

# Arterial Hypertension under Combined Effect of Metals in Experiments on Rats

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**Abstract** – In experiments on 120 Wistar rats, changes in systemic hemodynamics were studied under intragastric daily injections of cadmium sulfate (0.5 mg/kg per metal), cobalt chloride (4 mg/kg per metal), zinc chloride separately and together with cadmium or cobalt at a dose of 20 mg/kg or 1 mg/kg (per metal). Changes in average arterial pressure were studied by the method of electromanometry, and in the cardiac output – by the thermodilution method. Specific peripheral vascular resistance was calculated. The content of calcium and metals in the blood and bone tissue, and the content of ionized calcium in blood were determined. The correlation analysis of the parameters using the Pearson method was conducted. The results of the study revealed development of arterial hypertension after the injection of metals due to an increase in specific peripheral vascular resistance. Cardiac output decreased indicating a violation of the pumping function of the heart. The correlation between an increase in blood pressure and the content of ionized calcium was established. Accumulated metals in the bones causing their decalcification were identified. Under the combined injection of metals and zinc, it was found that high doses of zinc (20 mg/kg) do not prevent arterial hypertension from developing. Small doses (1 mg/kg) have a significant prophylactic effect.

**Keywords** – arterial hypertension, cadmium, cobalt, zinc, calcium.

## I. INTRODUCTION

The environment contains a huge amount of metals, some of which are vital (Zn, Fe, Mn, Ca, Mg), while the other ones are extremely toxic (Pb, Cd, Hg) [1–3]. The negative effect of these substances depends on the dose of the compounds entering the body, but even their low concentrations can accumulate in the tissues causing adverse effects.

Metals entering the body absorbed by the blood and accumulated by the tissues damage and change the functions of the cardiovascular system and kidneys [4], affect electrolyte metabolism, in particular calcium metabolism, leading to increased removal from the body [5]. Due to competitive relationships with calcium, metals are deposited in bone tissues [6]. The physiological antagonism of many heavy metals and calcium is known [5].

Modern conditions of anthropogenic pollution of the environment under the influence of industry and transport force several heavy metals to enter the body which requires studies on their joint influence on body functions. This applies primarily to the joint action of cadmium and cobalt with zinc. It is known that the oxidative stress is one of the pathogenetic mechanisms of heavy metals [7, 8], while zinc is able to exert an antioxidant protective effect [9]. Zinc intoxication has been little studied, and joint effects of zinc, cadmium or cobalt are understudied.

The article aims to study the effect of cadmium, cobalt and zinc on calcium metabolism and parameters of systemic hemodynamics as well as analyze the effects of long-term joint action of cadmium, cobalt and zinc on the state of systemic hemodynamics in experiments on rats.

**The hypothesis** Taking into account that cadmium and cobalt activate lipid peroxidation processes, and zinc activates the antioxidant system, it was assumed that toxic effects of cadmium or cobalt can be reduced when they are injected with zinc enter.

## II. METHODS AND MATERIALS

The experiments were conducted in compliance with Article 11 of Helsinki Declaration of the World Medical Association (1964), "International recommendations for conducting biomedical experiments on animals" (1985) and the Rules of Laboratory Practice in the Russian Federation No. 199. The object was 120 Wistar rats. During the experiment, the animals were on a standard diet, had free access to food and water during the day.

Hemodynamic parameters were determined during the experiment under thiopental anesthesia. Blood pressure was measured by inserting a plastic catheter into the femoral artery, filled with 10 % heparin solution and connected to the DDA electromanometer (Russia). The readings were recorded using an MX-04 monitor (Russia), data were printed using an Epson-1050 printer (USA). The mean arterial pressure (MAP) was calculated using the formula  $SAP = DP + 1/3 PP$ , where DP is the diastolic pressure, PP is the pulse pressure. To determine the minute blood volume, an MT-54M thermistor (Russia) was inserted through the left common carotid artery into the aortic arch. A physiological solution of a fixed temperature of 0.2 ml was injected into the right atrium through the catheterized right jugular vein. The thermodilution curves were recorded using an EPP-5 recorder (Russia). The cardiac index (CI), the beat index (BI) and specific peripheral vascular resistance (SPVR) were calculated using special formulas [12].

