

Research Article

Level of Osteotropic Elements in the Jaw Bones of Experimental Animals in Terms of the Impact of Xenobiotics

Mariana Kaminska, Mykola Rozhko, Hanna Ersteniuk

Abstract

Pollution of the environment with heavy metals, pesticides, nitrates leads to an unceasing deterioration of the population's health, in particular, the dental one. Excessive intake of salts of heavy metals and nitrites is accompanied by changes in calcium-phosphorus metabolism rates, indicating a disorder of metabolic processes in bone tissue. This necessitates the study of the level of osteotropic elements of the jaw bone tissue.

Materials and methods of research. In the experiment on 80 white outbred male rats, the effects of cadmium chloride, sodium nitrite and their combined action on the content of osteotropic elements in the jaw bone were studied. The control group consisted of 10 intact animals.

Results. It was determined that in the dynamics (1st, 14th, 28th days) of both cadmium-nitrite, nitrite and cadmium intoxication there was observed the accumulation of cadmium and decrease of calcium, copper, zinc levels in bone tissue of animal jaws compared to intact group.

Conclusions. The most significant changes in the level of osteotropic elements in the jaw bones are found in the combined effect of xenobiotics, which is important for understanding of the metabolic processes in the oral cavity.

Keywords

intact animals; cadmium intoxication; nitrite intoxication; nitrite-cadmium intoxication; macro- and microelements; bone tissue

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Problem statement and analysis of the latest research

Environmental pollution by xenobiotics leads to the deterioration of population's health. It is believed that 75% of diseases is caused by the environmental pollution [7]. Most often, according to the literature data, technogenic pollution is caused by salts of heavy metals, in particular cadmium, lead, zinc, nickel [1, 9]. In the organs and tissues of people and animals living in the areas contaminated by heavy metal salts, these substances accumulate and have a long half-life [3]. Admission of heavy metals to the body is performed in combination with

water, air, food products, and almost 70% of heavy metals comes into the human body from food [8]. Many regions are characterized by cadmium pollution, which is associated with the processing and storage of household and industrial wastes. Cadmium causes profound violation of hard tissues of the tooth in contact with it and calcium metabolism in the body as a whole. [4, 6] Toxic cadmium properties are due to the ability to interact with the vital elements such as: calcium, zinc, iron, copper, that in particular leads to disorder of calcium-phosphorus metabolism [2].

The most common environmental pollutants,

along with heavy metals and pesticides are nitrates. For decades nitrates and nitrites take priority among the pollutants of the environment. Nitrites – are an intermediate product in the chain of nitrates restoration, they are widely used, particularly as corrosion inhibitors, as well as for preserving of meat and fish products. Most of the nitrate loading on an organism has water containing an elevated levels of nitrates and which is used for food [10]. The vast majority of areas in Ukraine is environmentally unfavorable regions due to contamination of soil and groundwater with nitrates and nitrites [5]. According to SES (Sanitary Epidemiologic Service) chemical laboratories in some rural areas the level of nitrates in well-water exceeds 480 mg/l at maximum allowable concentration in Ukraine – 45 mg/l [11]. Nitrite intoxication is accompanied by changes in parameters of calcium-phosphorus metabolism in blood plasma of infected animals, in particular calcium, magnesium, phosphate and activity of alkaline and acidphosphatase, that indicates a profound disorders of metabolic processes in bone tissue under the conditions of xenobiotic's action [10]. However, in the available literature there are no data considering the influence of nitrites and cadmium compounds at the macro- and microelement composition of the jaw bone tissue.

Objective of the study was to investigate the level of osteotropic elements in the jaw bones of experimental animals under conditions of separate and combined actions of cadmium chloride (CdCl_2) and sodium nitrite (NaNO_2).

