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Original Research

Structural Changes in Femoral Bone Tissue of Rats after Intraperitoneal Administration of Nickel

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Abstract

The present study investigated the acute effects of nickel (Ni) on macroscopic and microscopic structure of femoral bone tissue in rats. For this purpose, ten 5-month-old male Wistar rats were injected intraperitoneally with a single dose of 15 mg $NiCl_2$ per kg of body weight. Ten 5-month-old males without Ni supplementation served as a control group. Forty-eight hours after Ni administration, all animals were killed, and their femora were collected for macroscopic and microscopic evaluation. We found that intraperitoneal application of Ni had no significant effect on femoral weight and femoral length in rats. On the other hand, cortical bone thickness was significantly higher in rats administered Ni ($P<0.05$). Also, a decreased number of primary and secondary osteons was observed in the microstructure of these rats' bones. Morphometrical measurements showed a significant increase in all variables (area, perimeter, maximum, and minimum diameter) of the primary osteons' vascular canals, Haversian canals, and secondary osteons ($P<0.05$) in rats from the experimental group. Our results suggest that intraperitoneal injection of $NiCl_2$ at the level used in this study had no impact on the macroscopic structure of femora of adult male rats; however, it significantly influenced the microscopic structure of their compact bone.

Keywords: bone tissue, femur, histomorphometry, rat, nickel

Introduction

Metal ions are ubiquitously distributed in the environment. Many of these, such as calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), cobalt (Co), nickel (Ni), chromium (Cr), and manganese (Mn), are essential components of biological systems and therefore constitute important micronutrients [1]. Within cells, they mediate oxygen transport and metabolism, catalyze electron transfer reaction, are involved in signal transduction processes, and establish functional structures of macromolecules [2]. Nevertheless, some trace elements may also be toxic for both humans and animals at high concentrations [3].

Nickel (Ni) may be both a micronutrient and a toxicant [4], and is often considered a contaminant [5]. Recent studies with rats and goats indicate that Ni deprivation depresses growth, reproductive performance, and plasma glucose, and alters the distribution of other elements in the body,

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including Ca, Fe, and Zn [6]. The results by Schnegg and Kirchgessner [7] showed that Ni-deficient rats experienced as much as a 35% decrease in body weight. Among the first reported signs of Ni deprivation were changes in Ca metabolism and bone structure and composition [8]. These changes included thickened legs, swollen hock joints, and reduced tibia length/width ratio in other animals [9]. According to Nielsen [10], diets low in Ni may impair bone development and strength in rats. The data of Wilson et al. [11] suggest that adding 25 mg/kg of dietary Ni to a poultry diet should improve broiler bone strength characteristics and performance. Bone, kidney, and liver are the main organs in which Ni accumulates [12]. As a result of dietary supplementation of 50 and 500 mg/kg body weight, Ni accumulated in the kidneys, rib bones, heart, and liver of rabbits [13]. After accumulation, Ni induces severe liver and kidney damage by altering several marker enzymes and the metabolism of ascorbic acid and cholesterol in experimental animals [14]. Sidhu et al. [15] found significant reductions in the body weight and hepatic protein contents in Ni-treated rats. Also, Ni-induced decreases in fertility and alterations of testicular steroidogenesis in male rats administered this metal orally have been well demonstrated [12]. Experimental studies focusing on the effects of Ni on bone have revealed that both a deficiency and a high concentration of this element have an adverse effect on bone development [16]. The results obtained by Wilson et al. [11] have shown that administration of Ni at a dosage above 50 mg $NiCl_2$ per kg feed mixture caused a decrease in radius diameter and cross-sectional area in the broilers. According to Chen et al. [17], Ni may have an oxidative effect on bone marrow. In the study by Martiniaková et al. [18], a decreased size of primary osteons' vascular canals was found in rabbits after peroral Ni application. Similar studies focusing on changes in compact bone microstructure after an intraperitoneal administration of Ni to rats had not been done prior to our experiment.

Therefore, this study was undertaken to analyze macroscopic and microscopic changes in femoral bone tissue of rats receiving a single supra nutritional intraperitoneal dose of nickel.

Materials and Methods

In our experiment, 20 Wistar rats obtained from the accredited experimental laboratory of the Slovak University of Agriculture in Nitra were analyzed. The animals (5-month-old males weighing approximately 400-600 g) were housed individually in plastic cages under controlled conventional conditions (22-24ºC, relative humidity 55%±5%), with unlimited access to drinking water and food (feed mixture M3, Bonargo).