The content of protein and calcium in the urine and blood plasma was determined spectrophotometrically (Solar-300, Belarus) using Agat-Med diagnostic devices (Russia). The level of ionized calcium in the blood plasma was determined by direct potentiometry using the Querty-Med ion-selective analyzer (Russia). To determine the content of cadmium and calcium in the bone tissue, the femur mineralization was carried out according to GOST 26929 and the test solution was prepared according to GOST 30178-96. Calcium was determined after preliminary dilution using a spectrophotometer "SOLAR-300" (Belarus). The cadmium content was determined using a Kvant-AFA atomic absorption spectrophotometer.

Statistical processing of the results, taking into account the number of samples and normal distribution of the comparison series according to the Shapiro – Wilk criterion ( $W\phi \gg W_m$ ), was carried out using Student's "t" criterion in GraphPad Prism 6 and Statistica 10 with a critical confidence level (p) less than 0.05. The degree of dependence of changes in hemodynamic parameters on the influence of heavy metal salts was analyzed and the Pearson correlation coefficient was calculated (r).

The experiments were conducted in twelve groups of rats (there were 10 rats in each group): Group 1 – daily injection of saline solution through the atraumatic probe into the stomach in a volume corresponding to the cadmium sulfate solution injected in the experimental series of rats; 2nd group – daily intragastric injection of cadmium sulfate in the form of an aqueous solution at a dose of 0.5 mg/kg (in terms of metal) for 30 days; Group 3 – daily intragastric injection of zinc chloride in the form of an aqueous solution at a dose of

20 mg/kg (in terms of metal) for 30 days; Group 4 – simultaneous intragastric injection of cadmium sulfate (0.5 mg/kg) and zinc chloride (20 mg/kg) daily for a month; Group 5 – intragastric daily injection of zinc chloride at a dose of 1 mg/kg; Group 6 – simultaneous intragastric injection of cadmium sulfate (0.5 mg/kg) and zinc chloride at a dose of 1 mg/kg daily for a month.

Similar six groups of rats were examined while injecting 4 mg/kg of cobalt chloride: Group 7 – daily injection of saline solution through the atraumatic probe into the stomach in a volume corresponding to the cobalt chloride solution injected in the experimental series of rats; Group 8 – injection of cobalt chloride at a dose of 4 mg/kg; Group 9 – injection of zinc chloride at a dose of 20 mg/kg; Group 10 – injection of cobalt chloride and zinc chloride at a dose of 20 mg/kg; Group 11 – injection of 1 mg/kg of zinc chloride; Group 12 – injection of cobalt chloride (4 mg/kg) and zinc chloride at a dose of 1 mg/kg.

## III. RESULTS AND DISCUSSION

Experimental studies established an increase in the SAP in a group of animals with experimental cadmium intoxication, relative to the values of intact control due to the growth of the SPVR. At the same time, both HI and BI decreased (Table 1). Correlation analysis of systemic hemodynamic parameters made it possible to establish that intragastric injection of cadmium sulfate (Group 2) increased the SAP due to changes in the SPVR ( $r = 0.94$ ;  $p < 0.001$ ) relative to the background. The inverse correlation between the level of average arterial pressure and the heart index was identified ( $r = -0.651$ ;  $p < 0.05$ ).

The study of the functional state of the cardiovascular system conducted under chronic intoxication with zinc chloride at a dose of 20 mg/kg, made it possible to establish that this dosage of zinc causes development of arterial hypertension (Table 1) which is less pronounced than that caused by cadmium sulfate injection. The main mechanism for the formation of hypertension was the growth of vascular resistance compared with the control indicators.

A high degree of direct correlation between increases in blood pressure and vascular resistance in animals treated with zinc was found ( $r = 0.76$ ;  $p < 0.001$ ). Simultaneously with this vascular effect, a significant change in the pumping function of the heart was identified. It was characterized by a decrease in the beat and heart indices relative to the values of the control group of animals. An inverse correlation between the level of average arterial pressure and heart index was detected ( $r = -0.39$ .  $P < 0.001$ ).