1. Materials and Methods

The objects of the study were 90 experimental animals (white outbred malerats weighing 180-200 g). The animals were kept on a standard diet of vivarium. Sodium nitrite and cadmium chloride intoxication of animals was carried out during 10 days. Calculation of the amount to introduce the toxicant-substances was performed on the basis of data on toxicity of cadmium chloride parameters per pure metal, and sodium nitrite (I.M. Trakhtenberg, 1991; H.I. Sydorenko et al., 1999; B.M. Shtabsky et al., 1990). Material (jaw bone tissue) sampling

was performed after decapitation under the mild ether anesthesia during the 1st, 14th and 28th day after the introduction of toxins. Decapitation was performed according to the rules of the European Convention for the humane treatment of the laboratory animals (Strasbourg, 1986). The animals were divided into the following experimental groups:

- Group 1 – control (intact) – who received physiological solution (0.9% sodium chloride solution);
- Group 2 – animals, whose intoxication was carried out by sodium nitrite at a dose of 1/10 LD_{50} (2.1 mg/kg of body weight per os) during 10 days;
- Group 3 – animals, whose intoxication was performed by cadmium chloride at a dose of 1/10 LD_{50} (1.2 mg/kg of animal body weight intramuscularly) for 10 days.
- Group 4 – animals whose intoxication was performed by cadmium chloride and sodium nitrite at a dose of 1/10 LD_{50} during 10 days.

Determination of macro- and microelements in the jaw bone tissue was performed by atomic-absorption method on the spectrophotometer C-115 C PC.

Statistical analysis of the obtained data was performed using the program "Statistica".

2. Results and Discussion

Study of osteotropic elements' levels in the jaw bones of experimental animals allowed to determine the different changes in depth and direction (Table 1).

In particular, calcium content has most significantly changed in the group of animals who were exposed to the combined influence of cadmium chloride and sodium nitrite (Figure 1) compared to intact ones: a decrease at 6.86% was observed in animals during the 1st day after the introduction of toxicants, during the 14th day – at 10.78%, and the most significant changes were observed during the 28th day – at 15.7%. In animals receiving sodium nitrite, we have noted quite a different tendency:

Table 1. The level of macro- and microelements in the jaw bones of animals affected by CdCl₂ and NaNO₂.

Investigated index	Groups of animals									
	Intact animals	CdCl ₂ +NaNO ₂			NaNO ₂			CdCl ₂		
		1 st day	14 th day	28 th day	1 st day	14 th day	28 th day	1 st day	14 th day	28 th day
Calcium mg/g of ash	22.17 ±0.51	20.65 ±0.18*	19.78 ±0.19*	18.69 ±0.18*	19.24 ±0.23*	19.85 ±0.07*	19.95 ±0.15*	20.77 ±0.22*	19.42 ±0.23*	19.93 ±0.28*
Copper mcg/g of ash	17.45 ±0.38	15.2 ±0.25*	13.91 ±0.39*	16.94 ±0.25	11.1 ±0.29*	9.38 ±0.46*	5.31 ±0.27*	14.79 ±0.37*	14.06 ±0.31*	16.07 ±0.27*
Zinc mcg/g of ash	462.7 ±3.66	335.36 ±6.65*	258.13 ±7.66*	406.33 ±6.54*	382.4 ±12.13*	365.44 ±7.82*	401.29 ±7.32*	348.8 ±6.76*	259 ±5.44*	290.25 ±3.53*
Cadmium mcg/g of ash	2.102 ±0.07	8.02 ±0.16*	9.19 ±0.13*	18.44 ±0.49*	2.069 ±0.08	2.23 ±0.13	3.78 ±0.12*	4.16 ±0.10*	5.6 ±0.21*	6.51 ±0.20*
Magnesium mg/g of ash	38.99 ±0.73	51.54 ±0.55*	30.91 ±0.46*	42.4 ±1.11*	46.1 ±1.23*	42.33 ±0.81*	41.12 ±0.77	50.60 ±1.06*	39.52 ±0.66	31.96 ±0.37*

Note: * - a statistically significant difference (p<0.05) relative to the corresponding group intact.

reduction of calcium level in the early period of observation at 13.22% followed by a slight growth during the 28th day, but at 10.01% lower than in the control group. In terms of cadmium intoxication the lowest calcium content was observed in animals' jaw bones during the 14th day after administration of cadmium – at 12.4%. The received data indicate that the dynamics of changes in calcium level depended on the chemical nature of the toxicant, which is important for understanding the mechanisms of metabolic disorders in the oral cavity organs.

