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Status of rats myocardium under subchronic mercury exposure and its pharmacological correction

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ABSTRACT

The article is devoted to the characteristics of the myocardium restructuring under the influence of 0.01 LD50 mercury (II) chloride, in rats, under subchronic exposure, and the correction of the detected changes. In the work, the structural and metabolic reorganization of myocardium was studied by way of general histological, histochemical and electron microscopic techniques. Herein, administration of Unithiolum reduces the severity of reactions to toxic effects of mercury salts: decreases the intensity of inflammation in the myocardium, improves microcirculation, connective tissue and mitochondrial membranes status and reduces cardiomyocytes apoptosis. Quercetinum has a normalizing effect on the myocardium structure, reduces energy imbalance in the myocardium, and stabilizes mitochondrial membranes. Both Unithiolum and Quercetinum promote intracellular ultrastructures recovery in cardiac myocytes and in endothelial cells, and enhance the regeneration of myofibrils.

INTRODUCTION

World-wide, today, cardiovascular diseases are considered a major problem. Annually, more than 9,4 million people worldwide die of these diseases; in Ukraine, statistically, the figure is more than 500,000 people [10]. The state of the cardiovascular system is affected both by lifestyle and environment [13,15]. One of the most dangerous pollutants, according to the WHO classification, is mercury [5,16], and the cardiovascular system is one of the main targets for the toxic effects of mercury and its compounds. Nowadays, searching for and finding pharmacological agents that could reduce the severity of pathological manifestations under exposure to heavy metals, remains an urgent problem [7].

The aim of this study was to evaluate both the morpho-functional changes of the myocardium under subchronic exposure to small doses of mercury (II) chloride, and the efficacy of cardioprotectors administration against a backdrop of mercury intoxication modeling. In our study, all the animal experiments were carried out in accordance with the Law of Ukraine "On protection of animals from cruelty" (2006), and the "General ethical principles of animal experiments", adopted by the First National Congress on Bioethics (Kyiv, 2001).

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MATERIALS AND METHODS

The experiments were carried out on 50 white male Wistar rats weighing between 100-150 g, with simulated toxic cardiomyopathy (using mercury (II) chloride solution at a dose of 0.01 LD50) brought about by intraperitoneal injection, as it was described earlier [12]. During the experiments, we studied the cardioprotective effect of Unithiolum (DMPS, Sigma-Aldrich) and/or Quercetinum (manufactured by PJSC SIC "Borshchahivskiy Chemical Pharmaceutical Plant", Ukraine). Unithiolum was chosen as it is a universal antidote in heavy metal poisoning. It contains two sulfhydryl groups and has low toxicity [4,6]. Quercetinum is nontoxic, having no cumulative effect. It affects key processes in pathogenesis of cardiomyopathies formation [11].

The animals were placed into five groups: one control group and four experimental that were subjected to subchronic intoxication against a backdrop of two-week exposure; such division allowed us to perform a correct comparative analysis. The groups of rats included: intact animals which were administered saline solution (C, control group, 10 animals), rats after subchronic exposure to a small dose of mercury (II) chloride without cardioprotective drugs administration (group I); rats under subchronic exposure to a small dose of mercury (II) chloride, with Unithiolum (0.01 mg/100 g of body mass) administration (group II); rats under subchronic exposure to a small dose of mercury (II) chloride, with Quercetinum (0.001 mg/100 g of body mass) administration (group III); rats under subchronic exposure to

a small dose of mercury (II) chloride, with both Unithiolum and Quercetinum administration in respective doses (group IV). The drugs were administered daily during two weeks following mercury (II) chloride exposure. The animals of both experimental and control groups were then withdrawn from the experiment under ether narcosis with subsequent decapitation. Their hearts were examined by histological, histochemical, morphometric and electron microscopic methods. Subsequently, statistical processing of the obtained numerical data occurred.

For the histological examination, the pieces of left ventricular myocardium were stained with hematoxylin and eosin, Weigert's and Heidenhain's iron hematoxylin, Van Gieson's picrofuchsin, and, afterwards, examined by way of an Olympus BX51 microscope on light-optical level. The images were taken using C-4040 zoom digital camera.

