Article

Studying the redox status of paramecium caudatum cells under influence of molybdenum, zinc, copper oxide nanoparticles and synthetic antioxidants

Olga Karpukhina [1, 3], Valeriya Dubova[2], A. Latanov[1], Anatoly Inozemtsev [1,*]

[1] Department of Higher Nervous Activity Lomonosov Moscow State University, Moscow, Russia;
[2] Department of Physiology RUDN University, Moscow, Russia;
[3] Semenov Institute of Chemical Physics RAS, Moscow, Russia;
* Correspondence: a_inozemtsev@mail.ru; Tel.: +74959395001;
karpukhina.msu@yandex.ru, https://orcid.org/0000-0002-4642-8366 (O.K.);
valeriya.dubova7525@yandex.ru, https://orcid.org/0000-0003-1318-5078 (V.D.)
latanov@neurobiology.ru, https://orcid.org/0000-0001-7388-3914 (A.L.)
a_inozemtsev@mail.ru, https://orcid.org/0000-0002-5059-3241 (A.I.).

Abstract: *Aims.* To study the effect of CuO, ZnO and MoO3 nanoparticles on the vital functions of the single cell organism Paramecium caudatum; to analyse the activity of antioxidant cellular enzymes: SOD and CAT, as well as to evaluate the possibility of cells protection against oxidative stress by endogenous antioxidants. *Methods.* Paramecium caudatum cells were cultured under standard conditions for this test subject [3-4]. The toxicity of metal nanoparticles, PMCO (20-60 nm), PM ZnO (40-80 nm) and PM MoO3 (5-20 nm) (Laboratory of Physical Modelling of Two-Phase Flows, United Institute of High Temperatures, Russian Academy of Sciences) - was evaluated by determining the LC50 lethality index of the concentration at which 50% of Paramecium caudatum population was killed. Free-floating cells were then exposed for 48 h to sublethal concentrations of NPs metal oxides in moderately hard water with dissolved antioxidant. The efficacy of emoxipine (10 mg/ml), mexidol (50 mg/ml) ascorbic acid AA (25 mg/ml) and nicotinic acid NA (10 mg/ml) was investigated; for this purpose cell number, cell membrane condition, changes of intracellular organelle shape were recorded (Levenhuk C310 digital camera microscope, 3.1 Mpixel). *Results.* We found that the presence of metal oxides induced a significant decrease ($p < 0.05$) in the number of viable Paramecium caudatum cells, in 24 h there were 58 % deaths from CuO, 43 % deaths from ZnO and 40 % deaths from MoO3, but in 48 h - 92 %, 88 % and 75 %, respectively. Copper oxide PM were the most toxic to cells, with an LC50 of 25 mg/l; for ZnO PM and MoO3 PM, the lethal concentration was equal and higher than 50 mg/l. negative pressure of metal-containing NPs led to osmotic disorganization, which was accompanied by an increase in vacuoles, abnormal bending and membrane rupture. *Conclusions.* Our results demonstrated that oxidative stress is one of the leading mechanisms of toxicity of PM oxides of transition metals such as copper, zinc and molybdenum. When the single-celled organism Paramecium caudatum was exposed to the studied NPs, destructive damages of membrane structures, changes in functions and morphology of organelles leading to cell death were observed. It should be noted that under such abiotic stress, the viability of Paramecium caudatum in general is the result of a complex interaction of the cells' own antioxidant system as well as the specific characteristics of NPs (size and solubility) and additional environmental factors, such as the presence of compounds with antioxidant activity in it.

Keywords: molybdenum, zinc, copper oxide nanoparticles and synthetic antioxidants.

Citation: Karpukhina O., Dubova V., Latanov A., Inozemtsev A. Studying the redox status of paramecium caudatum cells under influence of molybdenum, zinc, copper oxide nanoparticles and synthetic antioxidants. Journal of Clinical Physiology and Pathology (JCPP) 2023; 2 (1): 42-45.

https://doi.org/10.59315/JISCPP.2023-2-1.42-45

Academic Editor: Igor Kastyro

Received: 27.02.2023
Revised: 07.03.2023
Accepted: 18.03.2023
Published: 30.03.2023

Publisher's Note: International Society for Clinical Physiology and Pathology (ISCPP) stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

The modern application of nanotechnology is accompanied by problems of nanosafety and the assessment of the impact of nanomaterials on human health and the environment. Among the various biological reactions used to assess the potential effects of nanoobjects on biota, the generation of reactive oxygen species (ROS), which disrupts the pro- and antioxidant equilibrium in cells and thus causes oxidative stress (OS), is currently the most accepted paradigm for the toxicity of various nanoparticles (NPs) to humans and the environment [1-2; 5]. Metal-containing

nanoparticles are used in various fields from technical to medical, for example, copper and zinc nanoparticles are widely used biocides and molybdenum nanomaterials also have antibacterial properties. It should be noted that molybdenum, zinc and copper are essential trace elements for living organisms and each of them has a specific function in the redox state of the cell. For example, molybdenum is a promoter of antioxidants, particularly ascorbic acid (AA). Copper and zinc are found in the cellular enzyme superoxide dismutase (SOD), a catalyst for the dismutation of the aggressive AFC superoxide. But in excess, these transition metal ions have a serious toxic effect on cells, primarily caused by OS.

