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HARMONIZATION OF HEAVY METAL METABOLISM IN JAW ALVEOLAR BONE AND TEETH ENAMEL OF RATS UNDER THE EFFECT OF A-LIPOIC ACID

In the research with the help of atomic absorption spectrometry the concentration of heavy metals (Fe, Zn, Pb, Cu, Mn, Cr) in jaw alveolar bone and teeth enamel of rats and peculiarities of their accumulation under effect of α-lipoic acid were determined. It was proved that intake of α-lipoic acid simultaneously with heavy metal salts leads to harmonization of metal accumulation balance in tissues. α-lipoic acid increased the content of Cu, Mn and Cr in bone tissue, Cu and Mn in enamel, the concentration of which decrease under effect of heavy metals and decreased the content of Zn, Pb and Fe had accumulating in these tissues in oversize volume.

Keywords: salt of heavy metals, alveolar bone, teeth enamel, α-lipoic acid.

Foreword. In different spheres of life a man contacts with a great number of chemical elements which, influencing an organism for a long time in light dose and weak concentration, can accumulate in tissues and give noci-influence. One of the ecologically destructive factors influencing human's health is heavy metals (HM). As V.A. Isidorov (1999) wrote, phrase "heavy metals" is understood as synonym to "toxic metals" by most of people, but some metals are vital (essential) for life organisms [3]. That is why, in our opinion, there is an objective necessity to concentrate in some detail on characteristics of chemical elements from this group.

More the 40 chemical elements of Mendeleev periodic system with atomic mass over 50 atomic units are referred to heavy metals. In certain concentration (as microelements) they take an active part in biological process, being a part of many enzymes [9]. V.V. Gnidenko and his co-authors (2011) said about heavy metals: "Not all heavy metals are toxic, as cuprum, zinc, cobalt, mangan that are called microelements and have important biological meaning in the life of haematothermal, plants and microorganisms. That is why microelements and heavy metals are terms referring to one and the same elements, depending on their content in environment. It is true to use term "heavy metal" talking about hazard concentration of these elements, and to use term "microelements" when they are in weak concentration" [2].

Negative influence of single metal on an organism is more or less examined. Influence of heavy metals combination on a human body is the least examined issue. But it is known that different combination of chemical elements, their complicated interreacting, can have different influence in the result of summation effect, synergism and antagonism between them. During our examination it was established that after getting into the rats' bodies the excess of heavy metals (Fe, Zn, Pb, Cu, Mn, Cr) combination not the ordinary accumulation of metals in alveolar bone and teeth enamel is observed, but their selective accumulation. In other words, the concentration of one HV in mineralized tissues increased, and vice versa there was a deficit of other salts in comparison with reference group [6, 7, 12].

To correct abnormality caused by HM, a range of medication was offered which provide antioxidative effect. Among a great variety of this medication that can level down or reduce effect of HM intoxication, alpha lipoic acid (α-lipoic acid, ALA) has a special place. Our examination shows that ALA can give osteo-protection effect, reducing resorption of alveolar bone and sealing it, balances prooxidant-antioxidant system. [5, 13]. It has positive effect in treatment of heavy metals intoxicating. ALA is able to form other chelate bonds with metal ions and, thus, contributes to detoxication [14, 15].

Data about effect of α-lipoic acid on HM metabolism in bone tissue and teeth enamel was not found. That is why the goal of the research was to study peculiarities of HM (Cu, Cr, Mn, Zn, Pb, Fe) accumulation in these tissues under excessive intake of salt combination under the effect of α-lipoic acid.

Materials and Methods. The study was carried out on 30 mature outbred white male rats with initial weight ranging 180-200 g during 30 days. All animals were divided into 3 groups: group I (n = 10) united reference rats that were taking potable water. Animals in group II (n = 10) were taking potable water with SHM combined: zink ($ZnSO_4 \times 7H_2O$) – 5 mg/L, copper ($CuSO_4 \times 5H_2O$) – 1 mg/L, iron ($FeSO_4$) – 10 mg/L, manganese ($MnSO_4 \times 5H_2O$) – 0,1 mg/L, lead ($Pb(NO_3)_2$) – 0,1 mg/L, chrome ($K_2Cr_2O_7$) – 0,1 mg/L. Rats in group III beside the abovementioned SHM, were intragastrically administered with medication containing α-lipoic acid "Alfa-Lipon" (produced by OJSC Kyiv Plant of Vitamins, Ukraine) in amount of 100 mg/kg once a day. Rats had free access to water. In 30 days under ether anaesthesia animals were decapitated, jaws were skeletonised, alveolar bones were cleaned from muscle tissue, molar teeth enamel was sheared. The further examination of HM content in tissues of samples was performed on spectrophotometer C115-01 with flame and electro-thermal atomizer.

During the experiment, the laboratory animals were kept in compliance with rules adopted by the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986) and "General Ethical Rules for Experiments Using Animals", approved by the First Bioethics National Congress (Kyiv, 2001).

Study Findings and Discussion. Our previous researches showed that under excessive intake of Fe, Zn, Pb, Cu, Mn, Cr salt combination in 2nd group of rats such metals as Cu, Cr, Mn accumulated in alveolar bone and teeth enamel in less concentration in comparison with reference group [6, 7, 12].

Table 1, 2 shows that under simultaneous taking ALA and HM salts in 3rd group of rats there was increasing of Cu content in alveolar bone for 717% (p = 0,008) and in enamel for 62.8% (p=0.009) in comparison with 2nd group of animals. Thus, the concentration of cuprum in the bone was for 65,1% (p = 0,07) higher than in reference group, and in enamel it increased but was still lower then reference values and it was for 31,3% (p = 0,4) lower than reference.