The purpose of the histochemical examination was to investigate the activity of succinate dehydrogenase, EC 1.3.5.1 (SDH) by Nahlas et. al. Method; L-lactate dehydrogenase, EC 1.1.1.27 (LDH) by Gress, Scarpelli and Pierce method; and NADH dehydrogenase, EC 1.6.5.11 (NADH-DH) and NAD(P)H dehydrogenase, EC 1.6.5.2 (NADPH-DH) by Farber method. Moreover, the McManus PAS reaction was performed [14]. The intensity of the histochemical reactions was determined in activity units (a.u.) by semi-quantitative assay.

Ultrathin sections were obtained by means of utilizing a LKB ultratome III (Sweden) and a Reichert microtome (Sweden), and were contrasted by way of saturated 2% uranyl acetate and lead citrate. The slides were examined under the transmission electron microscope (TEM-125K, Ukraine).

For morphometric studies, we used the "Organelle" program developed by the Electron microscopy laboratory of the Institute of pathology problems of O.O. Bogomolets National Medical University. In the left ventricular cardiomyocytes, according to the principles of stereometric analysis [2], we measured mitochondrial volume (%) and quantitative (10^{-2} μm^2) density, the average area (10^{-2} μm^2) of mitochondria, the mitochondrial form factor, the average number of cristae in one mitochondrion, the cristae quantitative density (%), total (μm) and average (μm) length of cristae membranes in one mitochondrion, and the distribution of mitochondria (in area) in cardiomyocytes.

Statistical analysis of the results was conducted by means of Statistica for Windows 6.0 package (Microsoft Corporation, USA) using parametric and nonparametric methods of results evaluation. The differences with a significance level of more than 95% ($p < 0.05$) were considered to be reliable.

RESULTS AND DISCUSSION

The structure of the rats myocardium after subchronic exposure to small doses of mercury (II) chloride is characterized by signs of hypertrophy of the majority of cardiomyocytes, tendency to increased collagen formation in the intramuscular and perivascular interstitial compartments, focal expansion of the lumen of blood vessels, perivascular edema, increased blood supply to the microvessels and thickening of the walls of the intramural coronary arteries. Ultramicroscopic examination revealed shrinkage of myofibrils and damage to many mitochondria. The energy metabolism was also impaired: SDH and NADH-DH activity was significantly reduced, whereas LDH activity increased.

Having been administered Unithiolum, the rats with subchronic intoxication demonstrated a decreased level of pathological changes in their myocardial structures. Moreover, their myofibrils turned to be clear-cut and stained with acid dye. Through Heidenhain staining, single cardiomyocytes modified by contracture and cells with myofibrils swelling were identified. Vessels of hemomicrocirculatory bed had more even lumen diameter, and the morphological signs of endotheliocytes damage were less significant than in the group without drug correction. In addition, well-preserved middle-sized mitochondria were noted as being located among sarcomeres, some of them having hypertrophied forms that were confirmed by way of morphometric analysis. In this study group, we also noted the existence of phagosomes with lipid inclusions and inactive lysosomes. Moreover, granules of abnormal proteins, damaged by low dose mercury, had significantly smaller dimensions. Administration of Unithiolum was of great importance, as it did not only prevented the destruction of mitochondria, but contributed to their quantity increase (Table 1).

Table 1. Morphometric parameters (M±m) of mitochondria in cardiomyocytes of rats after subchronic exposure to small doses of mercury (II) chloride

Groups	Mitochondrial volume density, %	Mitochondrial quantitative density, 10^{-2}/μm^2	Mitochondrial sections area, 10^{-2} /μm^2	Mitochondrial form factor
C (n=10)	30.3±2.9	51.1±5.2	41.3±2.0	0.81±0.02
I (n=10)	12.6±3.5*	15.9±3.8*	53.6±3.8*	0.80±0.01
II (n=10)	25.7±3.3	27.0±3.6	57.8±3.5*	0.80±0.10
III (n=10)	21.6±2.4*,#	37.4±5,3#	49.5±3.0*,#	0.74±0.01*,#
IV (n=10)	25.8±3.0#	48.4±6.4#	45.9±2.2#	0.79±0.01