Regarding the research of another macroelement content – magnesium (Figure 2), it should be noted its significant (p<0.05) accumulation in the early period of the combined impact of xenobiotics (at 32.19%), followed by a sharp decrease compared to the intact group at 20.72% and increase at almost 8.74% by the end of the experiment. The introduction of sodium nitrite to animals has led to the increase of magnesium in the jaw bones during the whole observation period, the most significant during the 1st day – at 18.24%. In terms of cadmium intoxication we have observed the growth of

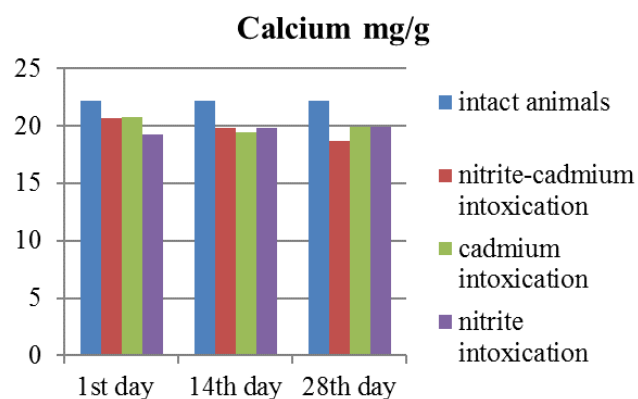


Figure 1. Dynamics of changes in the calcium level in the jaw bones under the conditions of separate and combined action of CdCl₂ and NaNO₂.

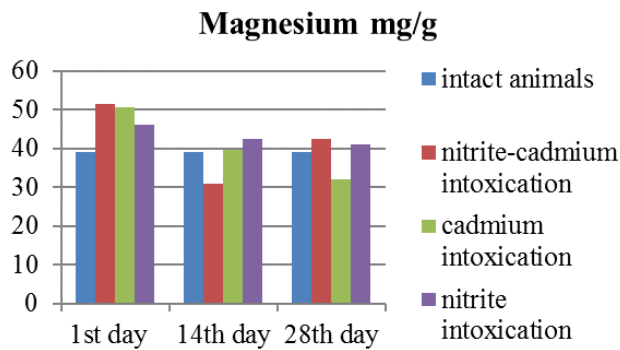


Figure 2. Dynamics of changes in the magnesium level in jaw bones under the conditions of separate and combined action of CdCl₂ and NaNO₂.

this macroelement at almost 29.78% during the 1st day after the introduction with a clear tendency to decrease and during the 28th day magnesium level was at 18.03% lower than data in the control group. Such changes in the content of magnesium in the jaw bone tissue can influence primarily the intensity of mineralization and the activity of energy metabolism enzymes.

Atomic-adsorption analysis of jaw bones allowed to determine serious violations of such microelements' content as Zn and Cu. In all experimental groups of animals it was found the significant ($p < 0.05$) reduction of zinc level (Figure 3), and it is worth noting that the lowest level was observed at the 14th day of intoxication: at 44.02% in terms of the impact of the cadmium and at 44.21% the combined effect of cadmium and nitrite, at 21.02% – in nitrite intoxication. In the later period of the experiment the most significant changes were marked in animals under conditions of cadmium ions influence. Reduction of zinc level can lead to the violation of mineralization processes due to insufficient collagen synthesis and the formation of organic matrix of bone, and on the other hand this microelement deficiency causes low activity of alkaline phosphatase, which in turn affects the level of phosphate ions.

Study of Cu level in the jaw bones (Figure 4) showed differently directed character of changes depending on xenobiotic that entered the animals' organisms. In terms of the combined effect, the lowest value of this indicator was marked during the 14th

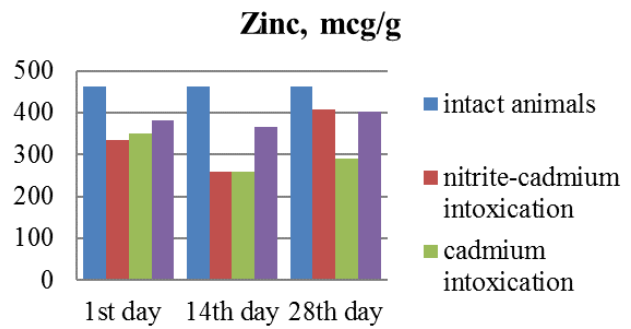


Figure 3. Dynamics of zinc level changes in jaw bones under the conditions of separate and combined action of CdCl₂ and NaNO₂.

