Influence of cadmium on blood and hair trace elements and biochemical markers

E V Kiyaeva¹, S V Notova¹

¹Federal Research Centre of Biological Systems and Agro-Technologies of the Russian Academy of Sciences, 29, 9 Yanvarya Str., Orenburg 460000, Russia
E-mail: elena_sap@mail.ru

Abstract. The aim of the present study was to investigate the effect of low doses of cadmium on the content of chemical elements in the blood and hair, blood biochemical parameters and the concentration of metallothionein-1 in the serum of laboratory animals. The study was conducted on male Wistar rats from two months of age (N = 20, weight=160-180 g). The rats of experimental group were fed with Cd chloride (0.01 mg/kg BW) for 12 weeks. The elemental composition of blood and hair was determined by atomic emission and mass spectrometry with inductively coupled argon plasma. Blood biochemical parameters were determined spectrometrically using a Clima MC-15 A/O Unimed analyzer. The concentration of metallothionein-1 was measured using ELISA kit. The content of copper, iron, zinc and cadmium significantly (p <0.001) changed in the blood of animals of the experimental group. In hair, the content of selenium, zinc and cadmium changed significantly (p <0.001). The concentration of ALT, AST and total bilirubin increased due to the toxic effects of cadmium. The concentration of MT1 in the blood of animals of the experimental group decreased (p <0.05).

1. Introduction
Cadmium is one of the most toxic ecopollutants in economically developed countries. Technological advances and industrialization during the twentieth century led to the increasing use of this element. Consumption of cadmium as well as soil, water and air polluted by it, as a result of production activities and tobacco smoking, is steadily increasing, creating a threat to the health of people and animals. [1, 2, 3]. Sources of most anthropogenic pollution are: the release of cadmium into wastewater, the production and use of phosphate fertilizers, waste incineration, coal, gasoline, etc. [4]. As a result, in areas exposed to the intense impact of technogenic factors, the concentration of cadmium in the atmospheric air, soil and snow cover is several times higher than the permissible standards [5, 6]. Soil contamination with cadmium is a serious problem from the point of view of the environment and public health, since heavy metals tend to bioaccumulate throughout the food chain — soil-plant-animal-man [7, 8, 9].

Under natural conditions, most organisms encounter non-toxic doses of various heavy metals and pollutants. A substantial range of studies show that exposure to even very low doses of cadmium over a lifetime can damage kidneys, liver, bone system, cardiovascular and endocrine systems. Along with this, cadmium intoxication can contribute to the development of lung cancer, breast cancer, prostate cancer, pancreas, bladder and nasopharynx.

The group of proteins metallothioneins takes an active part in the metabolism of heavy metals, while MT1 is the most common isoform because it is expressed in all tissues. Metallothioneins take part in various physiological processes in the body: detoxification of metals, transport of essential elements,
protection against oxidative stress, regulation of cell proliferation and apoptosis, maintenance of intracellular redox balance, regulation of inflammation. Linde-Arias and co-authors suggest using metallothioneins as biomarkers of the regional environmental situation [10].

The purpose of this research work was to study the effect of low doses of cadmium on the metabolism of chemical elements, blood biochemical parameters and the concentration of metallothionein I in the serum of laboratory animals.

2. Materials and methods

2.1 Animals

The study was conducted on male Wistar rats from two months of age (N = 20, weight=160-180 g). The animals were kept in clinical and biological laboratory “Vivarium” (Orenburg State University). They were maintained in controlled environment (12:12 h light/dark cycle). All procedures used in the study were performed in accordance with the protocol approved by the Institutional Animal Care and Use Committee of the Federal Research Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences, Orenburg, Russian Federation and complied with Directive of the European Parliament and the Council of the European Union 2010/63 / EU.