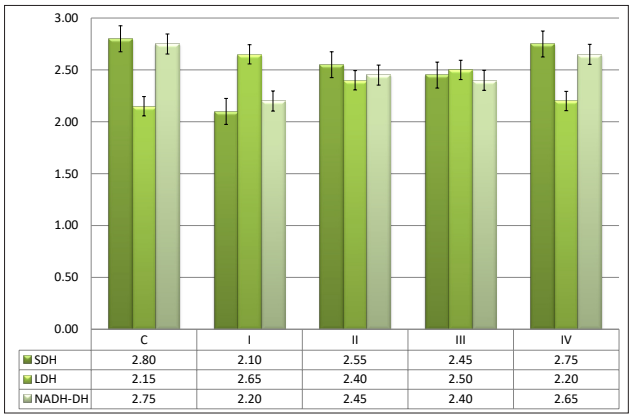
Note:
* - significant differences in relation to control ($p < 0.05$); # - significant differences in relation to group of rats after exposure to small dose of mercury (II) chloride without cardioprotective drugs administration ($p<0.05$)

Table 2. Morphometric parameters (M±m) of cristae in cardiomyocytes mitochondria of rats after subchronic exposure to small dose of mercury (II) chloride

Groups	Quantity of cristae in one mitochondrion	Quantitative density of cristae, %	Total length of cristae membranes in one mitochondrion, μm	Average length of crista membrane, μm
C (n=10)	26.9±1.1	5.4±0.9	48.6±3.6	18.8±0.2
I (n=10)	19.7±3.3*	2.4±0.7*	34.0±3.0*	10.6±0.2*
II (n=10)	21.9±2.1*	4.8±0.7*	41.5±2.7#	16.3±0.4#
III (n=10)	33.2±2.1*,#	2.4±0.7*	35.3±2.9*	11.0±0.3*
IV (n=10)	23.3±2.5	5.0±0.6#	45.2±3.0#	15.6±0.4*,#

Note:
* - significant differences in relation to control ($p < 0.05$); # - significant differences in relation to group of rats after exposure to small dose mercury (II) chloride without cardioprotective drugs administration ($p<0.05$)

Administration of Unithiolum (group II) also resulted in normalization of energy metabolism; we observed the significantly increased activity of SDH and NADN-DH, and decreased activity of LDH (Fig. 1), in comparison to corresponding parameters of the studied rats without cardioprotection (group I).



	C	I	II	III	IV
SDH	2.80	2.10	2.55	2.45	2.75
LDH	2.15	2.65	2.40	2.50	2.20
NADH-DH	2.75	2.20	2.45	2.40	2.65

Figure 1. Histochemical parameters (M±m) of redox enzyme activity in the rats myocardium

The myocardium examination after administering Quercetinum showed that the drug had partially prevented pathological changes under subchronic exposure to low doses of mercury (II) chloride. After Quercetinum administration, however, some morphological signs of myocardial pathology were preserved both in the bloodstream, interstitium and in the cardiomyocytes; there was a combination of microscopic signs of edema, contracture and degenerative changes. Still, the blood capillaries maintained their structural organization, but with thinned endothelium; there was an uneven narrowing of the lumen in some capillaries.

In the cardiomyocytes, we observed the activation of protein synthesis processes, well developed granular endoplasmic reticulum and accumulation of free ribosomes; however, there were altered myofibrils contracture and dystrophically modified mitochondria; also lysis of the sarcolemma and somewhat increased amount of micropinocytotic vesicles were present.

In the case of Quercetinum administration, we observed mitochondrion with preserved external membrane and cristae in cardiomyocytes. There was an increased amount and decreased average area of mitochondrial sections, when compared to the mitochondria of cardiomyocytes in animals without correction, due to an increased number of small and large forms in relation to the distribution in animals without pharmacological intervention (Fig. 2).

Beyond the aforementioned, the total length of cristae in one mitochondrion was increased, in comparison to both untreated and control animals. In addition, histological examination showed Quercetinum to bring about a decrease in the intensity of cardiomyocytes energy disturbances (Fig. 1). Literature overview suggests this drug to inhibit phosphodiesterase activity and contribute to the accumulation of the main intracellular mediator, i.e. cyclic adenosine monophosphate (cAMP) in the cells [9].

