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INFLUENCE OF ALPHA-LIPOID ACID ON THE CONDITION OF PROOXIDANT-ANTIOXIDANT HOMEOSTASIS IN BLOOD SERUM AND GUMS OF RATS UNDER THE EFFECT OF HEAVY METALS

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The study focused on the impact of alpha lipoic acid on content of diene conjugates, malondialdehyde and activity of catalase, superoxide dismutase, glutathione peroxidase in rats' homogenates gums and blood serum under the heavy metals salts effect. It was established that salts of heavy metals cause activation of lipid peroxidation in gums and inhibit the antioxidative protection system. Administration of alpha lipoic acid contributes to reduced content of lipid peroxidation products and enhances activity of antioxidative enzymes.

Keywords: gum, blood serum, salts of heavy metals, diene conjugates, malondialdehyde, catalase, superoxide dismutase, glutathione peroxidase, alpha lipoic acid

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Foreword. Due to the intensive development of industry, agriculture, transport, chemical elements became widespread pollutant of the environment. Their excessive accumulation in human body can be the reason of technogenetic hypermicroelementosis development [5]. Detail study that was performed during the last two decades showed that heavy metal salt (HMS), their disturbance can lead to the induction of oxidative stress with active forms of oxygen production and suppression of antioxidant protection system (AOP) [7]. It can play an important role in the pathogenesis of inflammatory diseases of periodontium [1].

Series of medications having antioxidant properties are suggested for the correction of disturbed prooxidant-antioxidant balance. Analysing literature data concerning antioxidants, we paid attention to α-lipoid acid (ALA). It is an important component of biological membranes and protects them from peroxide oxidation of lipids (POL). It appears as bidirectional antioxidant, that can react either in water-soluble or in fat-soluble areas of cells and tissues. Moreover, α-lipoid acid helps to enlarge positive effect of other important body antioxidants, such as glutathione, coenzyme Q_{10}, contributes the regeneration of vitamins C and E [8]. But there is no data concerning influence of ALA on induced by heavy metals oxidative stress in gums and blood serum.

Goal of our research was to study the influence of α-lipoid acid on prooxidant-antioxidant homeostasis in gums and blood serum of rats under the effect of Fe, Zn, Pb, Cu, Mn, Cr heavy metals.

Materials and Methods. The study was carried out on 70 outbred white male rats (initial weight 180-200g). All animals were divided into 3 groups. Group I united reference rats that were taking usual potable water. Animals in group II were taking potable water with HMS combined: zinc ($ZnSO_4$ x $7H_2O$) – 5 mg/L, copper ($CuSO_4$ x $5H_2O$) – 1 mg/L, iron ($FeSO_4$) – 10 mg/L, manganese ($MnSO_4$ x $5H_2O$) – 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 except above mentioned HMS were taking intragastrically medication with α-lipoid acid "Alpha-lipon" ("Kyivskyi vitaminnyi zavod" Ltd., Ukraine) at the rate 100mg/kg once per day. Free excess to water. Every group of animals were taken off the experiment on 30, 60 and 90 day.

Under ether anaesthesia rats were decapitated. Samples of gum tissue and blood were made. The content of diene conjugate (DC), malondialdehyde (MDA), strength of enzyme superoxide dismutase (SOD), catalase (CT) and glutathione peroxidise (GPO) was determined in homogenates of gums in 26 rats from I group, 21 from II group, 23 from III group, and in blood serum in 21 rats from I group, 14 from II group, 17 from III group. All study were hold in laboratories of biochemistry of State University "Institute of dentistry at National Academy of Medical Science of Ukraine" (Odessa)

Statistical processing of material was made according to parametrical criterion (mean value – M, error of mean value – m) and statistical significance of differences between data of two independent groups according to nonparametric criteria (W-test of Wilcoxon) with the help of AtteStat 10.8.4. for MS Excel. Statistical significant were differences while $p < 0.05$.

During the experiment, the laboratory animals were kept in compliance with the 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. Let's consider data of POL process in gums tissue for 3 months of study. Findings show that average content of POL products in gums is much different between groups (Table 1). So, concentration of DC increased in II group on 50.4% (p=4E-06), and MDA on 42.3% (p=5E-06) in comparison with reference group.

