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Mechanisms of morphogenetic disorders in the lower jaw under the influence of heavy metal salts on the body

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Abstract: In the experiment on the 36 mature rats, the peculiarities of morphological disorders in the lower jaw of rats under the influence of salts of chromium, lead, zinc, iron, copper, and manganese are showed. Profound morphological changes in bone and cartilaginous tissues of lower jaw are followed by disorders of bone mineral content. The importance of the S100 protein in morphological changes in bone and cartilaginous tissues under the influence of heavy metal salts is shown.

Keywords: morphology, lower jaw, bone tissue, heavy metal salts, S100 protein

Introduction

One of the urgent issues in modern medicine is the increase of population sickness rate due to the environmental degradation which is connected with anthropogenic environment pollution caused by harmful wastes of transport, various types of industrial and agricultural activity of man, and heavy metal salts [1–5].

Hard tissues of the oral cavity are firstly affected by harmful influence of hazardous exogenous and endogenous factors while alimentary intake. An excess intake of salts of zinc, copper, manganese, lead, and chromium can cause the features of osteoporosis and bone tissues destruction in alveolar process of lower jaw [6, 7].

It was found that excess intake of heavy metals into the body is followed by their accumulation in hard tooth structures. The result of these processes is the changes in morphological structure of enamel and dentin [8, 9]. It is known that cations and anions of heavy metal salts can affect the hard tissues of the tooth in different ways causing the enamel strength inhibition [10]. Increased level of lead, magnesium, and manganese in enamel reduces the caries resistance. The imbalance of certain elements, Al, F, P, Zn, Fe, Si, Mg, and Cu, is followed by the development of pathological abrasion of hard tooth tissue [11, 12].

At the same time, the destructive changes in odontoblasts are developed, collagen fibers are destroyed in bone tissue, the hemorrhage appears, and enzymes activity is changed [13].

Thus, under the effect of heavy metal salts in hard tissues of oral cavity, the profound morphological changes are developed which cause the increase of morbidity of these organs. At the same time, the morphogenetic mechanisms of these disorders in bone tissue of the lower jaw are not studied enough. Due to this, the objective of this research is to find out the mechanisms of morphological disorders in the lower jaw under the influence of heavy metal salts on the body.

Materials and Methods

The research was carried out on 36 white mature rats – males, divided into two groups: control and experimental. During 1 month, the testing animals of the second group had been taking water with the excessive amount of HMs: zinc – 5 mg/L, copper – 1 mg/L, iron – 10 mg/L, manganese – 0.1 mg/L, lead – 0.1 mg/L, and chromium – 0.1 mg/L. The material was examined in 1, 15, 30, and 60 days after discontinuance of taking the HMs. The cuts were

stained with hematoxylin–eosin and Van Gieson's picro-fuchsin.

The material for the immunohistochemical study was fixed in 10% neutral formalin for 24 h, and then paraffin blocks were made of it. Then, sections with the thickness of 3–4 mm were made, and they were subjected to the standard process of dehydration in xylol and alcohols of rising concentration. Immunohistochemical reaction consisted of 2 stages. During the first stage, incubation with primary mouse antibodies in dilution of 1:150 took place (4C4.9 clone was used for determination of S100 protein). During the second stage, incubation with secondary antibodies (UltraVision ONE HRP Polymer) took place. We visualized the cell structural components using diaminobenzidine, which painted them in a brown color.

Quantitative chemical analysis of lower jaw and incisors was carried out with the help of atomic absorption spectrophotometer C-115M1. By drying in drying chamber at 105 °C to constant weight, the moisture content was determined. The dried tissue was burning in porcelain crucibles in a muffle furnace at 450 °C for 48 h. The total amount of organic and mineral substances in the solid residue was determined. The received ash was dissolved in 10% hydrochloric and nitric acids. According to the standard procedure, the amount of zinc (wavelength – 213.9 nm), copper (wavelength – 324.7 nm), lead (wavelength – 283.3 nm), manganese (wavelength – 279.5 nm), chromium (wavelength – 357.9 nm), and iron (wavelength – 248.3 nm) was determined.

