The effect of Cd^{2+} ions on the passive permeability of the mitochondrial membrane of liver cell of Rats

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Abstract. This article describes the results of a study conducted on rats on the effects of heavy metal salts, including Cd^{2+} ions, on mitochondrial membranes. The experiments showed that heavy metals are one of the most dangerous sources of environmental pollution, and that they have a strong genotoxic, tumor-causing and cytotoxic effect when ingested. Cd^{2+} poisoning occurs in the metallurgical industry through inhalation of atmospheric air, consumption of contaminated food and water, and cigarette smoke. The studies showed that the studied heavy metal salts, Cd^{2+} ions, negatively affect the structure of the mitochondrial membrane, increasing the passive permeability of the membrane for monovalent H^+, K^+ and Na^+ cations.

1 Introduction

Cadmium and lead are widespread and non-biodegradable pollutants of great concern to human health. In real life scenarios, we are exposed to mixtures of chemicals rather than single chemicals, and it is therefore of paramount importance to assess their toxicity [1].

The element Cd^{2+} occurs naturally in the Earth's crust in the form of oxides, chlorides and sulfates. The element Cd^{2+} is released into the environment as waste in the manufacturing industry, in the production of mineral fertilizers, various batteries, paints and pigment products, plastic products, and in the mining and metallurgical industry. Cd^{2+} is found in the form of compounds in drinking water [2,3]. Heavy metal compounds are observed to enter the natural environment - soil, water, and accumulate in plants and animals, and salts in this form have the property of being stored in environmental objects for a long time, and by accumulating in the human and animal body, they cause serious pathological conditions in organs and tissues [4]. Heavy metals occupy one of the leading places in environmental pollution from various sources. Heavy metals entering the human and animal body lead to disruption of homeostasis in the body [5]. Experts are concerned about the contamination of food products and drinking water [6] with mineral fertilizers,

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toxic substances, heavy metals, and the negative impact of xenobiotics on the human and animal body. It should be noted that in soil and water, including drinking water, the content of mineral salts is often higher than the norm, as well as the presence of organic compounds. Among the chemicals classified as global environmental pollutants, heavy metals constitute a special group of anthropogenic toxicants, which determine the unfavorable ecological situation and the increase in morbidity, mainly in industrialized regions [7, 8].

Analysis of the available literature shows that heavy metals, when ingested, have a negative effect on various systems of the body. Currently, the mechanisms of action of heavy metals at the membrane level are being studied in many laboratories around the world [9-10]. Heavy metals are considered one of the most dangerous sources of environmental pollution and, when ingested, exhibit strong genotoxic, tumor-causing and cytotoxic effects [11-17].

2 Methods

2.1 Preparation of Samples

The experiments were conducted on outbred white male rats weighing 180-200 g. Rat liver mitochondria were isolated using the Schneider [18] differential centrifugation method. The composition of the separation medium: 250 mM sucrose, 10 mM tris-chloride, 1 mM EDTA, pH 7.4. For this, after decapitating the rat, the liver was placed in a previously prepared separation medium and its mass was weighed on a scale. For grinding, it was passed through a micropress with holes of 1 mm. The ground liver tissue was placed in a molybdenum glass homogenizer, the separation medium was added to it in a 1:6 ratio and homogenized using a Teflon pestle. Homogenization was carried out using a pestle mounted on an electric motor at a speed of 600-800 rpm. The resulting homogenate was poured into a centrifuge tube. An SLP-1 centrifuge was used to separate mitochondria from the homogenate. Centrifugation was carried out at a temperature of 0-2°C. The centrifugation process was carried out in 2 stages. In the first stage, centrifugation lasted 7-8 minutes at a speed of 1500 rpm (relative centrifugal acceleration 600 g). During this, large cellular components of the tissue that were not broken down were precipitated. The supernatant was transferred to another clean test tube and used for a second centrifugation. In the second stage, centrifugation was carried out at a speed of 6000 rpm for 15 minutes. After centrifugation, liquid residues and fat particles on the walls of the test tube were removed using filter paper. Mitochondria purified from the separation medium were taken into a glass using an autopipette. For experiments, mitochondria were diluted in a 1:1 ratio in EDTA-free separation medium and stored in a glass with ice in a refrigerator.

2.2 Determination of ionic conductivity

The passive ion permeability of the mitochondrial membrane for various cations was measured by the Brierley method [19]. The passive ion permeability of the inner membrane of liver mitochondria was measured using isoosmotic media prepared from the corresponding metal salts KNO_3, $NaNO_3$, $NH4NO_3$ for monovalent K^+, Na^+ and H^+ ions, and from $Ca(NO_3)_2$ and $Mg(NO_3)_2$ for divalent Ca^{2+} and Mg^{2+} ions. The rate of mitochondrial swelling was determined when the protein content in the medium was 0.3-0.4 mg/ml. The passive ion permeability of mitochondria was measured using an LMF 69 photometer at a wavelength of 540 nm.

