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Zinc Properties in Weakening the Pathogenic Effects of Excess Cobalt Intake

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***Abstract* – The high level of anthropogenic environmental pollution in urban areas leads to the accumulation of the body and the development of diseases. In connection with the foregoing, the study focuses on the features of changing the parameters of systemic hemodynamics in conditions of combined and isolated administration of cobalt and zinc salts. Cobalt chloride (4 mg/kg) and zinc (20 mg/kg) were administered intragastrically to Wistar rats via an atraumatic probe daily for one month. Blood pressure was determined by catheterization of the femoral artery. The catheter was filled with a 10 % heparin solution and connected to the DDA electrometer of the monitor MX-04 (Russia). Minute blood volume was measured by thermodilution, for which an MT-54M thermistor was inserted through the left common carotid artery into the aortic arch. A saline solution of fixed room temperature with a volume of 0.2 ml was injected into the right atrium through a catheterized right yarmine vein. The mean arterial pressure (MAP), cardiac index (CI), stroke index (SI) and specific peripheral vascular resistance (SVR) were calculated. The concentration of ionized calcium was determined using an AEK-01 electrolyte analyzer (Russia). The concentration of total calcium was determined spectrophotometrically using the sets "Calcium Arsenazo", "LLC" "Agat-Med" (Moscow, Russia). The cobalt content was determined after preliminary sample preparation on a Kvant-AFA atomic absorption spectrophotometer (Russia). Studies have shown monthly intragastric administration of cobalt or zinc chloride can observe hypokinetic arterial hypertension. According to the analyses zinc weakens the pathogenic effect of cobalt on the parameters of systemic hemodynamics within their combined introduction. The study also revealed that cobalt with increased intake accumulates in bone tissue, leading to its decalcification, while the content of ionized calcium increases in the blood.**

Key words – zinc, pathogenic effect, cobalt salts, environmental pollution

I. INTRODUCTION

Anthropogenic environmental pollution has led to the irreversible deformation throughout the world; There is a change of basic processes of the adequate formation and metabolism of ecological systems, including living organisms as a key link. The recent intense environmental pollution in North Ossetia-Alania, especially in Vladikavkaz, is greatly influenced by the industrial factories and vehicles. There have been many factories extracting and processing non-ferrous metal ores. According to the data provided by I.D. Alborov, the president of International Academy of Ecology and Life Protection Sciences, there are serious environmental pollution issues in the region processing non-ferrous metal ores than in the regions with no factories.

According to the studies of the ecological state of North Ossetia-Alania the territory of Vladikavkaz is experiencing an extremely high technogenic load, largely associated with pollution by heavy metals (zinc, cadmium. Lead, cobalt). Moreover, the incidence of the North Ossetia-Alania population depends on environmental factor. Vladikavkaz leads the top list regions with heavy oncological diseases, heart failures and kidney diseases [1].

Cobalt and zinc as essential elements are considered metals accumulating in the environment and effecting negatively when releasing into a living organism. According to the study cobalt precipitates in the lysosome matrix as a result of a complex formation with anionic groups and fall into a competitive reaction with Ca2 + and Mg2 + ions for correlating with the proton pump active centers [2]. The experiment proves positive effect of the nanoscale cobalt particles on the body: it accumulates essential macrocells into the body, including calcium. [3]. However, excessive cobalt

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intake in the body when exceeding the MPC leads to excessive accumulation of xenobiotics in organs and tissues (liver, heart, kidneys, bones) and damage the structural and functional system of the body [4].

The mechanism of cobalt toxic effect on the body is not yet fully proved, however, some negative effects are associated with its ability of replacing divalent positive ions in metal-active enzymes. The pathogenic effect depends on the high affinity of cobalt for sulfhydryl groups of protein molecules leading to inhibition of the main mitochondrial respiration enzymes and to the launch of the "hypoxia activator factor" existing in all cells and leading to the development of numerous adverse effects [5, 6].

Zinc – is one of the common industrial agents; it belongs to the substances of the second hazard class. As a cofactor of a large group of enzymes, zinc is involved in all types of metabolism; it is necessary for the normal course of about two hundred biochemical processes [7]. Zinc takes an active part in the process of stabilization of the cell membrane and is a powerful component of the antioxidant system. In addition, the metal is a component of enzymatic activators of secretion and reabsorption of substances in the kidneys [8].