Clinically healthy rats were randomly divided into two groups of 10 animals each. Adult males in the experimental group (EG) were injected intraperitoneally with a single dose of nickel (15 mg $NiCl_2 \cdot kg^{-1}$ body weight). The second group (n=10), without nickel application, served as a control (control group: CG). Forty-eight hours after experimental nickel uptake, all animals were killed and their femora were used for macroscopic and microscopic analyses. All procedures were approved by the Animal Experimental Committee of the Slovak Republic. After removal of all soft tissue, the femora were immediately weighed on analytical scales and their length was measured with a sliding instrument. Values for macroscopic analysis were expressed as mean±standard deviation. The unpaired T-test was used to distinguish possible changes in bone length and bone weight between analyzed groups of rats. The significance level was accepted at $p<0.05$.

For histological analysis, each right femur was sectioned at the midshaft of its diaphysis. The obtained segments (approx. 0.5 cm) 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, Germany). Transverse thin sections (70-80 μm) were prepared with a sawing microtome (Leitz 1600, Germany) and affixed to glass slides by Eukitt (Merck, Germany), as previously described [19, 20]. The qualitative histological characteristics of the compact bone tissue were determined according to the classification systems of Enlow and Brown [21] and Ricqlés et al. [22]. The quantitative 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 maximum and minimum diameters of primary osteons' vascular canals and Haversian canals, and secondary osteons in all views of thin section(s) in order to minimize inter-animal differences. Secondary osteons were distinguished from primary osteons (i.e. primary vascular canals) on the basis of the formers' well-defined peripheral boundary (cement line) between the osteon and the surrounding tissue. The measured values were expressed as mean±standard deviation. The differences in the quantitative histological characteristics of the compact bone between Ni-exposed rats and those of the control group were determined using the unpaired T-test. The criterion significance level was set at $p<0.05$. Diaphyseal cortical bone thickness was also measured by the Motic Images Plus 2.0 ML software. Twenty random areas were selected, and average thickness was calculated for each femur. The unpaired T-test was used to determine statistical significance between experimental and control groups. The significance level was accepted at $p<0.05$.

Results

Femoral length and femoral weight were greater in rats from the experimental group; however, these differences were not statistically significant. On the other hand, cortical bone thickness was significantly increased in Ni-injected rats (Table 1).

The femora of all the animals had the following microstructure in common. An internal layer surrounding the medullary cavity was consisted of a zone of non-vascular bone tissue in all views (anterior, posterior, medial, and lateral) of thin section(s). This type of bone tissue was composed

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

Rat's group	n	Femoral length (cm)	Femoral weight (g)	Cortical bone thickness (mm)
CG	20	4.02±0.11	1.13±0.15	0.540±0.088
EG	20	4.11±0.13	1.22±0.12	0.618±0.070
T-test		NS	NS	+

P<0.05: +; NS: non-significant changes

of concentric lamellae with osteocytes. Primary and/or secondary osteons were absent. In addition, there were also some areas of primary vascular radial bone tissue observed in lateral, anterior, and posterior views. This tissue created vascular canals (branching or unbranching) radiating from marrow cavity. Moreover, some primary and secondary osteons were exceptionally found in anterior and posterior views near endosteal surfaces. In the middle parts of the compact bone, a few primary and secondary osteons were identified. Finally, the periosteal border of investigated bones was again composed of non-vascular bone tissue, mainly in anterior and posterior views (Fig. 1). Although there were no statistically significant differences in qualitative histological characteristics of compact bone tissue between experimental and control groups, we identified a smaller number of primary and secondary osteons near the periosteum and in the middle parts of compact bone in Ni-exposed rats (Fig. 2).

For quantitative histological characteristics, 713 vascular canals of primary osteons, 323 Haversian canals, and 323 secondary osteons were measured. The results are summarized in Tables 2, 3, and 4. We have found that the values of vascular canals of primary osteons, Haversian canals, and secondary osteons were higher for all variables (area, perimeter, maximum and minimum diameters) in rats from the experimental group (P<0.05).

Discussion

Macroscopic analysis of examined femora showed no significant effect of Ni on bone weight or bone length in adult male rats. Similarly, Martiniaková et al. [18] did not find any demonstrable changes in these variables in rabbits after peroral administration of Ni.