The similar situation is observed in blood serum. Findings shown in Table 2 testifies that the content of DC in blood serum of I group of rats has probably increase in 1.7 times. The level of DC increases if the period of research is increased. Its content increases on 70.9% (p=0.02) on the 30th day of the research, there is gradual increase of its content on 72.2% (p=0.01) and 83.3% (p=0.03) in comparison with reference values on the 60th and the 90th day

Table 1

Content of POL products in rats' gums while HMS intake (M±m)

POL products	Group		
	I (n=26)	II (n=21)	III (n=23)
Diene conjugate (DC), mmole/kg	3.77±0.20	5.67±0.30*	4.43±0.19** ***
Malondialdehyde (MDA), mmole/kg	11.31±0.51	16.09±0.57*	11.95±0.55**

Note. Difference between groups with a probability p<0.05: *II-I, **III-II, ***III-I

Table 2

Content of POL products in rats' blood serum while HMS intake (M±m)

POL products	Day	Group of rats		
		I n=21	II n=14	III n=17
Diene conjugate (DC), mmole/l	30	0.86±0.08	1.47±0.17*	1.17±0.12
	60	0.90±0.12	1.55±0.12*	0.97±0.13**
	90	0.90±0.14	1.65±0.08*	1.02±0.06**
Malondialdehyde (MDA), mmole/l	30	0.878±0.044	1.213±0.045*	1.116±0.009***
	60	0.911±0.068	1.248±0.028*	1.068±0.038**
	90	0.897±0.060	1.313±0.047*	1.014±0.044**

Note. Difference between groups with a probability p<0.05: *II-I, **III-II, ***III-I

correspondingly. Dynamics of MDA content has the same tendency, on the back of HMS influence its concentration increases in 1.4-1.5 times. After 30 and 60 days the statistically significant increase of its level in comparison with the referent group on 38.2% (p=0.008) and 37.0% (p=0.005) correspondingly was registered and maximum increase was registered on the 90th day – 46.4% (p=0.01).

As we can see, Fe, Zn, Pb, Cu, Mn, Cr salt combination causes induction of lipid peroxidation processes in tissues of gums and blood serum. The main mechanism of initiation of POL processes by this metals lies in formation of superoxide radicals, hydroxyl radicals (namely according to Fenton reaction) and regeneration of active forms of oxygen [9], and is accompanied by modification or destruction of bilayer membrane that is agreed with literature data [6].

It is known that the intensity of POL processes in the body is determined not only by factors initiating it but also the condition of antioxidant system [2]. Under physiologic conditions, POL process is under constant control of enzyme and nonenzym systems of cells. In reaction to toxic factors the activation of protective-compensatory mechanisms takes place, that leads to changes in activity of such strong enzyme-antioxidant as SOD, CT, GPO. They have definite specialization concerning specific radicals and peroxides [6].

Determination of gum AOP status under the influence of HMS indicates on changes in activity of antioxidant enzymes (Table 3). So, sharp decrease below the referent values of catalase activity on 25.2% (p=1E-07), SOD activity on 36.9% (p=0.0006) and GPO activity on 23.4% (p=0.0002) is observed.

Influence of law intensity HMS induces reduction of the activity of AOP enzyme system and in blood serum (table 4). Activity of catalase in rats blood serum under the influence of HMS statistically significant reduces on the 30th day on 49.9% (p=0.051), on the 60th day on 44.0% (p=0.003), on the 90th day on 49% (p=0.025) in comparison with the referent group. Activity of SOD reduces on the 30th day on 43.2% (p=0.1), but evidential reduction takes place from the 60th day on 40.7% (p=0.01), the 90th day on 39.0% (p=0.02). Among all enzymes of AOP glutathione peroxidase has the least variation of activity. Its activity reduces only in 1.2 times in average. While HMS intake the level of GPO activity reduces on 7.6% (p=0.3) on the 30th day, on 17.4% (p=0.007) on the 60th days, on 14.8% (p=0.045) on the 90th day in comparison with the reference values.

Increase of POL intensity during decrease of AOP functioning in gums tissues and blood serum of experimental animals testifies that intake of Fe, Zn, Pb, Cu, Mn, Cr salts combinations leads to the development of oxidative stress that can produce structural-metabolic changes in biomembranes, and later become the inducer of progressive deterioration of periodontium tissues [1, 11]. Due to that fact it is cannot be ruled out that determined disorders of prooxidant-antioxidant homeostasis in gums tissues and in blood serum under the effect of environmental HMS can be one of the main causes of the increase of periodontium tissues diseases on specified territories, as indicated by our previous epidemiological studies [3].