Under the joint injection of cadmium and zinc at a dose of 20 mg/kg, an increase in blood pressure was observed under cadmium sulfate injection. A high degree of direct correlation between the blood pressure index and the increase in vascular resistance in animals injected with cadmium and zinc ( $r = 0.89$ ;  $p < 0.001$ ) was revealed. At the same time, the hemodynamic structure of an increase in blood pressure has changed: there was a less significant increase in the SPVR, and the degree of reduction in cardiac output was less pronounced.

**TABLE I. THE EFFECT OF CADMIUM SULFATE (0.5 MG/KG) ON THE HEMODYNAMIC PARAMETERS (M ± M) IN RATS UNDER INTRAGASTRIC INJECTION OF ZINC CHLORIDE OF 20 MG/KG AND 1 MG/KG**

Conditions	The average arterial pressure (mmHg.)	Peripheral vascular resistance (RU)	Heart index (ml/kg)	Beat index (ml/kg)
Group 1 – Background	114.6±0.93	1.51±0.08	55.09±1.19	0.127±0.003
Group 2 Cadmium 0.5 mg/kg injection	130.1±0.88*	2.40±0.05*	42.8±0.91*	0.108±0.002*
Group 3 Zinc 20 mg/kg injection	119.1±1.01*	1.75±0.03*	48.2±1.17*	0.114±0.003*
Group 4 Cadmium 0.5 mg/kg and zinc 20mg/kg injection	130.5±1.8*	2.18±0.02* <sup>#</sup>	48.74±0.87* <sup>#</sup>	0.121±0.002 <sup>#</sup>
Group 5 Zinc 1 mg/kg injection	105.4±3.1*	1.49±0.056	56.22±2.20	0.149±0.007
Group 6 Cadmium 0.5 mg/kg and Zinc 1 mg/kg injection	123.8±1.46* <sup>#</sup>	2.10±0.03* <sup>#</sup>	47.86±0.85* <sup>#</sup>	0.111±0.004*

<sup>a</sup>. Note: \*) – reliable changes compared to the background;

<sup>b</sup>. #) – compared to isolated cadmium injection

Thus, under the joint action of cadmium and a high dosage of zinc, there is no hypertensive effects of metals, or the effect is weak, although severity of the hypokinetic type of hypertension is weakened. Being powerful cumulative poison, cadmium accumulated in the bone tissue of experimental animals and increased the concentration of ionized calcium in the blood plasma of animals that received only cadmium, while its total fraction decreased, compared with similar indicators in the control group of animals (Table 2). A high degree of inverse correlation between the level of cadmium accumulation and a decrease in the calcium content in the bone tissue was detected ( $r = -0.9$ ,  $p < 0.01$ ). An increase in the

fraction of free ionized calcium in the blood plasma of animals with cadmium intoxication is a consequence of a decrease in the protein content due to leaching of calcium from the bones during their decalcification. The literature contains information on the role of interaction of cadmium and calcium in the genesis of cadmium intoxication [10].

A decrease in total plasma protein concentration in animals that received only cadmium relative to the control values (Table 2) could be a consequence of proteinuria, as well as a decrease in the formation of proteins in the liver associated with the direct toxic effect of cadmium on the liver.