Digital data were processed by computer program AtteStat 12.0.5. The experiments on testing animals were carried out in accordance with the regulations adopted by the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), "General ethic rules about experiments on animals" approved by the I National Congress of Bioethics (Kyiv, 2001) and the Law of Ukraine "On protection of animals from cruelty" No. 3477-IV from 21.02.2006.

Results

Under the influence of combination of heavy metal salts on the body, the growth processes inhibition in incisor and lower jaw by 5.03–8.55% ($p < 0.05$) was found. In bone tissue of lower jaw, the features of significant inhibition of bone formation *processes* in compact and cancellous substances were determined. Deceleration of *appositional growth* and disorders of bone matrix ossification, and emersion of significant hypomineralized areas in ground substance were observed *(Fig. 1)*. Compactness of bone tissue of the lower jaw was significantly disordered because of profound dystrophic and osteoporotic changes both in intercellular substance and in osteogenetic cells with nucleus pycnosis and even complete destruction in some areas. In readaptation period during all terms of the research, the destructive morphological changes in bone tissue of the lower jaw did not totally vanish. Even after 60 days of observation, the hypomineralized areas of compact with its swelling remained in bone tissue *(Fig. 2)*.

During the histologic research of condylar cartilage, the features of growth processes inhibition were also found as well as intensification of resorption of cancellous bone tissue and unevenness of mineralization of the ground substance *(Fig. 3)*. Morphometric results showed that the most significant changes were observed

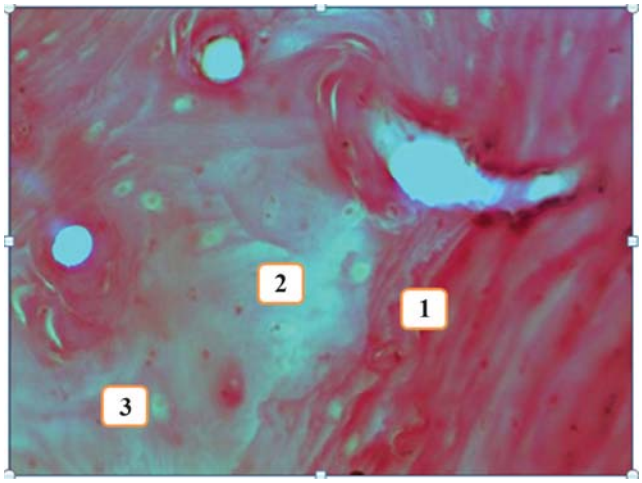

Fig. 1. Bone tissue of the lower jaw of testing animals. After a month of influence of heavy metals: 1 – uneven lines bonding, 2 – the hypomineralized areas of compact, 3 – swelling of the cell. Stained with Van Gieson's picro-fuchsin. Zoom: ×360

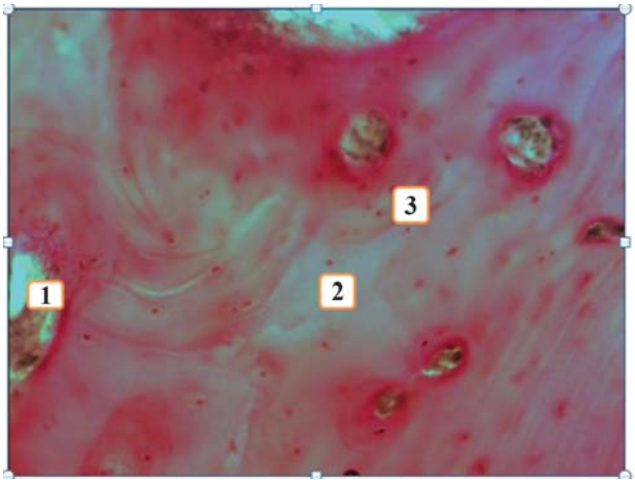

Fig. 2. Bone tissue of the lower jaw of testing animals. After 30 days of readaptation period: 1 – resorption of bone tissue, 2 – the hypomineralized areas of compact, 3 – pycnosis of the cell. Stained with Van Gieson's picro-fuchsin. Zoom: ×320