Table 3

Activity of AOP enzymes in rats' gums while HMS intake (M±m)

Enzyme	Group of animals		
	I (n=26)	II (n=21)	III (n=23)
Catalase, mkat/kg	7.280±0.178	5.442±0.150*	6.585±0.146** ***
Superoxide dismutase, IU/g	0.412±0.028	0.260±0.028*	0.388±0.023**
Glutathione peroxidase, ikat/kg	10.93±0.45	8.37±0.24*	10.07±0.44**

Note. Difference between groups with a probability p<0.05: *II-I, **III-II, ***III-I

Table 4

AOP enzymes activity in rats' blood serum, M±m

Enzymes	Day	I group n=21	II group n=14	III group n=17
Catalase, mkat/l	30	0.315±0.031	0.180±0.003*	0.271±0.018**
	60	0.309±0.031	0.173±0.008*	0.288±0.09**
	90	0.314±0.035	0.160±0.012*	0.283±0.030**
Superoxide dismutase, IU/g	30	0.361±0.05	0.205±0.02	0.288±0.02
	60	0.327±0.03	0194±0.02*	0.303±0.016**
	90	0.328±0.02	0.200±0.03*	0.310±0.02**
Glutathione peroxidase, μkat/l	30	1.44±0.04	1.33±0.01	1.48±0.01
	60	1.49±0.05	1.23±0.02*	1.32±0.03**
	90	1.42±0.07	1.21±0.05*	1.40±0.04**

Note. Difference between groups with a probability p<0.05: *II-I, **III-II, ***III-I

Intake of alpha-lipoid acid changes prooxidant-antioxidant imbalance in III group of rats. Under its effect POL products content in gums reduces respective III group (Table 1) – DC on 21.9% (p=0.001), MDA on 27.7% (p=5E-06). Fall of the level of POL metabolites and in blood serum takes place (Table 2). So, there is the decrease of DC content on 20.4% (p=0.2) on the 30th day, on 37.4% (p=0.02) on the 60th day, on 38.2% (p=0.01) on the 90th day. Statistically significant reduction of DC concentration in blood serum is registered after 60 days of α-lipoid acid intake. The level of MDA in blood serum also reduces on 8.0% (p=0.2), 14.4% (p=0.01), 22.8% (p=0.01) in 30, 60 and 90 days correspondingly. Evidentiary decrease of MDA content in serum was observed on the 60th day of ALA intake.

In III group of rats the intake of alpha-lipoid acid favours the increase of the activity of all AOP system enzymes in gums homogenes (Table 3) – CT on 21.0% (p=2E-05), SOD on 49.2% (p=0.004) and GPO on 20.3% (p=0.003) in comparison with the activity values of these enzymes in rats intaking potable water with HMS. The same happens in blood serum (Table 4). So, there is the increase of CT activity in 1.5-1.8 times. Moreover with extension of ALA intake term its activity increases: in 30 days on 50.6% (p=0.03), in 60 days – on 66.5% (p=0.02), in 90 days – on 76.9% (p=0.1). SOD activity also increases in 1.4-1.6 times. Its activity level is higher on 40.5% (p=0.4), 56.2% (p=0.008) and 55.0% (p=0.03) correspondingly on the 30th, 60th, 90th day of the study in comparison with II group of animals. The least increase of activity in 1.1-1.2 time is observed in GPO. It is higher on 11.3% (p=0.2) on the 30th day, on 7.3% (p=0.01) on the 60th day, 15.7% (p=0.007) on the 90th day after ALA intake.

Antiradical effect of ALA can be conditioned by the list of its properties. It intensifies efficiency of oxidative phosphorylation [4]; fixes Cu^{2+} in lipoproteins, inhibiting Cu-inducted peroxidation of low density lipoproteins [15]; intensifies cell capacity to produce active forms of oxygen [16]; produces stable complexes with metal ion and favours their detoxication [14, 15]; plays an important role in mitochondrial dehydrogenase reactions, being the co-factor of such enzymes as piruvate dehydrogenase and α-ketoglutarate dehydrogenase [13]; it is effective in treatment of different pathological conditions accompanied by oxidative damage [12]. Our least publications proved one more its property. That is the capacity to harmonize in teeth enamel and bone tissue of alveolar bone the content of heavy metals under its excess intake [10].

Thus, according to the results of our studies we can make the following **summary**.

1. Fe, Zn, Pb, Cu, Mn, Cr salt combination causes oxidative stress in blood serum and gums of rats due to activation of processes of lipids peroxidation and suppression of AOP system.

2. Intake of alpha-lipoid acid normalizes the balance of prooxidant-antioxidant system under the influence of heavy metal salts.

3. Statistically significant reduction of the content of products of lipid peroxidation and increase of the activity of antioxidant system protection enzymes in blood serum take place in 2 months after ALA intake.