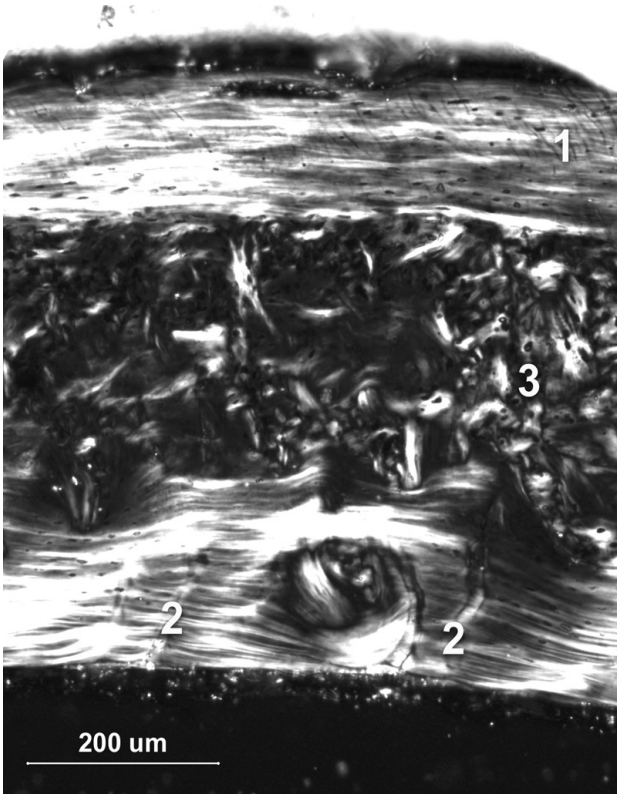

Fig. 1. Microscopic structure of compact bone tissue in rat from the control group.
1 – non-vascular bone tissue
2 – vascular canals radiating from marrow cavity
3 – primary and secondary osteons in middle part of compact bone

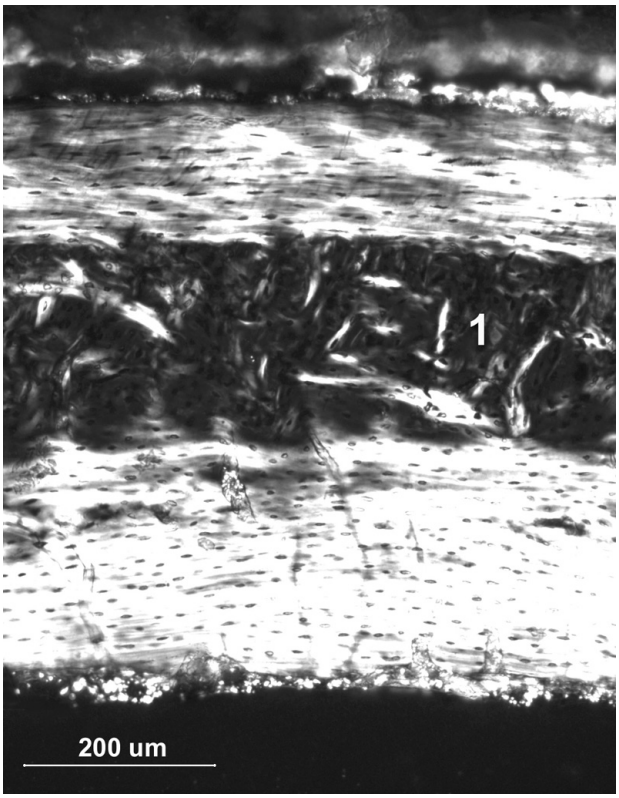

Fig. 2. Microscopic structure of compact bone tissue in rat from the experimental group.
1 – smaller number of primary and secondary osteons in middle part of compact bone.

Table 2. Data on primary osteons' vascular canals in rats from control (CG) and experimental (EG) groups.

Group of rats	n	Area (μm²)	Perimeter (μm)	Max. diameter (μm)	Min. diameter (μm)
CG	10	386.32±78.60	70.57±7.43	12.31±1.57	9.99±1.41
EG	10	448.56±72.18	76.37±6.61	13.37±1.70	10.73±1.21
T-test		+	+	+	+

P<0.05: +

Table 3. Data on Haversian canals in rats from control (CG) and experimental (EG) groups.

Group of rats	n	Area (μm²)	Perimeter (μm)	Max. diameter (μm)	Min. diameter (μm)
CG	10	457.10±69.12	76.46±5.58	12.97±1.24	11.27±1.34
EG	10	626.88±117.46	89.28±8.37	15.18±1.67	13.10±1.57
T-test		+	+	+	+

P<0.05: +

Table 4. Data on secondary osteons in rats from control (CG) and experimental (EG) groups.

Group of rats	n	Area (μm²)	Perimeter (μm)	Max. diameter (μm)	Min. diameter (μm)
CG	10	6,392.40±1,656.98	287.48±36.68	50.97±7.30	39.53±6.43
EG	10	8,359.29±1,715.11	330.77±33.97	59.53±7.09	44.45±5.61
T-test		+	+	+	+

P<0.05: +

The thickness of cortical bone is an important parameter in the evaluation of cortical bone quality and strength. We observed significantly increased cortical bone thickness in Ni-injected rats. The value of cortical bone thickness in rats from the control group was higher in comparison with the value reported by Comelekoglu et al. [23]. However; this discrepancy may be influenced by the different age of the rats in the two studies. We could not compare the value of cortical bone thickness in rats from the experimental group with published data, since it was not reported in previous studies.