2.2 Experimental design

During the accounting period (12 weeks), the animals were divided into 2 groups. The rats of both groups received basic diet, compiled in accordance with the recommendations of the Institute of Nutrition of the Russian Academy of Sciences and contained corn starch (58 g), casein (25 g), unrefined sunflower oil and lard, 4% salt mixture, 1% mixture of vitamins, 2% microcrystalline cellulose. The rats of experimental group were treated with Cd chloride (0.01 mg/kg BW). Metal solution was prepared by dissolving the equivalent weight of the metal in its salt in deionized water according to the approved standard operating procedures for handling the metals in the laboratory. The concentrations prepared for cadmium chloride was 10 μg/ml. The rats of control group were administered with deionized water at 2 ml/kg. All the treatments were administered by gavage once daily between 08.00 h and 09.00 h for 12 weeks. After 12 weeks of the investigation rats were euthanized under deep anesthesia. The whole blood was collected by cardiac puncture.

2.3 Analytical procedures

The elemental composition of blood and hair was determined in the laboratory of the ANO "Center for Biotic Medicine" (registration number in the state register - Ross.Ru 0001. 513118 dated May 29, 2003; Registration Certificate of ISO 9001: 2000, Number 4017-5.04.06) using atomic emission and mass spectrometry with inductively coupled argon plasma (Optima 2000 DV and ELAN 9000 (Perkin Elmer, USA)).

Biochemical parameters assessing liver functions (alanine aminotransferase (ALT), aspartate aminotransferase (AST) total protein and total bilirubin) were determined spectrophotometrically using commercial kits by biochemical analyzer (Clima MC-15 A / O Unimed). The concentration of metallothionein-I was measured using rat specific ELISA kit (Cloud-Clone Corp, Houston, TX, USA), according to the manufacturer’s instructions.

2.4 Statistical analysis

Mathematical processing of data was carried out using the program "Statistica 10.0". Parameters of descriptive statistics for quantitative indicators are given in the form of a median (Me) and interquartile latitude (25th, 75th percentile - Q1; Q3). Since n does not exceed 30, the Mann-Whitney U test was used to estimate the significance of the similarity (difference) of two independent samples. The significance level was considered reliable at p <0.05. Correlation analysis was carried out using the Spearman correlation coefficient.
3. Results and discussion

3.1 Effects of treatments on elemental composition of blood and hair of laboratory animals

The effect of cadmium on the elemental composition of the blood of laboratory animals is presented in Table 1. The content of copper, iron, zinc, and cadmium changed significantly (p < 0.001). Against the background of an increase in the content of the toxic element cadmium, a decrease in the content of the essential elements was observed: copper by 1.3 times, iron by 1.2 times and zinc by 1.4 times.

Table 1. Elemental composition of blood of laboratory animals (μg/ml)

| Elements | Control Group | Experimental Group | MWU | p   |
|----------|---------------|--------------------|-----|-----|
| Ca       | 38.9 (32.6-54.8) | 29.12 (22.43-41.91) | 0.075 |
| K        | 2041 (1948-2065) | 1999 (1968-2056) | 0.850 |
| Mg       | 46.6 (28.7-58.9) | 39.4 (25.8-52.3) | 0.307 |
| Na       | 1997 (1969-2049) | 1996.8 (1948-2026) | 0.850 |
| P        | 473.1 (407.3-524.01) | 431.2 (412.8-443.4) | 0.273 |
| As       | 1.015 (0.9-1.19) | 0.99 (0.98-1.08) | 0.426 |
| Co       | 0.007 (0.004-0.009) | 0.004 (0.002-0.005) | 0.064 |
| Cr       | 0.186 (0.151-0.202) | 0.171 (0.096-0.243) | 0.520 |
| Cu       | 1.158 (1.153-1.167) | 0.857 (0.801-0.880) | 0.000183 |
| Fe       | 455 (432-485) | 379 (365-402) | 0.000282 |
| Li       | 0.044 (0.029-0.052) | 0.033 (0.023-0.039) | 0.185 |
| Mn       | 0.019 (0.013-0.029) | 0.017 (0.017-0.018) | 0.427 |
| Ni       | 0.019 (0.018-0.021) | 0.021 (0.017-0.025) | 0.677 |
| Se       | 1.02 (0.97-1.12) | 0.98 (0.98-1.05) | 0.401 |
| Si       | 1.77 (1.59-2.16) | 1.61 (1.02-2.28) | 0.496 |
| V        | 0.036 (0.025-0.051) | 0.022 (0.016-0.025) | 0.075 |
| Zn       | 7.1 (7.06-7.18) | 4.89 (4.84-4.94) | 0.000180 |
| Al       | 0.173 (0.13-0.222) | 0.206 (0.155-0.251) | 0.384 |
| Cd       | 0.0003 (0.0003-0.0003) | 0.004 (0.0038-0.0041) | 0.000173 |
| Pb       | 0.003 (0.002-0.004) | 0.003 (0.002-0.004) | 0.909 |
| Sn       | 0.013 (0.008-0.022) | 0.015 (0.008-0.024) | 0.705 |