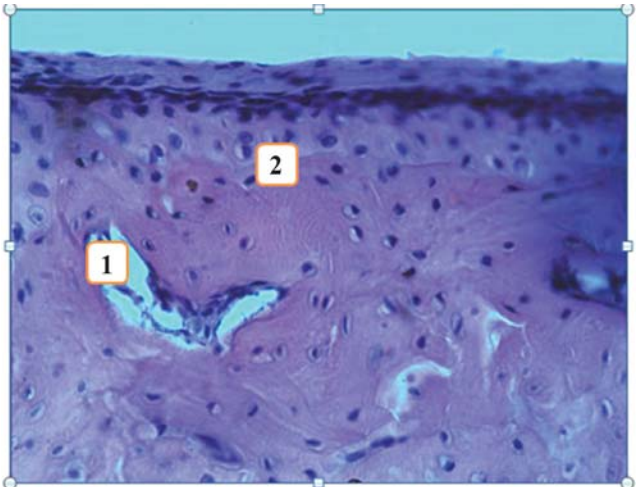

Fig. 3. Condylar cartilage of the lower jaw of testing animals. After a month of influence of heavy metals: 1 – resorption of bone tissue, 2 – unevenness of mineralization of the ground substance. Stained with hematoxylin–eosin. Zoom: ×320

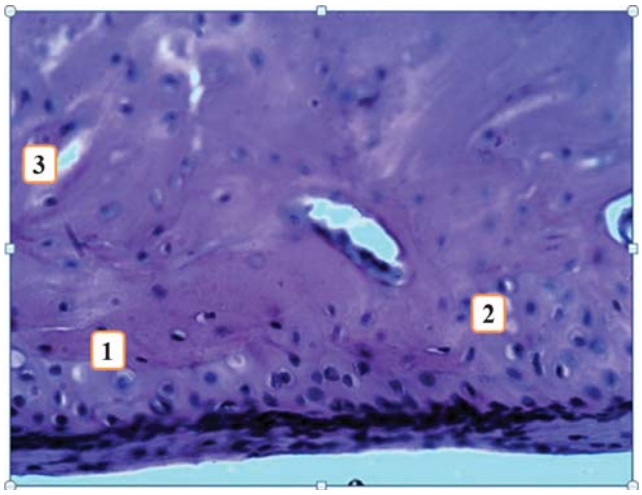

Fig. 4. Condylar cartilage of the lower jaw of testing animals. After 15 days of readaptation period: 1 – cytoplasm vacuolation of the cells, 2 – unevenness of mineralization of the ground substance, 3 – resorptive and osteoporotic changes remained in cartilage. Stained with hematoxylin–eosin. Zoom: ×360

in subchondral osteogeny area which narrowed to 9.78% ($p < 0.05$) with a simultaneous decrease of cells and volume of primary spongiosis. After 60 days of readaptation period, the features of resorptive and osteoporotic changes remained in cartilage, as well as dystrophy of cartilaginous cells in the form of cytoplasm vacuolation and nucleus pycnosis *(Fig. 4)*.

The research of S100 protein expression in osteogenetic cells in lower jaw of testing animals showed a significant reduction *(Fig. 5)*, which was not restored to control values in readaptation period *(Fig. 6)*.

Analysis of metabolic processes in bone tissue and incisors of lower jaw of testing animals showed the mineral disorders in bone and incisor due to the reduction of calcium in hydroxyapatite crystal lattice by 12–14% and of basic osteotropic microelement zinc by 8–9%. At the same time, the total amount of organic and inorganic substances also decreased, but the water contents increased by 15–17%. In intercellular space, the dissociation of connective tissue with its swelling was developed as a result of the imbalance of mineral component in the observed organs with a significant accumulation of heavy metal ions up to 14–23%. Readaptative period after completion of taking the heavy metal salts was characterized by a slight decrease of metabolic disorders in the mineral component of the lower jaw.