To evaluate the ability of NM of these transition metals to cause OS in various ecologically relevant organisms, this work used biomarkers such as increased activity (SOD) and catalase (CAT), the most sensitive elements of the antioxidant system, the so-called first-line defense against AOS damage. In addition, since lipid molecules constitute approximately 30-80% of biological membranes by mass, lipid peroxidation in response to reactive oxygen species is a very likely scenario of OS, therefore, we additionally evaluated the condition of cell membranes by accounting for the secondary product of peroxidation, malondialdehyde MDA.

Our study was undertaken to better characterize the mechanism of action of metal-containing NPs at different levels of organization of living matter. The free-living freshwater infusoria Paramecium caudatum was chosen as a test object in this work, as this cell-organism provides an opportunity to study both cellular and organismal forms of response to chemical stressors simultaneously.

Aims. To study the effect of CuO, ZnO and MoO3 nanoparticles on the vital functions of the single cell organism Paramecium caudatum; to analyse the activity of antioxidant cellular enzymes: SOD and CAT, as well as to evaluate the possibility of cells protection against oxidative stress by endogenous antioxidants.

2. Methods

Paramecium caudatum cells were cultured under standard conditions for this test subject [3-4]. The toxicity of metal nanoparticles, PMCO (20-60 nm), PM ZnO (40-80 nm) and PM MoO3 (5-20 nm) (Laboratory of Physical Modelling of Two-Phase Flows, United Institute of High Temperatures, Russian Academy of Sciences) - was evaluated by determining the LC50 lethality index of the concentration at which 50% of Paramecium caudatum population was killed. Free-floating cells were then exposed for 48 h to sublethal concentrations of NPs metal oxides in moderately hard water with dissolved antioxidant. The efficacy of emoxipine (10 mg/ml), mexidol (50 mg/ml) ascorbic acid AA (25 mg/ml) and nicotinic acid NA (10 mg/ml) was investigated; for this purpose cell number, cell membrane condition, changes of intracellular organelle shape were recorded (Levenhuk C310 digital camera microscope, 3.1 Mpixel).

Activity of biomarkers SOD (by reduction of tetrazolium blue), CAT (by hydrogen peroxide reduction rate) and MDA (by Uchiyama-Michara method) was determined by spectrophotometric methods (SPECORD 50 plus). All data were statistically processed and valid (Statistica 12.6).

3. Results

We found that the presence of metal oxides induced a significant decrease ($p < 0.05$) in the number of viable Paramecium caudatum cells, in 24 h there were 58 % deaths from CuO, 43 % deaths from ZnO and 40 % deaths from MoO3, but in 48 h - 92 %, 88 % and 75 %, respectively. Copper oxide PM were the most toxic to cells, with an LC50 of 25 mg/l; for ZnO PM and MoO3 PM, the lethal concentration was equal and higher than 50 mg/l. It was shown in a number of works [1-2; 5] that metal oxide particles may deposit on cell wall causing mechanical damage; change of cell morphology, deformation of membranes and intracellular structures. In our experiments on Paramecium caudatum infusoria, negative pressure of metal-containing NPs led to osmotic disorganization, which was accompanied by an increase in vacuoles, abnormal bending and membrane rupture (Fig.). These results were consistent with the experimental literature [2; 5], which reported that in unicellular organisms, ZnO NPs increase membrane permeability, depolarize cells and/or perforate cell walls, allowing NPs to penetrate into the cytoplasm. The survival of Paramecium caudatum cells was dependent on the amount of NPs and their degree of aggregation within the cells. NPs were detected in the food vacuoles and cytoplasm but their accumulation varied depending on the size, concentration of NPs and time of exposure. The amount of accumulated PM in the cell was higher after 2 h of exposure than after 24 and 48 h. It is shown that the increase of CAT activity in the cells was observed after 2 h of incubation of Paramecium caudatum with metal-containing PM. This is due to the fact that catalase is considered a first-line

defense enzyme against the damaging effects of AFC. Indeed, catalase activity is the conversion of hydrogen peroxide (H2O2) into water and molecular oxygen (O2) initiated by the presence of exogenous components such as metal ions in the body.

