-vascular bone tissue. 2 vascular canals radiating from marrow cavity. 3 primary and secondary osteons in middle part of compact bone. 4 non-vascular bone tissue

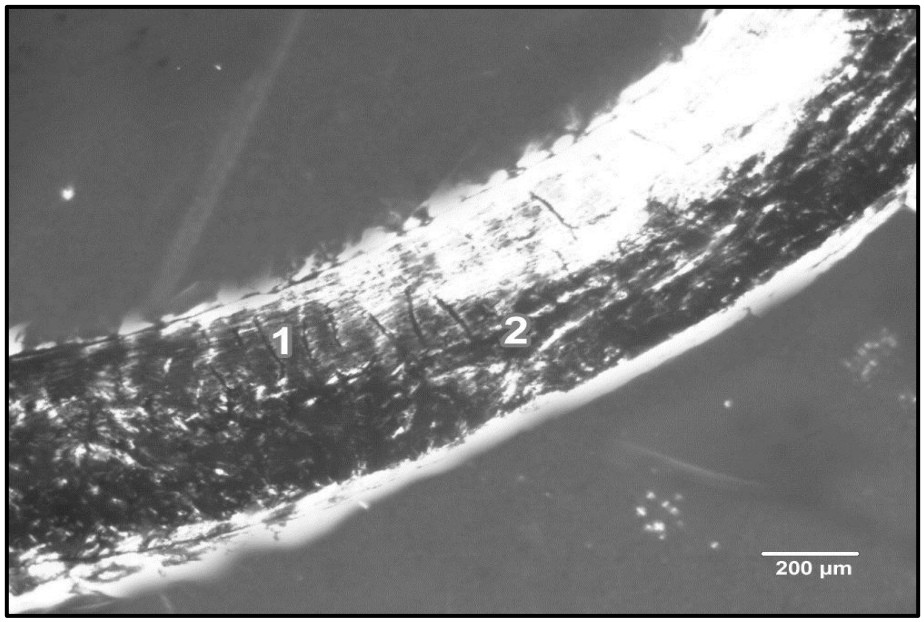

Fig. 2 Microscopic structure of compact bone in Cd-DZN-exposed rats:
1 Enormous vascular canals radiating from marrow cavity. 2 Smaller number of primary and secondary osteons in middle part of compact bone.

Discussion

The results of the qualitative histological analysis of femurs from the control rats correspond with previous works (Enlow a Brown, 1958; Martiniaková *et al*., 2005; Reim *et al*., 2008; Martiniaková *et al*., 2009). The basic structural pattern of compact bone tissue was non-vascular. In addition, there were some areas of primary vascular radial and/or 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 (Erben *et al*., 1996; Reim *et al*., 2008). 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*. (2008) in 13 month-old male rats. Prolonged intake of Cd and DZN mixture resulted in induction of demonstrable changes in the middle part of compact bone where vascular canals expanded from endosteal border and led to the formation of primary vascular radial bone tissue. In some cases, vascular canals were also present near periosteal surfaces. The final result of this process was a smaller number of primary and secondary osteons indicating the reduced bone mechanical properties. In general, bone is dynamic tissue that is continuously remodeled to remove microfractures, to adapt to changing mechanical strains and metabolic demands (Hofstetter, 2007; Chen *et al*., 2009). Disappearance of the Haversian canal system, which was replaced by a large quantity of degenerated, necrotic, and restorative tissues have been demonstrated in the study by Li *et al*. (1997) for ovariectomized rats after a long-term Cd administration. Also, Cd-induced apoptosis of bone cells was documented in many studies (Chen *et al*., 2009; Smith *et al*., 2009; Arbon *et al*., 2012; Brama *et al*., 2012). Furthermore, decreased number of active osteons in broiler chicks was found after exposure to OP pesticides (Garg *et al*., 2004). In general, DZN exerts its toxicity through inhibition of AChE. According to Genever *et al*. (1999) and Inkson *et al*. (2004), this enzyme is also expressed by osteoblasts suggesting a role for AChE (i.e. bone matrix protein) in bone tissue. Thus, the expression of high levels of AChE in bone-forming osteoblasts

and their progenitors supports a toxic effect of AChE inhibitors (including DZN) on these cells (Genever *et al*., 1999; Grisaru *et al*., 1999; Inkson *et al*., 2004; Hoogduijn *et al*., 2006). On the basis of all mentioned findings we propose that the formation of primary vascular bone tissue, mainly in the central area of the femur, could be explained as an adaptive response of bone to Cd-DZN toxicity, in order to protect the tissue against death of cells and subsequent necrosis.

Conclusions

Simultaneous peroral exposure to Cd and DZN had the osteotoxic effect on femurs in adult male rats. Co-administration to Cd and DZN affected mainly the middle part of rats' bones where primary vascular radial bone tissue was identified as a result of adaptive response to xenobiotic-induced osteonecrosis. On the other hand, the vascular canal expansion into central area of *substantia compacta* led to a smaller number of primary and secondary osteons signalizing weakened mechanical properties of the bones.

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