Data presented as median (25-75); MWU = p values as assessed by Mann-Whitney U-test; * = difference significant at p<0.05

In the wool of laboratory animals exposed to low doses of cadmium, the content of selenium, zinc and cadmium differed significantly. (Table 2).

The concentration of selenium decreased 1.4 times compared with the control while the zinc content increased 1.2 times. The concentration of cadmium also increased by 1.6 times.

3.2 Effect of treatments on serum liver enzyme activities and total bilirubin

The effect of treatments on activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) is shown in Table 3. There was a significant (P < 0.001) increase in the AST, ALT and total bilirubin concentration in the Cd group compared to that of the controls. Such changes can be characteristic for toxic liver damage.
Table 2. Elemental composition of hair of laboratory animals (µg/g)

| Elements | Control Group | Experimental Group | MWU p |
|----------|---------------|-------------------|-------|
| Ca       | 428.2 (394.7-457.5) | 426.4 (337.5-458.7) | 0.850 |
| K        | 1683 (1615-1746)   | 1743 (1676-1859)   | 0.344 |
| Mg       | 126.2 (93.7-140.5) | 131.9 (79.5-155)  | 0.791 |
| Na       | 311.38 (299.7-319.7) | 297.55 (289.06-312.8) | 0.161 |
| P        | 275.7 (216.01-311.04) | 306.8 (247.1-335.2) | 0.623 |
| As       | 0.079(0.077-0.082) | 0.071(0.059-0.08) | 0.088 |
| Co       | 0.009(0.008-0.011) | 0.008(0.007-0.009) | 0.075 |
| Cr       | 0.429(0.305-1.08) | 0.343(0.155-0.611) | 0.212 |
| Cu       | 11.72 (10.85-13.22) | 11.065 (9.92-11.8) | 0.185 |
| Fe       | 15.89 (13.31-16.24) | 13.4 (11.95-13.94) | 0.212 |
| Li       | 0.080 (0.074-0.085) | 0.065(0.06-0.086) | 0.140 |
| Mn       | 1.42 (1.12-1.5) | 1.135(0.89-1.38) | 0.161 |
| Ni       | 0.23 (0.192-0.253) | 0.186(0.157-0.269) | 0.344 |
| Se       | 1.145 (0.95-1.2) | 0.8 (0.76-0.82) | 0.0002 a |
| Si       | 6.11 (5.29-6.91) | 7.36 (5.89-8.46) | 0.121 |
| V        | 0.04 (0.038-0.042) | 0.038(0.031-0.051) | 0.969 |
| Zn       | 156 (143-172) | 187 (173-194) | 0.0017 a |
| Al       | 0.903 (0.794-1.102) | 1.13 (0.94-1.21) | 0.140 |
| Cd       | 0.051 (0.043-0.06) | 0.082 (0.067-0.088) | 0.0004 a |
| Pb       | 0.06 (0.04-0.075) | 0.042(0.026-0.057) | 0.053 |
| Sn       | 0.061(0.041-0.079) | 0.084(0.069-0.104) | 0.053 |

Data presented as median (25-75); MWU p=p values as assessed by Mann-Whitney U-test; a - difference significant at p<0.05

Table 3. Biochemical indicators of laboratory animals

| Indices | Units | Control Group | Experimental Group | MWU p |
|---------|-------|---------------|-------------------|-------|
| Total protein | g/l | 81.7 (76.2-89.4) | 76.3 (67.1-79.4) | 0.069 |
| ALT     | U/l  | 24.8 (22.5-26.7) | 44.25 (40.1-49.5) | 0.000 a |
| AST     | U/l  | 21.6 (18.6-26.3) | 36.9 (34.2-45.4) | 0.000 a |
| Total bilirubin | μmol/l | 5.8 (4.3-6.5) | 12.3 (10.2-14.3) | 0.000 a |