Zinc enters into a competitive reaction with other metals. Particularly, zinc becomes the main antagonizer reducing the toxic effect on the animal tissues. Zinc changes the nature of the distribution of lead between organs and tissues, reducing its content in the skeleton and increasing it in the kidneys and liver [9]. The decrease in the toxic effect of lead by zinc is explained by the ability of the latter to initiate the synthesis of metallothionein protein, which binds excess lead, providing its detoxification.

One of the known mechanisms of the toxic effect of heavy metals on the body is the ability to cumulate: they can accumulate in organs and tissues, integrate into various biochemical processes, and replace some cell components. The scientific sources present the data information about cobalt and zinc effects on organism systems and on the body as a whole. However, there are no studies connected to the combined effect analysis of cobalt and zinc chloride on systemic hemodynamics, as well as an experiment with rats.

II. Problem statement

The questions clarified in the study are about the effect of excess cobalt chloride introduced into the body on systemic hemodynamics, accumulation in bone tissue and the effect on calcium metabolism in Wistar rats, the effect of zinc chloride when combining with cobalt.

III. The research purpose

the research purpose is to study the pathogenic effect of long-term co-administration of cobalt and zinc salts into the body on the parameters of systemic hemodynamics

IV. Materials and methods

The work was performed on 48 sexually mature male Wistar rats with an average weight of 280 g ± 20 g. The experiments were carried out in 4 experimental groups of animals: the first group – control; 2nd group – animals with isolated intragastric (iv) administration of zinc chloride dosing 20 mg/kg (daily dose); 3rd group – animals with isolated administration of cobalt chloride dosing 4 mg/kg (daily dose); 4th group – animals with the combined introduction of metals (zinc + cobalt).

The total concentration of cobalt chloride administered over the entire period (30 days) of the experiment was 120 mg/kg per animal, and zinc chloride was 600 mg/kg. The animals were on a standard diet, had free access to water and food, under natural light conditions. The study was conducted in autumn. In carrying out the experiments, they were guided by the 11th article of the Helsinki Declaration of the World Medical Association, "International Recommendations for Biomedical Research Using Laboratory Animals" (1985) (revision 2008) and the rules of laboratory activity in the Russian Federation (order of the Ministry of Health of the Russian Federation from 01.04. 2016 No. 199). All studies were performed under zoletil anesthesia (5 mg per 100 g of general weight).

At the end of the experiment (30 days), blood pressure was determined by direct means by catheterization of the femoral artery. The catheter was filled with a 10 % heparin solution and connected to the DDA electrometer of the MX-04 monitor (Russia). The minute of blood volume through the left common carotid artery was measured by MT-54M thermistor introduced into the aortic arch. A saline solution of fixed room temperature with a volume of 0.2 ml was injected into the right atrium through a catheterized right yarmine vein. Thermal dilution curves were recorded on an EPP-5 recorder. The analysis has calculated the mean arterial pressure (MAP), cardiac index (CI), stroke index (SI) and specific peripheral vascular resistance (PVR). Heart rate (HR) was determined by the MX-04 monitor [10].

The concentration of ionized calcium was determined using an AEK-01 electrolyte analyzer (Russia). The content of ionized calcium in blood was determined with the help of a pre-prepared heparinized syringe was and a transdermal blood sample drained from the heart proximately. After blood recovery, the material is immediately placed into the analyzer in ten seconds to record the level of calcium. The level of ionized calcium in the plasma was determined with the help of hermetically sealed tube in a centrifuge for 8 minutes at 3000 rpm to precipitate the formed elements. It contains the whole blood samples (with low molecular weight heparin). In the resulting plasma, the amount of calcium was immediately determined. The concentration of total calcium was determined by spectrophotometer (on a PV1251C spectrophotometer, Belarus) using the "Calcium Arsenazo-Agat", "LLC" "Agat-Med" kits (Moscow, Russia).