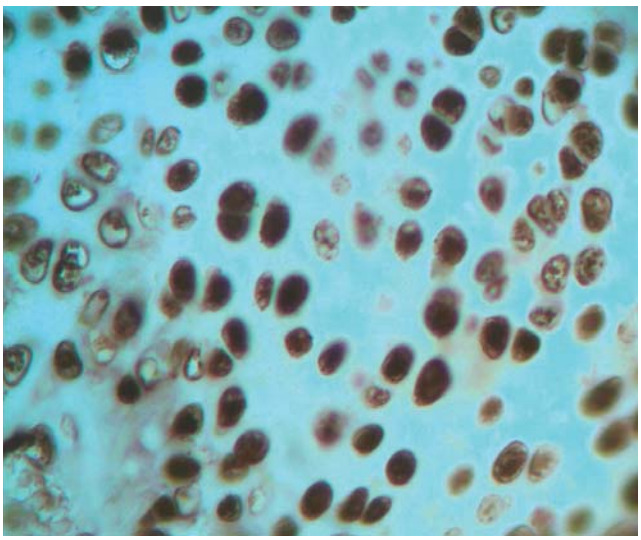

Fig. 5. S100 protein expression in osteogenetic cells in lower jaw of testing animals. After a month of influence of heavy metals. Stained with diaminobenzidine. Zoom: ×480

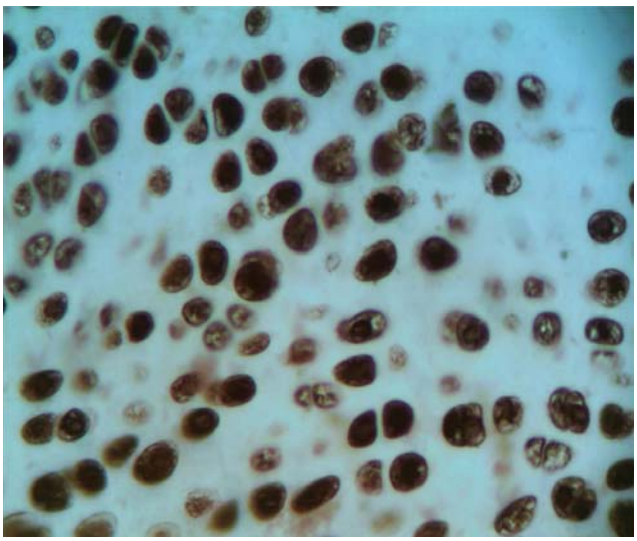

Fig. 6. S100 protein expression in osteogenetic cells in lower jaw of testing animals. After 30 days of readaptation period. Stained with diaminobenzidine. Zoom: ×480

Discussions

In our opinion, the inhibition of protein synthetic function in osteogenetic cells plays a significant role in the mechanism of profound regressive morphological changes in bone and cartilaginous tissue of the lower jaw under the influence of heavy metal salts. One of the proofs of this hypothesis is the low expression of S100 proteins in these cells. According to the opinions of many authors [13], these proteins are actively involved in bone tissue formation by mineralization of cartilage by their ability to bind with calcium ions [10]. Due to the formation of both homo- and heterodimers, the S100 proteins can form the proteins complexes with Ca^{2+} and Zn^{2+}. Trapping these ions allows changing the spatial organization of S100 protein and provides the ability to be bound to different target proteins and to intensify their biological effect [6]. The results of chemical analysis of bone tissue showed the reduction of calcium ions and basic osteotropic microelement zinc in hydroxyapatite crystal lattice, which are displaced from hydroxyapatite crystals by cations of heavy metals due to their accumulation. This causes the depletion of bone inorganic substances. By reducing of the calcium expression of S100 binding protein in osteogenetic cells, the mineralization disorders in bone tissue occur with the regressive morphological changes.

Conclusions

The heavy metal salts cause the deep morphological and metabolic disorders in bone tissue of lower jaw and incisor dentin, which is expressed by growth processes inhibition, dystrophic and osteoporotic changes in compact and cancellous substance of bone, reduction of organic and inorganic substances, calcium and zinc, and accumulation of ions of heavy metals in the hydroxyapatite crystal lattice.

Among the mechanisms of the profound morphological changes in the lower jaw under the influence of heavy metal salts which is followed by the disorders of bone formation processes, it is necessary to point out the protein synthetic function inhibition of osteogenetic cells, namely, the reduction of the S100 protein expression in these cells.

Prospects for further developments in this area are to study the ways of correction of the revealed changes.

* * *

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