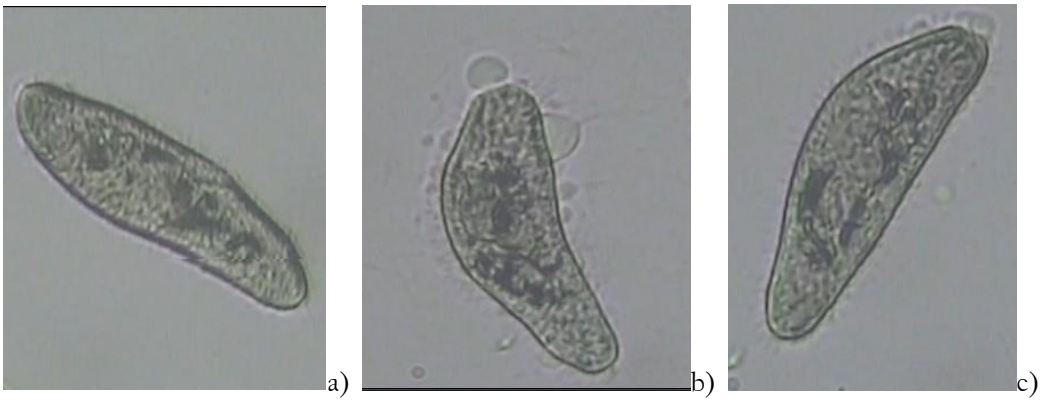

Figure. Changes in Paramecium caudatum cell shape: a - cell in normal condition; b - disruption of the outer cell membrane by ZnO NPs; c - cell condition in the presence of the antioxidant emoxipine

Increased concentrations of NPs metal oxides (50 mg/l) in the environment contributed to the development of OS. At the same time, the level of MDA increased, while the levels of SOD and CAT activity decreased by about half. This indicates an intensive process of free oxygen radical formation and related cell damage.

The addition of the synthetic antioxidant emoxypine to the experimental medium with a population of Paramecium cauda tum (Fig.) reduced the toxic effect of NPs metal oxides, and the same positive effect on cells was exerted by mexidol. It is indicative that the effect of PM on cells cultured in medium with emoxipin or mexidol, which was evaluated by MDA level, was 3.5 times lower than that of cells that were in medium with metal-containing PM only. Nicotinic acid in our experiments proved to be ineffective in protecting the cells from exposure to the metal oxide NPs under study.

Earlier by us [3-4] on cells of Paramecium caudatum in model of OS was determined a strict concentration dependence of pro/antioxidant effect of AA. It was found that AA in the presence of Cu+2 and Zn+2 inhibited the activity of infusoria and promoted the development of OS resulting in cell death. The results of experiments with nanosized particles of copper, zinc, and molybdenum were similar to those obtained earlier [3-4].

5. Conclusions

Our results demonstrated that oxidative stress is one of the leading mechanisms of toxicity of PM oxides of transition metals such as copper, zinc and molybdenum. When the single-celled organism Paramecium caudatum was exposed to the studied NPs, destructive damages of membrane structures, changes in functions and morphology of organelles leading to cell death were observed. It should be noted that under such abiotic stress, the viability of Paramecium caudatum in general is the result of a complex interaction of the cells' own antioxidant system as well as the specific characteristics of NPs (size and solubility) and additional environmental factors, such as the presence of compounds with antioxidant activity in it.

We have developed an approach to assess the impact of nanomaterials on the living organism by monitoring the most sensitive biomarkers of oxidative stress such as superoxide dismutase, catalase and malondialdehyde - characteristic indicators of chemical pollution.

The research was carried out within the framework of a scientific project under the state assignment of Lomonosov Moscow State University (subject No. 121032500080-8).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Chevallet G, Veronesi A, Fuchs E, Mintz I, Michaud-Soret A. Deniaud. Impact of labile metal nanoparticles on cellular homeostasis. Currentdevelopments in imaging, synthesis and applications Impact of labile metal nanoparticles on cellular homeostasis. Biochimica et Biophysica Acta 2017, 1861: 1566–1577.
2. Du J, Tang J, Xu S, Ge J, Dong Y, Li H, Jin M. ZnO nanoparticles: recent advances in ecotoxicity and risk assessment. Drug Chem Toxicol 2020, 43(3): 322–333.
3. Karpukhina OV, Gumargalieva KZ, Inozemtsev AN. The effect of antioxidant compounds on oxidative stress in unicellular aquatic organisms. Materials Science and Engineering: Physiochemical Concepts, Properties, and Treatments 2016, 323–329.
4. Morgunov I, Karpukhina O, Kamzolova S, Samoilenko V, Inozemtsev A. Investigation of the effect of biologically active threo-Ds-isocitric acid on oxidative stress in Paramecium caudatum. Preparative Biochemistry and Biotechnology 2018, 48(1): 1–5.
5. Moos N, Slaveykova V. Oxidative stress induced by inorganic nanoparticles in bacteria and aquatic microalgae – state of the art and knowledge gaps. Nanotoxicology 2014, 8(6): 605–630.