To determine the calcium content in bone tissue, tissue samples were mineralized according to GOST 26929-94 (introduced on 01.01.1996), the preparation of the test solution was carried out according to GOST 30178–96 (introduced on 01.01.1998). After preliminary dilution in the resulting solution, calcium was determined using a PV 1251C spectrophotometer, cobalt – on a quantum-AFA atomic absorption spectrophotometer (Russia).

Statistic result processing within samples and the normal distribution of the comparison series, established by the Shapiro-Wilk test (Wf >> Wm), was carried out using the "t" student criterion by the STATISTICA 10 program. The

Pearson correlation coefficient was calculated. The presence of significant differences and factor influences was judged at a critical level of confidence (p) less than 0.05.

V. DISCUSSION OF FINDINGS

The main parameters of systemic hemodynamics (Table 1) contributed to determine the result of isolated intragastric zinc chloride administration increasing the average blood pressure. The change of blood pressure was due to an increase in specific peripheral resistance compared to the control group of animals. During intoxication with zinc chloride, a change in the parameters characterizing the pumping activity of the heart was noted – the cardiac index decreased as a result of a decrease in the stroke index compared to the control group. At the same time, there was an increase in heart rate.

According to the scientific researches an increased concentration of zinc causes the death of numerous body cells [11], including cell death in heart [12]. This effect of zinc most likely leads to the problems of the heart's pumping function.

The systemic hemodynamics changes were similar to the effects of zinc chloride administration during intragastric administration of cobalt salts. There was a more significant increase in mean arterial pressure compared with the control group. The increase in pressure was facilitated by an increase in vascular resistance. At the same time intoxication with cobalt salts effects myocardial damage and diseases of the heart pumping activity (shock and cardiac indices). Thus, excessive intake of heavy metals cause hypokinetic arterial hypertension, decreasing cardiac output and increasing vascular resistance.

Significant increase of mean arterial pressure in the group of two metal intoxication was compared to the control group: the pressure was lower when compared with the isolated administration of both zinc and cobalt. There is an increase of the specific peripheral vascular resistance observed in the same group as in the control group. As isolated administration of metals shows a tendency to restore the parameter was noticeable. The baseline of prolonged intoxication of two metals shows the cardiac index decrease due to the strike index reactions. When compared to the control group, the changes were smaller than with separate administration of metals. Scientific researches suggest that zinc deficiency in vascular endothelial cells enhances the inflammatory response [11] through mechanisms associated with increased cellular oxidative stress, but zinc supplements in the diet protect the vascular system from oxidative damage [13].

TABLE I. PARAMETERS OF SYSTEMIC HEMODYNAMICS UNDER THE CONDITIONS OF COMBINED INTOXICATION OF COBALT AND ZINC CHLORIDE.

Experiment conditions	**Statistical indicators.**	**MAP (mm Hg.)**	**HR (beat per min.)**	**CI (mg/100g)**	**SI (mg/100g)**	**PVR (relative value unit)**
Baseline values (group 1)	M±m, Me, Pr.[25–75]	103.9±1.07 105 [100-106]	365±8.17 359 [349-382]	55.27±2.35 55.17 [51.59-58.11]	0.151±0.007 0.154 [0.135-0.163]	1.47±0.05 1.43 [1.32-1.58]
Injection Zn (group 2)	M±m, Me, Pr.[25–75]	127.4±0.97 128 [126.26-129]	389.5±4.41 397 [393-402]	46.99±1.29 48.35 [43.32-47.82]	0.126±0.001 0.125 [0.125-0.126]	2.51±0.05 2.61 [2.44-2.63]
Injection Co (group 3)	M±m, Me, Pr.[25–75]	136±3.9 136 [129.25-142]	400±8.7 402 [389-415]	42.60±2.09 43.36 [37.41-46.03]	0.109±0.006 0.114 [0.109-0.115]	2.67±0.107 2.67 [2.52-2.76]
	$P_{baseline}$	<0.05	<0.05	<0.05	<0.05	<0.05
	P_{Co}	<0.01	<0.005	—	—	<0.005
	P_{Zn}	<0.01	<0.001	—	<0,005	<0.01
Injection Co + Zn (group 4)	M±m Me, Pr.[25–75]	130.8±2.02 131.50 [122.50-126]	405±6.36 404 [396-415]	40.63±0.98 40.24 [39.56-43.17]	0.101±0.002 0.103 [0.097-0.107]	2.59±0.095 2.61 [2.41-2.70]
	$P_{baseline}$	<0.05	<0.05	<0.05	<0.05	<0.05
	P_{Co}	—	—	—	—	—
	P_{Zn}	—	—	<0.001	<0.05	—