Under intake of HM, Cr as well as Cu accumulated in bone and enamel in less volume in comparison with values of 1st group. Under ALA effect the level of its accumulation in bone increased for 35,6% (p = 0,2), but there was the decreasing of its content for 21.1% (p = 0,4) in enamel in comparison with 2nd group. But Cr concentration in bone did not reach the value of reference group, it was lower for 26,4% (p = 0,3), and for 45,5% (p = 0,01) lower in enamel.

Accumulation of Mn in tissues under excess intake of HM salts decreased. α-lipoic acid caused increasing of its level in bone tissue for 24.2% (p = 0,4) and in enamel for 32,7% (p = 0,2). But content of this element under effect of ALA also was lower for 36,5% (p = 0,2) in bone and for 1,4% (p = 0,8) in enamel than reference values.

We established that Zn, Pb, Fe under their excess intake in the form of Fe, Zn, Pb, Cu, Mn, Cr salt combination accumulated in alveolar bone and teeth enamel in larger quantities in comparison with reference values [6, 7, 12].

ALA intake contributed to decrease of Zn in alveolar bone for 21,2% (p = 0,8) and enamel for 57% (p = 0,04). Nevertheless, its concentration exceeded the value of reference group. It was higher for 37,2% (p = 0,07) in bone and for 243% (p = 0,01) in enamel.

ALA intake contributed to decrease of Pb in alveolar bone for 90,5% (p = 0,0008) and enamel for 64,4% (p = 0,009) in comparison with 2nd group of animals. But its concentration exceeded the value of reference group for 192% (p = 0,09) in bone and for 85,7% (p = 0,1) in enamel.

ALA caused complex effect on accumulation of Fe. Its concentration decreased for 66,6% (p = 0,03) in bone and for 45.0% (p = 0,04) in enamel. This complex effect was in the fact that the decrease was so expressed, that there was the further decrease of element concentration in comparison with reference group for 26,2% (p = 0,5) in bone and for 9,4% (p = 0,6) in enamel.

It is known, that main component of nonorganic part of enamel is hydroxyapatite crystals able to accumulate metals. Apatites can have a great amount of vacancies and thereby can have some ion replacements, which determine their reactivity and biological properties. As contrasted with other biominerals they have unique adaptiveness to different biological functions. Special mechanisms of charge compensation make possible molecular and ion insertions and replacements that determine properties of apatite crystal surface with relation to chemical (solvability, capacity to ion replacement, ion insertions, adsorbation and molecular insertions) and physical (surface charge of interphase energy) properties [10]. There are more than 40 elements in hydroxyapatite crystals of enamel [16]. Analysis of hydroxyapatite structure, when central Ca atom is displaced by one of the metals (Mg, Cu, Zn, Fe, Cr, Mn) ions, shows that heteroion replacement in apatite crystals influences its composition and leads to crystal deformation. The replacement

Table 1

Content of heavy metals in enamel, mcg/g (M ± m)

Metals	Experimental groups		
	1 (n=10)	2 (n=10)	3 (n=10)
Cu	35,99±9,25	15,18±2,08*	24,72±2,27**
Zn	69,66±10,05	555,00±98,80*	238,95±47,84** ***
Cr	575,39±70,38	397,56±59,75	313,66±44,40***
Mn	58,74±4,53	43,65±5,15*	57,91±18,79
Pb	0,14±0,05	0,73±0,09*	0,26±0,06**
Fe	616,48±91,59	1013,4±141,60	558,64±21,46**

Note. * – statistically significant difference between 2nd and 1st group, ** – 3rd and 2nd, *** – 3rd and 1st

Table 2

Content of heavy metals in alveolar bone of rat jaw, mcg/g (M ± m)

Metals	Experimental groups		
	1 (n=10)	2 (n=10)	3 (n=10)
Cu	10,05±1,80	2,03±0,52*	16,59±1,90** ***
Zn	194,19±22,55	336,02±56,37*	266,44±29,57
Cr	251,17±38,44	136,36±14,35*	184,85±33,13
Mn	18,10±3,53	9,25±1,40*	11,49±1,90
Pb	0,13±0,04	4,02±0,68*	0,38±0,12**
Fe	136,37±28,16	300,98±43,27*	100,63±48,65**

Note. * – statistically significant difference between 2nd and 1st group, ** – 3rd and 2nd, *** – 3rd and 1st is accompanied with bond distance change between oxygen and atoms of Ca in molecule of hydroxyapatite and migration of atomic groups encircling central atom of Ca [11].

It was determined, that the most quantity of biometals accumulated in bone tissue. Jaw alveolar bone is one of the main parts of tissue complex forming paradontium. Its condition determines the level of inflammatory diseases in paradontium tissues and their destruction. As some microelements take part in catalase of enzyme reactions in osteogenic cells, their lack or imbalance interrupt osteoblast and osteoclastic differentiation, processes of apatite crystallization, cell cooperation, that leads to disorders of physiological and reparative bone turnover [1].

Bone tissue and teeth enamel are similar with chemical similarity of their nonorganic component – apatite crystals. So, normalization of biometal balance in crystals is essential for prophylaxis of chemical and physical properties damage. α-lipoic acid to some extent corrects imbalance in metal metabolism in mineralized tissues.

Conclusion. Intake of α-lipoic acid simultaneously with Fe, Zn, Pb, Cu, Mn, Cr salt combination harmonizes the balance of metal accumulation in jaw alveolar bone and teeth enamel of rats. α-lipoic acid increases the content of Cu, Mn and Cr in bone tissue, Cu and Mn in enamel, the concentration of which decrease under effect of HM; and decreases the content of Zn, Pb and Fe had accumulating in these tissues in oversize volume. α-lipoic acid does not provide complete normalization of the said elements concentration.

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