**TABLE II. THE EFFECT OF 20 MG/KG CADMIUM SULFATE AND ZINC CHLORIDE INJECTIONS ON BIOCHEMICAL PARAMETERS**

Conditions	Statistical indicator	Ca in blood mmol/l	Ca <sup>2+</sup> In blood mmol/l	protein in blood g/l	Cd in bone tissue mg/100g dry bone weight	Ca in bone tissue g/100g dry bone weight	Zn in bone tissue mg/100g in dry bone weight	Cd in blood mg/g	Zn in blood mg/kg
Group 1 Control	M±m	2.390±0.0420	1.090±0.0330	73.30±1.2400	0.013±0.00120	250.60±9.85	65.20±3.62	0.0085±0.00082	3.90 ±0.32
Group 2 cadmium injection	M±m	2.020±0.0550	1.282±0.035	67.00±1.8500	0.260±0.022	126.35±9.85	60.30±4.36	0.122±0.012	3.10±0.32
	p under control	<0.001	<0.001	<0.05	<0.001	<0.001	н/д	<0.001	н/д
Group 3 zinc 20 mg/kg injection	M±m	2.100±0.0450	1.200±0.032	69.00±1.3600	0.0095±0.00099	153.60±7.65	138.40±8.70	0.0077±0.00077	6.10±0.60
	p under control	<0.001	<0.05	<0.05	<0.05	<0.001	<0.001	н/д	<0.01
Group 4 cadmium and zinc 20 mg/kg injection	M±m	1.960±0.0490	1.390±0.032	60.40±1.9700	0.200±0.020	87.60±6.50	111.60±8.95	0.0985±0.0068	5.60±0.54
	p under control	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05
	p Cadmium	H	<0.05	<0.05	0.1	<0.01	<0.001	H	<0.001
	p Zinc	<0.05	<0.001	<0.01	<0.001	<0.001	<0.05	<0.001	H

Under prolonged intragastric injection at a dose of 20 mg/kg, zinc showed natural competition with calcium which was characterized by a decrease in plasma calcium concentration and an increase in zinc levels compared with the control indicators (Table 2). A high degree of inverse correlation between the level of zinc in the blood plasma and a decrease in the plasma calcium concentration was found ( $r = -0.72$ ,  $p < 0.001$ ).

Zinc injection caused demineralization of bone tissues which was manifested by an increase in the concentration of ionized calcium in the blood plasma in combination with a decrease in the calcium content in the bone tissue. These

changes were determined with a simultaneous increase in the concentration of zinc in the bones of animals. In addition, in animals that received only zinc, there was a decrease in plasma protein concentration compared with intact control indicators.

Cadmium and zinc (Table 2) caused the most significant changes in calcium metabolism – the total calcium content in the blood decreased, and the concentration of ionized calcium was the highest among all the groups of rats. These results were due to the lowest protein content in the blood and the most pronounced decalcification of the bone tissue.

A high degree of inverse correlation between the level of accumulation of cadmium, zinc in the bone tissue and a decrease in the calcium content in the bone tissue was detected ( $r = -0.91, p < 0.001$ ). The level of cadmium in the blood and the bone tissue was lower than in the group of rats with its isolated injection.

The hemodynamic effects of prolonged intoxication with cobalt chloride were similar to cadmium intoxication (Table 3). Arterial hypertension occurred in rats of the 8th group, caused by an increase in the SPVR with a decrease in the pumping function of the heart.

Isolated 20mg/kg zinc injection of rats of the 9th group caused a month hypertension, similar to the effects of cobalt chloride, but its severity was less. An increase in blood pressure was also associated with an increase in the SPRV, and cardiac output decreased, but to a lesser extent than under the action of cobalt (Table 3).

Under the joint injection of cobalt chloride and zinc chloride at a dose of 20 mg/kg, the hypertensive effect of chronic cobalt intoxication wakened, although blood pressure increased. The hemodynamic structure of arterial hypertension differed little from the identified hypokinetic type of hypertension under cobalt or zinc injection.

According to literature data, zinc suppresses lipid peroxidation processes [11], which play a leading role in the genesis of arterial hypertension during intoxication with heavy metals [12]. However, the use of high dosage of zinc together with cobalt does not prevent hypertension. Moreover, in the literature there is information about the synergistic cytotoxic effect of cobalt and zinc [13] due to a high dosage of zinc. In this regard, we conducted experiments using a lower dosage of zinc chloride – 1 mg/kg.

The study of the isolated use of zinc chloride at a low dose showed zinc injection caused a slight decrease in blood pressure and no changes in the SPRV parameters and the cardiac output (Table 1, group 5).