[a] Note: M ± m is the arithmetic mean value of sample, Me-median, Pr- [25–75] percentile of sample, P – statistical significance level of differences of compared indicators

TABLE II. CHANGES IN THE CONTENT OF CALCIUM AND COBALT IN TISSUES.

Experiment conditions	**Statistical indicators**	**Ionized Ca concentration in plasma (mmol/L)**	**Total calcium in plasma (mmol/L)**	**Calcium content in bones of rat (g/kg dry wt)**	**Bone cobalt content (µg/g wt)**
Baseline value (group 1))	M±m	0.838±0.041	2.186±0.087	244.6 ± 1,8	0.033±0.005
Injection Co (group 3)	M±m	1.047±0.033	3.030±0.036	118.3±3.3	0.469±0.034
	p	**	**	**	**

[b] Note: (*) – accurate indicator (p <0.001) change from baseline;

[c] (**) – accurate indicator (p <0.05) change from baseline;

The animals' experiment group had combined introduction of metals (zinc + cobalt), prolonged intake of zinc in the body most likely had a protective effect on the cardiovascular system because of cobalt intoxication reactions. Perhaps the interaction of zinc with cobalt in the body is similar to the results of a study of the interaction of zinc with cadmium, where it was shown that zinc either displaces cadmium or is a competitor for cadmium binding sites when it enters the body [14].

There is a fact that cobalt leads to the activation of lipid peroxidation, and zinc effects antioxidant activity [15].

The determination of indicators of cobalt and calcium in the bones and the plasma and total ionized calcium was put for a thorough study of cobalt accumulation in the body with its increased oral intake and its reaction with calcium.

The analyses of the results in an experiment animal group treated with cobalt chloride in isolation, an increase of both total and ionized calcium level in the blood was noted in relation to the baseline values (Table 2).

There is an increase of mean arterial pressure noted along with an increase of total and ionized calcium (Table 1), a heart rate increase, and an increase of vascular resistance. Obviously, these changes show the reaction of the body to the damaging effect of cobalt directly on the myocardium and vascular wall. The content of cobalt in the bones during its intragastric intake exceeded the initial value by 14 times (Table 2). According to this fact, increased intake of metal in the body causes accumulation of cobalt in the bones replacing calcium.

A correlation is established between a change in systemic hemodynamic parameters and an increase in ionized and total blood calcium when reacting with cobalt.

When analyzing the Pearson coefficient in the control group of animals, a moderate inverse relationship is established between the level of total calcium and vascular resistance. ($r = –0.676$; $p > 0.005$) in the same group, a pronounced positive relationship is found between the content of ionized calcium and heart rate ($r = 0.899$; $p > 0.005$) and a negative increasing relationship between cardiac, stroke indices and ionized calcium ($r = –0.788$; $p > 0.005$). ($r = –0.643$ $p > 0.05$).

In the group with isolated cobalt administration, a moderately negative relationship was found between blood pressure ($r = –0.579$; $p > 0.005$) and total calcium. A similar relationship was also observed with heart rate and total calcium. A direct positive reaction ($r = 0.605$; $p > 0.05$) was found between the content of total calcium in the blood and two indicators of cardiac activity (stroke and cardiac indices). Similar changes in the correlation values were also observed with ionized blood calcium.

VI. Conclusion

1 Excessive intake of cobalt chloride or zinc chloride in rats causes hypokinetic arterial hypertension.

2 The combined introduction of zinc and cobalt salts weakens the effect of cobalt on the parameters of systemic hemodynamics

An increased intragastric intake of cobalt into the body causes accumulation mainly in the bones, at the same time leading to the decalcification and an increase of calcium in blood.

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