Histological examination of rats myocardium in combined administration of Unithiolum and Quercetinum following subchronic exposure to small doses of mercury (II) chloride has shown a significant decrease in the degree of myocardial damage. The structure of the vessel walls of the hemomicrocirculatory bed and the perivascular connective tissue in its structure were similar to those in the control group, and were only slightly locally changed due to a small focal edema of endothelium and basement membrane.

Electron microscopic examination revealed a thinning of the endothelial lining, a significant number of micropinocytotic vesicles and the preservation of the integrity of the cellular components. There were also signs of edematous swelling of cardiomyocytes with altered myofibrils contracture and solitary hyperchromatic and pycnotic nuclei. Muscle fibers were stained moderately with acid dye, the nuclei of majority of cells were elongated, and somewhat enlarged with predominant euchromatin. Moreover, some of them had deep invaginations of the nuclear membrane and paranuclear cytolysis. We observed mitochondria of different forms and size in combined therapy with Unithiolum and Quercetinum. Combined administration of Unithiolum and Quercetinum did not result in complete restoration of mitochondrial quantitative parameters, however, they were approaching those of the control.

Quantitative density and the total length of the cristae membranes in one mitochondrion were also close to that of the control values. Such parameters confirmed a clear tendency towards normalization of energy metabolism in the myocardium in the case of combined administration of Unithiolum and Quercetinum, as well as reduced signs of tissue hypoxia. We also observed the level of activity of the terminal oxidation enzymes to approach the control

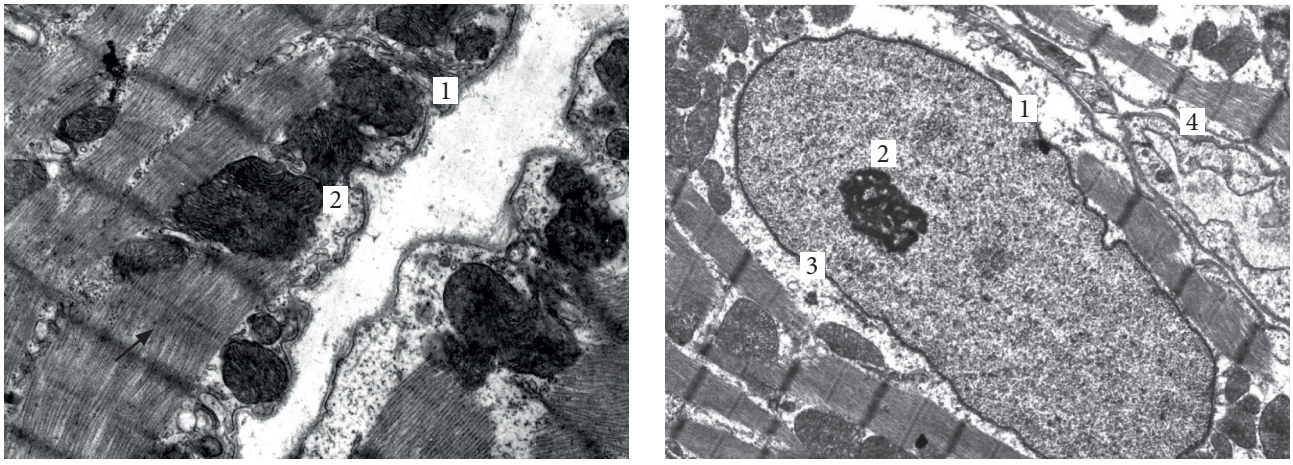


Figure 2. Left ventricular myocardium of rats under subchronic mercury (II) chloride exposure and pharmaceutical protection with Quercetinum (on the left: T-tubule system (#) smooth endoplasmic reticulum (1), mitochondria (2)) and with both Unithiolum and Quercetinum (on the right: neucleus (1) with neucleolus (2), mitochondria (3), myofibrils (4)). Electron microscopic picture. Magnification: 16000 and 14000

parameters. Herein, activity of SDH and NADN-DH were of the highest value (group IV), while, the activity of LDH decreased, in comparison to the group without cardioprotection (group I).