The toxic effects of cadmium and zinc at a dose of 1 mg/kg were characterized by a less significant increase in blood pressure compared with similar indicators of a group of animals that received only cadmium. It is due to a less pronounced change in the SPRV, the Pearson correlation coefficient  $r = 0.59$  ( $p < 0.01$ ). In addition, there was a significant recovery in the pumping function of the heart of animals, compared with those in animals that received only cadmium (group 6, Table 1).

Thus, the use of zinc at a dose of 1 mg/kg under conditions of cadmium poisoning reduces severity of toxic effects of cadmium.

Biochemical symptoms of arterial hypertension under chronic intoxication with cobalt chloride were similar to those under cadmium intoxication. Increased blood pressure was combined with an increase in ionized calcium (from  $0.971 \pm 0.042$  in control to  $1.297 \pm 0.017$  mmol/l;  $p < 0.001$ ) and had a clear correlation ( $r = 0.517$   $p < 0.005$ ). There was a relation between an increase in ionized calcium and a decrease in cardiac output ( $r = 0.654$ ;  $p < 0.05$ ). The calcium content in bone tissues decreased from  $244.6 \pm 1.8$  g/kg dry weight in control rats to  $118.3 \pm 3.3$  g/kg dry weight in experienced rats of the 8th group ( $p < 0.001$ ) which was associated with the accumulation of cobalt in bones (with  $0.033 \pm 0.005$   $\mu\text{g/kg}$  in the control groups up to  $0.469 \pm 0.034$   $\mu\text{g/kg}$  after the monthly cobalt injection ( $p < 0.001$ ).

TABLE III. THE EFFECT OF COBALT CHLORIDE (4 MG/KG) ON THE HEMODYNAMIC PARAMETERS ( $M \pm M$ ) IN RATS UNDER INTRAGASTRIC INJECTION OF ZINC CHLORIDE AT DOSAGES OF 20 MG/KG AND 1 MG/KG

Conditions	The average arterial pressure (mmHg.)	Peripheral vascular resistance (RU)	Heart index (ml/kg)	Beat index (ml/kg)
Group 7 -control group	103.8±1.01	1.46±0.05	55.24±2.35	0.151±0.007
Group 8 cobalt injection 4 mg/kg	135±3.8*	2.66±0.106*	42.50±2.08*	0.109±0.006*
Group 9 zinc injection 20 mg/kg	127.3±0.98*	2.49±0.04*	46.89±1.29*	0.126±0.001*
Group 10 cobalt injection 4 mg/kg zinc injection 20 mg/kg	123.8±2.27*#	2.28±0.11*#	44.09±2.15*	0.104±0.003*
Group 11 zinc injection 1 mg/kg	105.4±3.1	1.49±0.056	56.22±2.20	0.149±0.007
Group 12 cobalt injection 4 mg/kg zinc injection 1 mg/kg	110.6±4.1#	1.76±0.046*#	50.23±2.09#	0.130±0.008*#

Note: \*) – reliable changes compared to the background;  
#) – compared with isolated cobalt injections

Isolated injection of zinc chloride at a dose of 1 mg/kg (Table 3, group 11) did not change the state of systemic hemodynamics compared with the control group of rats. There was no arterial hypertension.

Experiments with combined injection of cobalt chloride (4 mg/kg) and zinc chloride (1 mg/kg) (group 12, Table 3) showed that a small dosage of zinc can eliminate the hypertensive effect of chronic cobalt intoxication. Zinc exercises this effect on the SAP, the SPRV and the pumping function of the heart.

#### IV. CONCLUSION

Under prolonged injection, heavy metals cause arterial hypertension. An increase in blood pressure is due to an increase in specific peripheral vascular resistance. Arterial hypertension is accompanied by a decrease in the pumping function of the heart. Impaired calcium homeostasis increasing ionized calcium plays an important role in the development of arterial hypertension caused by metal intoxication. The combined effect of zinc with cadmium or cobalt can suppress severity of toxic arterial hypertension when using low

concentrations of zinc, while high concentrations of zinc do not exercise this effect.

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