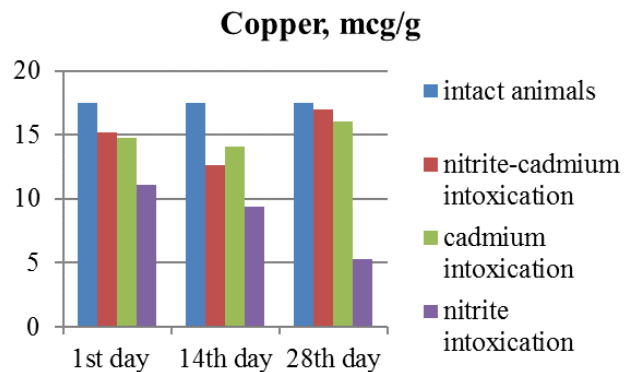


Figure 4. Dynamics of changes in the copper level in jaw bones under the conditions of separate and combined action of CdCl₂ and NaNO₂.

day (at 20.29%) compared to the control group, in nitrite intoxication the tendency of copper content decrease was clearly traced and during the 28th day its 3.3 times decrease was marked. Quite a different direction of changes was observed in conditions of cadmium ions influence – during the total period of observation the accumulation of Cu was marked, at 7.94 - 19.43%, this index was higher than in intact animals. Violation of copper exchange in the organisms of experimental animals can lead primarily to the formation of collagen matrix, because copper ions are activators of prolyl hydroxylase and lysyl hydroxylase which provide hydroxylation of proline and lysine in tropocollagen structure.

Research of cadmium content in the jaw bones showed accumulation of this heavy metal both in

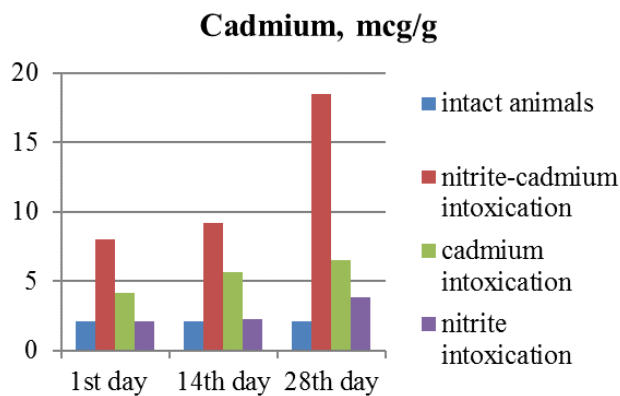


Figure 5. Dynamics of changes in cadmium level in jaw bones under the conditions of separate and combined action of CdCl_2 and NaNO_2

combined and in separate action of the studied xenobiotics. It should be noted that in groups one and two of animals, the level of this heavy metal increased by the end of the experiment and exceeded the indexes of the control group at 3 and 8.7 times in conditions of cadmium and combined intoxication, respectively. In nitrite intoxication there were not observed significant differences from intact animals during the 1st-14th day of the experiment, the accumulation of cadmium was observed only during the 28th day.

The obtained data indicate that the combined effect of cadmium chloride and sodium nitrite on the body of experimental animals is accompanied by a significant accumulation of toxic metal in the jaw bone tissue, compared with a separate action of investigated xenobiotics. The accumulation of cadmium may cause violation of mineralization processes as cadmium can replace calcium ions both in amorphous calcium phosphate and in hydroxyapatite structure. In addition to this, it is known about cadmium antagonism regarding essential micronutrients – such as zinc and cuprum, which act as alkaline phosphatase and hydroxylase activators, which have an important influence on the formation of inorganic and organic bone tissue matrix.

Thus, the results of the experimental studies indicate significant changes in the content of osteotropic macro- and micronutrients in the jaw bones under the influence of the studied xenobiotics.

3. Conclusions

1. In nitrite, cadmium and combined intoxication the accumulation of cadmium in the jaw bone tissue, violation of content of macro- (Ca and Mg) and microelements (Cu, Zn), which are important for the formation of collagen matrix and mineralization processes, are observed. Such violations are the most pronounced in conditions of nitrite-cadmium intoxication.
2. The obtained results are the basis in the search of preparations for the correction of metabolic disorders in the oral cavities of the population living in areas with a high content of cadmium compounds and nitrites in the soil and water, as well as undergoes nitrite loading.

4. Prospects of Further Researches

Further research are directed at deepening of understanding of the damage to the bone tissue and periodontal tissues and also the search of preparations that promote the prevention and correction of disorders caused by cadmium and nitrite intoxication.

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