Quercetin is a flavonol with anti-inflammatory and antioxidant effects, and is also an activator of the peroxisome proliferator-activated receptor γ coactivator 1α that is capable of antioxidant upregulation, mitochondrial biogenesis and prevention of cardiac complications [3]. Several authors have reported that Quercetinum administration as feed has increased in young mice, mitochondrial biomarkers, antioxidant protein and utrophin, and brought about a decreased matrix metalloproteinase. Given that these adaptations are associated with attenuated cardiac pathology and damage, the present findings may indicate that dietary quercetin enrichment attenuates dystrophic cardiac pathology.

Our results demonstrate Quercetinum to be antioxidant and membrane stabilizing, as well as a cell regeneration stimulating drug, while Unithiolum was seen to be a universal antidote [1,8].

CONCLUSIONS

1. Under subchronic exposure to small doses of mercury (II) with Unithiolum administration, we observed the activation of regenerative processes in the myocardium.
2. Under subchronic simulation of cardiomyopathy with a small dose of mercury (II) chloride, Quercetinum had a membrane stabilizing effect on myocardium; its administration resulted in the improvement of the morphological and functional state of mitochondria.
3. Combined administration of Quercetinum and Unithiolum as cardioprotectors following the exposure to small dose of mercury (II) chloride brought about the most significant decrease of intensity of cardiomyocytes energy and structural impairments.

REFERENCES

1. Aaseth J. et al.: Chelation in metal intoxication-Principles and paradigms. *J Trace Elem Med Biol*., 31, 260-266, 2015.
2. Avtandilov G.G. (1990): *Medical morphometry*. Guide. Medicine. (in Russian)
3. Ballmann C. et al.: Histological and biochemical outcomes of cardiac pathology in mdx mice with dietary quercetin enrichment. *Exp Physiol*, 100, 12-22, 2015.
4. Bernhoft R.A.: Mercury toxicity and treatment: a review of the literature. *J Environ Public Health*,. 2012, 2012.
5. Busch J. et al.: The heavy metals cadmium, lead and mercury in raw materials of animal origin: evaluation of data from practice. *Pharm Bio Sci Notes*, 2015, 150-165, 2015.
6. Cao Y. et al.: Chelation therapy in intoxications with mercury, lead and copper. *J Trace Elem Med Biol*., 31, 188-192, 2015.
7. Dziegiel P. et al.: Metallothioneins: Structure and Functions. *Adv Anat Embryol Cell Biol*, 218, 1-117, 2016.
8. Figueredo F.G. et al.: Cytoprotective Effect of Lygodium venustum Sw. (Lygodiaceae) against Mercurium Chloride Toxicity. *Scientifica* (*Cairo*). 2016, 4154265, 2016.
9. Foras L.D.: Quercetin in treatment cardio-vascular. *The radical Research*., 39, 2005.
10. Graham I. et al.: Fourth joint task force of European society of cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). European guidelines on cardiovascular disease prevention in clinical practice: full text. Fourth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). *Eur J Cardiovasc Prev Rehabil*., 14, 2, S1-113, 2007.
11. Jain A.K., Mehra N.K., Swarnakar N.K.: Role of Antioxidants for the Treatment of Cardiovascular Diseases: Challenges and Opportunities. *Curr Pharm Des*., 21(30), 4441-4455, 2015.
12. Kaminsky R. et al.: A study of impact of mercury chloride on myocardium in experiment. *Georgian Med News*., 251, 64-70, 2016.
13. Kim K.H. et al.: A review on the distribution of Hg in the environment and its human health impacts. *J Hazard Mater*, 306, 376-385, 2016.
14. Pierce E. (1962). *Histochemistry theoretical and applied*. M Moscow: Univ. foreign. Literature.
15. Rafati-Rahimzadeh M. et al.: Current approaches of the management of mercury poisoning: need of the hour. *Daru*, 22, 46, 2014.
16. Sokurenko L.M. et al.: Mildronate protects neuroblasts against toxic influence of mercuric chloride in cell culture. *Neurophysiology*, 46(3), 271-273, 2014.