Data presented as median (25-75); MWU p=p values as assessed by Mann-Whitney U-test; a - difference significant at p<0.05

3.3 Effect of treatments on serum metallothionein-1 concentrations

The serum metallothionein-1 concentration significantly (p<0.05) decreased in experimental group by 1.2 times. Figure 1 shows the change in the concentration of metallothionein 1 in the serum of laboratory animals depending on the concentration of cadmium.
The content of chemical elements in our body is very closely connected with the peculiarities of nutrition, lifestyle, professional activities and of course the biogeochemical and environmental factors of the area in which people live. Toxicants, including cadmium, enter our body with food, water, inhaled air, cigarette smoke. The study showed that cadmium has an ambiguous effect on the metabolism of chemical elements in the blood and hair of laboratory animals. In the blood of laboratory animals of the experimental group there was a decrease in the content of zinc, iron and copper. The decrease in zinc content is possibly due to the ability of cadmium to displace Zn$^{2+}$ in Zn-binding proteins (in Zn-binding proteins). Cadmium can replace zinc in the active sites of enzymes leading to their dysfunction [11]. In addition, it has been demonstrated that cadmium toxicity decreases in the presence of zinc [12,13]. The reduction in cadmium toxicity in the presence of zinc may also be due to the fact that Zn reduces Cd-dependent oxidative stress [14].

The results of the study on the decrease in the iron content in the blood against the background of cadmium intoxication are consistent with those of Olusola O. O. and co-authors [15]. They found a decrease in the iron content amid exposure to toxicants (lead, cadmium and manganese).

The decrease in the copper content in the blood of the experimental group animals is consistent with previous studies. Erdem O. with co-authors found a decrease in the content of copper and zinc in the tissues of laboratory animals after prolonged oral administration of cadmium [16].

In the wool of laboratory animals against the background of cadmium intoxication, the concentration of selenium decreased and the concentration of zinc increased. Cadmium has a pronounced effect on the exchange of many essential trace elements - selenium and zinc, including as a result of changes in their homeostasis and disruption of their biological functions, acting as an antimetabolite. The increase in the concentration of zinc in wool rather indicates a state of pre-deficiency of this element against the background of cadmium intoxication.

The obtained data on the content of ALT and AST are consistent with the research of Saleh R. M. [17,18]. The increased activity of these enzymes in the blood may indicate damage to the liver cells during cadmium intoxication.

The decrease in serum antioxidant protein metallothionein 1 is due, most likely, to a reduced concentration of zinc and iron in the blood. Zn plays a vital role in maintaining and strengthening the antioxidant status, protecting cell membranes from oxidative damage. Our findings are consistent with Olusola O. O.[15], who suggested that reducing the level of metallothionein in the blood against the toxic effects of various metals seems to reflect an inadequate response to oxidative stress due to a decrease in the level of essential elements in the blood.
4. Conclusion
The results of the study showed that even low doses of cadmium cause metabolic disturbances of chemical elements in the blood and hair of laboratory animals. The imbalance of microelements was accompanied by an increase in hepatic transaminases and total bilirubin. In addition, the concentration of the antioxidant protein metallothionein 1 in the blood was reduced against the background of cadmium intoxication.

Acknowledgements
The studies were carried out in accordance with the research plan for 2019–2020 of the Federal Research Center for Biological Systems and Agrotechnology’s of the Russian Academy of Sciences (Project No. 0526-2019-0001).