3 Result and Discussion

It is known that a large number of toxicants, including, first of all, heavy metal ions, form bonds with the SH-group in cells. However, it is noted that some metals (lead, cadmium, nickel, copper, manganese, cobalt) also actively interact with the carboxyl group. Some heavy metals interact with parts of individual enzymes located outside the active center and, as is observed in competitive inhibitors, do not affect their inhibitory properties under conditions of high substrate concentrations (non-competitive inhibition). These inhibitors interact with certain specific groups of the enzyme, for example, heavy metals form bonds with the SH-group or, more often, bind to the regulatory center and, in turn, reduce the degree of binding of the active center.

In the conducted studies, it was proven in experiments that the heavy metal salts $Pb^{2+,}$ As^{2+} and Cd^{2+} ions studied negatively affected the structure of the mitochondrial membrane, increasing its permeability to monovalent and divalent cations. Since the toxic effect of Cd^{2+} salts among the heavy metals studied in the experiments was the highest, in our next stage of experiments, the experiments were continued to study the effect of Cd^{2+} salts on the permeability of the mitochondrial membrane to monovalent H^+, K^+, Na^+ cations at different concentrations (Figures 1-3).

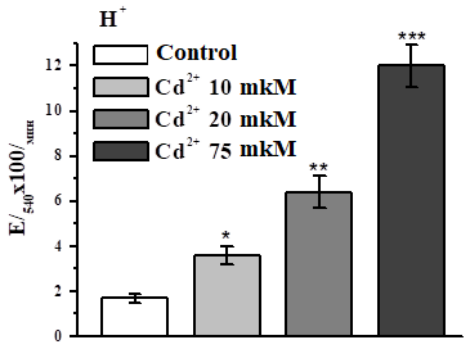

Fig. 1. Effect of Cd^{2+} ions on the passive permeability of the mitochondrial membrane for H^+ ions (*P<0.05; **-P<0.01; ***-P<0.001; (n=5))

The results of experiments conducted to study the effect of Cd^{2+} ions on the energy-independent decay of mitochondria in an isoosmotic solution of nitrate salts showed that these heavy metals also increased the passive permeability of the mitochondrial membrane for monovalent cations H^+, K^+, Na^+, depending on the applied concentrations.

In the experiments, it was noted that a concentration of 75 μM of Sd^{2+} ions increased the permeability of the mitochondrial membrane for H^+, K^+, Na^+ ions by 7 times compared to the control value.

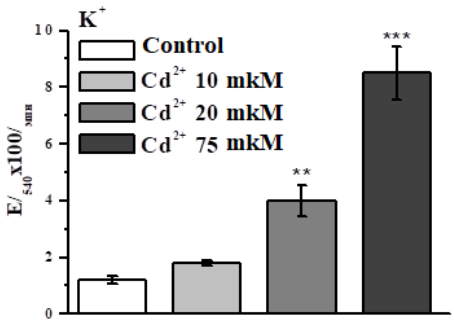

Fig. 2. Effect of Cd^{2+} ions on the passive permeability of the mitochondrial membrane for K^+ ions (**-P<0.01, ***-P<0.001, (n=5)).

Also, based on the comparison of the effect of each metal used at a concentration of 75 μM on the permeability of the mitochondrial inner membrane, it can be concluded that all the studied metals cause proton permeability to one degree or another, as well as permeability for K^+ and Na^+. The efficiency of the property of various heavy metals to cause the permeability of the mitochondrial membrane for monovalent cations can be represented in the form of the following sequence: Cd^{2+} – H^+ : K^+ : Na+ = 1:0.7:0.6.

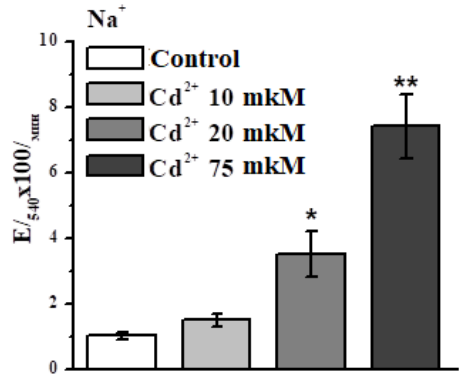

Fig. 3. Effect of Cd^{2+} ions on the passive permeability of the mitochondrial membrane for Na^+ ions (*P<0.05; **-P<0.01; (n=5))

In these calculations, the maximum value of the mitochondrial membrane in an isoosmotic solution of nitrate salts is taken as the unit.