Our findings from the qualitative histological analysis correspond with those reported by other researchers in rats [24-27]. The basic structural pattern of compact bone tissue was non-vascular. In addition, primary vascular radial and/or irregular Haversian bone tissues were found in both groups. No significant differences in qualitative histological characteristics of compact bone tissue between rats from experimental and control groups were observed in our study. We found only a decreased number of primary and secondary osteons near the periosteum and in middle parts of compact bone in Ni-exposed rats. The same finding has also been documented in rabbits perorally administered with $NiCl_2$ [18]. This fact could indicate that bone remodeling (i.e. formation of new primary and secondary osteons) is reduced in the rats from our experimental group. The results obtained by Mabilleau et al. [28] have demonstrated that short-term exposure to Ni could reduce the release of pro-inflammatory markers, known to activate both osteoclastic resorption as well as osteoclast precursors. Also, Ni is known to induce severe osteoblast apoptosis and dysfunction. In vitro, osteoblasts exposed to Ni^{2+} (10 μM) showed significantly suppressed alkaline phosphatase activity, which is known to be a marker of osteoblasts in a proliferative stage [29]. In the study by Gough and Downes [30], osteoblasts cultured with $NiCl_2$ (100 μM-1 mM) showed typical apoptotic morphology. Similarly, osteoblast-like ROS-17 cells of rats cultured 48 h on commercially pure Ni discs exhibited a few typical adhesion plaques or focal contacts that are disrupted in cells undergoing apoptosis [31]. In general, rats lack true Haversian intracortical bone remodeling under physiological conditions [26, 32]. According to Reim et al. [26] the newly formed remodeling units within compact bone originate from the endocortical surface, from where they extend deeply into the underlying compact bone. Considering these facts, the decreased number of primary and secondary osteons in Ni-injected rats could be explained by the effect of Ni on the activity of osteoblasts and osteoclasts in the endocortical surface of compact bone.

Morphometrical measurements showed a significant increase in all variables (area, perimeter, maximum, and minimum diameter) of the primary osteons' vascular canals, Haversian canals and secondary osteons (P<0.05) in rats from the experimental group. Similarly, higher values of Haversian canals and secondary osteons were reported in rabbits after peroral Ni application in the study by Martiniaková et al. [18]. Generally, it is known that animal

bone is able to adsorb Cu and Ni ions from their single aqueous solutions [33]. Raisz [34] has suggested that the vast surface area of bone mineral can adsorb toxins and heavy metals and minimize their adverse effects on other tissues. More recently, these findings were confirmed by the results of Mabilleau et al. [35]. These authors have shown that some metal ions, such as Cr^{2+}, Ni^{2+}, and Co^{2+}, can be incorporated into the hydroxyapatite crystals during mineralization, and they could affect the crystal lattice parameters as well as the size of bone minerals. Hydroxyapatite crystals, as a major mineral component of bones, are aligned with their long axis parallel to the collagen fiber axis [36], creating concentric lamellae of secondary osteons. On the basis of this knowledge and the results of Mabilleau et al. [35], we can speculate that an increase of hydroxyapatite crystals could partially contribute to the changes in the size of secondary osteons. The above-mentioned authors also found decreased levels of Ca and P in the bone mineral. Calcium was progressively substituted by metal ions during the mineralization process. We assume that disturbance of bone mineralization with the following Ca reduction could lead to increased size of primary osteons' vascular canals and Haversian canals in Ni-injected rats. The vascular (also Haversian) canal extension in these rats could also be associated with better bone vascularization. In contrast, changes in the size of bone minerals (and subsequently secondary osteons) could be related to an increased occurrence of bone fractures. It has been suggested that bones with a preponderance of larger crystals have reduced resistance to load [37].

The measured values of primary osteons' vascular canals of rats from the control group were higher than those reported by Martiniaková et al. [27]. This fact; however, might in part reflect different ages of the rats in the two studies. According to classifications of Rämsch and Zerndt [38], and Gladuhsew [39], secondary osteons of rats from the control group had short Haversian canals (12.12±1.29 μm).

In conclusion, our results clearly demonstrate that a single intraperitoneal administration of 15 mg $NiCl_2 \cdot kg^{-1}$ body weight induces changes in cortical bone thickness and compact bone microstructure of femora in adult male rats.

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