References
[1] Satarug S, Garrett S H, Sens M A and Sens D A 2010 Cadmium, environmental exposure and health outcomes J. Environ. Health Perspect. 118 182–90
[2] Wu S, Peng S, Zhang X, Wu D, Luo W, Zhang T and Wu L 2015 Levels and health risk assessments of heavy metals in urban soils in Dongguan, China J. Geochem. Explor. 148 71–8
[3] Notova S V, Kireeva G N, Zhukovskaya E V, Grabeklis A R, Kiyaeva E V, Skalny A V and Deryagina L E 2017 The influence of anthropogenous and geochemical environmental factors on the elementary status of children of Chelyabinsk region J. Human Ecol. 11 23–8
[4] Barsova N, Yakimenko O, Tolpeshta I and Motuzova G. 2019 Current state and dynamics of heavy metal soil pollution in Russian Federation. A review J. Environ Pollut. 249 200–7
[5] Kumar V, Sharma A, Kaur P, Sidhu G P S, Bali A S, Bhardwaj R and Cerda A 2018 Pollution assessment of heavy metals in soils of India and ecological risk assessment: A state-of-the-art J. Chemosphere. 216 449–62
[6] Wieczorek J, Baran A, Urbański K, Mazurek R and Klimowicz-Pawlas A 2018 Assessment of the pollution and ecological risk of lead and cadmium in soils J. Environ. Geochem. Health. 40(6) 2325–42
[7] Wu J, Song J, Li W and Zheng M 2016 The accumulation of heavy metals in agricultural land and the associated potential ecological risks in Shenzhen, China J. Environ Sci Pollut Res Int. 23(2) 1428–40
[8] Huang Y, He C, Shen C, Guo J, Mubeen S, Yuan J and Yang Z 2017 Toxicity of cadmium and its health risks from leafy vegetable consumption J. Food. Funct. 8(4) 1373–401
[9] Rehman K, Fatima F, Waheed I and Akash M S H 2018 Prevalence of exposure of heavy metals and their impact on health consequences J. Cell. Biochem. 119(1) 157–84
[10] Linde-Arias A R, Inácio A F, Novo L A, de Alburquerque C and Moreira J C 2008 Multibiomarker approach in fish to assess the impact of pollution in a large Brazilian river, Paraiba do Sul J. Environ. Pollut. 156(3) 974–9
[11] Tinkov A A, Filippini T, Ajsuvakova O P, Aaseth J, Gruhcheva Y G and Nemeshina O N 2017 The role of cadmium in obesity and diabetes J. Sci. Total Environ. 601 741–55
[12] Bernotiene R, Ivanoviene L, Sadauskiene I, Liekis A and Ivanov L 2012 Influence of cadmium ions on the antioxidant status and lipid peroxidation in mouse liver: protective effects of zinc and selenite ions J. Trace Elem. Ecol. 29(2) 137–43
[13] Brzoska M M, Rogalska J, Galažyn-Sidorczuk M, Jurczuk M, Roszczenko A, Kulikowska-Karpinska E and Moniuszko-Jakoniuk J 2007 Effect of zinc supplementation on bone metabolism in male rats chronically exposed to cadmium J. Toxicology 237(1–3) 89–103
[14] Matović V, Buda A, Bulat Z and Đukić-Ćosić D 2011 Cadmium toxicity revisited: focus on oxidative stress induction and interactions with zinc and magnesium J. Arh. Hig. Rada. Toksikol. 62(1) 65–76
[15] Oladipo O O, Ayo J O, Ambali S F, Mohammed B and Aluwong T 2017 Dyslipidemia induced by chronic low dose co-exposure to lead, cadmium and manganese in rats: the role of oxidative
stress J. Environ. Toxicol. Pharmacol. 53 199–205

[16] Erdem O, Yazihan N, Kocak M K, Sayal A and Akcil E 2016 Influence of chronic cadmium exposure on the tissue distribution of copper and zinc and oxidative stress parameters in rats J. Toxicol Ind. Health. 32(8) 1505–14

[17] Saleh R M and Awadin W F 2017 Biochemical and histopathological changes of subacute cadmium intoxication in male rats J. Environ. Sci. Pollut. Res. Int. 24(32) 25475–81

[18] Lebedev S, Gavrish I, Rusakova E, Kvan O and Gubaidullina I. 2018 Influence of various chromium compounds on physiological, morpho-biochemical parameters, and digestive enzymes activity in Wistar rats Trace elements and electrolytes 35(4) 242–5

[19] Sheida E, Sipailova O, Miroshnikov S, Sizova E, Lebedev S, Rusakova E and Notova S 2017 The effect of iron nanoparticles on performance of cognitive tasks in rats Environmental Sci. and Pollution Res. 24(9) 8700–10