Thus, in our experiments, it was noted that Cd^{2+} increases the permeability of the mitochondrial membrane, possibly depolarizing the membrane. In this case, membrane depolarization under the influence of Cd^{2+} ions, as noted in other scientific laboratories, causes the release of cytochrome c from mitochondria and the activation of caspase-3 in all types of mammalian cells - including kidney cells, hepatocytes, neurons and lymphocytes. This is assumed to be a universal mechanism of apoptosis under the influence of Cd^{2+} in mammals [20]. In studies conducted by Ye.A. Belyaeva et al., it was noted that the swelling caused by the influence of Cd^{2+} in energized mitochondria isolated from rat liver in the presence of sucrose in the incubation medium and in the absence of Ca^{2+} and P(i) was Ca^{2+} dose-dependent [11]. Chemical agents - ADF, SsA, EGTA, DTT, ruthenium red, atractyloside and Ca^{2+} in NH_4NO_3, KCl and sucrose media - have been found to have varying degrees of effect on mitochondrial membrane permeabilization induced by Cd^{2+}. It

is known that apoptosis is an energy-intensive process, and in studies conducted in mammalian models, ATP depletion at the cellular level is noted as one of the reasons for the activation of the cell death program from apoptosis to necrosis.

4 Conclusion

It can be concluded that the destructive effect of Cd^{2+} on the mitochondrial membrane on oxidative phosphorylation (OP) and the decrease in membrane potential may play a certain role in the occurrence of apoptosis and necrosis. Our results suggest that the differences in the characteristics of the effects of heavy metals on mitochondrial membrane permeability identified in this study may be due to their effects on mitochondria through different mechanisms.

References

1. M. Andjelkovic, A. B. Djordjevic, E. Antonijevic, et alInt. J. Environ. Res. Public Health **16**, 274 (2019) DOI:10.3390/ijerph16020274
2. ATSDR. Draft toxicological profile for cadmium. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services. Atlanta, USA (2008)
3. M. Karaca, Biosorption of aqueous Pb2+, Cd2+, and Ni2+ ions by Dunaliella salina, Oocystis sp., Porphyridium cruentum, and Scenedesmus protuberans prior to atomic spectrometric determination. PhD Thesis master of science in Chemistry, pp. 3-10 (Izmir., 2008)
4. N. P. Setko, E. A. Zakharova, Hygiene and Sanitation **6**, 65-67 (2005)
5. V. A. Stezhka, E. G. Lampeka, N. N. Dmitrukha, Gig. truda Issue **32**, 219-230 (2001)
6. Sh.T. Iskandarov, Regional sanitary and hygienic problems of protection of water sources and water supply of the population in specific conditions of the Republic of Uzbekistan, p. 2008 (Tashkent, 2001)
7. M. V. Skachkov, M. A. Skachkova, N. N. Vereshchagin, Gig. i Sanitation **5**, 39-42 (2002)
8. A. R. Gutnikova, K. O. Makhmudov, B. A. Saidkhanov, et al., Toxicological Bulletin **3**, 21- 27 (2009)
9. D. Khayitov et.al., Biodiversitas Journal of Biological Diversity **25** (2024) DOI: 10.13057/biodiv/d250414.
10. E. A. Belyaeva, S. M. Korotkov, N. E. Saris, *In vitro modulation of heavy metal-induced rat liver mitochondria dysfunction: a comparison of copper and mercury with cadmium*. Trace. Elem. Med. Biol., pp. 163-173 (2011)
11. I. Hernandez-Ochoa, et al., Reprod Toxicol. **20(2)**, 221-228 (2005)
12. D. Khojakulov, K. Khaydarov, A. Rabbimov, et.al., Plant Science Today **11(3)** (2024) DOI: https://doi.org/10.14719/pst.3180
13. G. F. Nordberg, Toxicol Appl Pharmacol. **238**, 192-200 (2009)
14. M. P. Waalkes, Mutat Res. **533**, 107-120 (2003)
15. M. Waisberg, P. Joseph, B. Hale, D. Beyersmann, Toxicology **192**, 95-117 (2003)
16. F. Rayimova, G. Dushanova, B. Alikulov, et.al., Biodiversitas **25**, 1329-1336 (2024)
17. W. C. Schneider, G. H. Hogeboom, Cancer.res. **11**, 1-22 (1951)

18. G. P. Brierley, Ann. N.Y. Acad. Sci. **227**, 398-411 (1974)
19. I. Martinou, S. Desagher, R. Eskes, B. Antonsson, E. André, S. Fakan, J.C. Martinou. J Cell Biol. **144(5)**, 883-9 (1999)