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CARDIOVASOTOXIC EFFECT OF DIFFERENT SIZES LEAD NANOPARTICLES INTRODUCTION

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Cardiovascular toxicity of Pb compounds NP (nanoparticles) 26-34 nm and 50-80 nm in size was investigated in the experiments. Morphological and morphometric methods were used to detect structural changes in the heart and aorta after 30 and 60 injections of Pb compounds NP. The results of the experiments indicate damage to the myocardium, which consists in increasing of the interstitial space between the fibers of cardiomyocytes, dystrophy of cardiomyocytes, stasis of blood in the microvessels of the heart` ventricles. A greater sensitivity of the atrial myocardium to the toxic effects of Pb compounds NP was detected. Structural changes of the aorta consisted in a stratification of elastic membranes, a decrease in the density of the connective tissue of the adventitious layer, which tended to progressive changes.

Key words: lead, Pb, nanoparticles, morphological changes, aorta, myocardium.

The work is a fragment of the research project "Investigation of the heavy metal nanoparticles toxic effects, search and substantiation of preventive measures", state registration No. 0116U000497, and "Changes in internal organs and regulatory systems under the conditions of experimental damage and historical aspects of histology, cytology and embryology development in Ukraine", state registration No. 0116U000121.

The development of new technologies and nanomaterials is a prerequisite for technological advancement in various industries [7]. The significant increase in environment and workspaces pollution by heavy metal nanoparticles, in particular the lead PbS NP (nanoparticles), one of the most common and highly toxic metals, is worrying [10]. The small size, shape, chemical composition, charge, structure of NP as well as the large surface area determine their unique properties as a promising material for the manufacture of temperature-sensitive sensors, detectors, photoresistors, selective sensors; in flexible optoelectronics - as high-performance photodetectors; in the third generation solar elements as quantum dots, which greatly increases the efficiency of solar energy conversion; as well as in the composition of various polymer films and nanoporous matrices, abrasive treatment of lead materials, etc [12, 14].

Lead compounds are characterized by pronounced neurotoxicity, hepatotoxicity, nephrotoxicity, gonadotoxicity and cardiotoxicity and are indicated by blood indices [6]. Lead can have direct and indirect toxic effects on cardiovascular system [13]. Experimental studies have shown its high affinity to be accumulated in blood vessels, stimulate the production of reactive oxygen species, the development of oxidative stress and impaired nitric oxide metabolism, resulting in the development of endothelial dysfunction [15].

The nanoscale and the properties of the surfaces of NP allow coming into direct contact with proteins and individual cell structures of the body at the molecular level. Overcoming cellular barriers and damaging cell structures, NP can disrupt functions and even lead to cell death of all organs and tissues. At the same time, numerous experimental data do not give an unambiguous answer to the dangerous effects of NP on human health, since NP toxicity depends on their characteristics (including size, method of stabilization, etc.) [2]. This determines the relevance of the study of the effects of nanoparticles on the body and the assessment of potential risks

The purpose of the study was to examine features of morphological changes in the organs of the cardiovascular system under the action of lead sulfide nanoparticles of different sizes and lead nitrate (ionic form) in the experimental model of intoxication.

Materials and Methods. In this work lead compounds are used in nanoform: NP of lead sulfide (PbS NP) with 26-34 nm and 50-80 nm average size and in ionic form: lead nitrate ($Pb(NO_3)_2$) which is readily soluble in water. Lead sulfide NP were obtained by chemical synthesis using a sodium polyphosphate stabilizer $(NaPO_3)_n$. NP size was determined by electron microscopy.

The study was conducted on mature male Wistar rats 160-180 g. The animals were kept in vivarium on a standardized diet with free access to drinking water. The rats were divided into 3 experimental groups and control group. The first experimental group was injected with 26-34 nm PbS NP ($PbS_{26-34nm}$ NP group), the second – 50-80 nm PbS NP ($PbS_{50-80nm}$ NP group), the third – with $Pb(NO_3)_2$ ionic form ($(NO_3)_2$ group); the control group received a physiological solution. These substances were administered intraperitoneally daily 5 times a week (simulation of a working week) in 0,94 mg/kg/day dose adjusted to lead [1]. Histological examinations were performed after 30 injections (1.5 months) and 60 injections (3 months). To study the morphological changes, the internal organs of the experimental rats were taken after decapitation under mild ether anesthesia. The heart and aorta were fixed in 10% neutral formalin (phosphate buffer, PBS). The fixed frontal tissues were dehydrated and embedded in paraffin. Paraffin sections were made on a Thermo Microm HM 360 microtome. The sections were deparaffinized and stained with hematoxylin and eosin, using Picro-Mallory method, which can reveal collagen. The micropreparations were examined on an Olympus BX51 microscope. Morphometric analysis was performed using Carl Zeiss software (AxioVision SE64 Rel.4.9.1), magnification × 200, × 400. Aorta wall thickness (mkm), tunica adventitia of aorta thickness (mkm), comparative amount of collagen fibers in tunica adventitia (%), number of elastic membranes in tunica media (conventional units) were examined. The statistical study was performed in Origin Lab version 8.0 using the non-parametric Kruskal-Wallis test, becous normal distribution of data was not proven. Data are presented as medians with smaller and larger quartiles (M [Q1-Q3]). The difference was considered statistically significant at $P < 0.05$.

All manipulations with animals were carried out in accordance with the provisions of European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1985). The study meets bioethical requirements. The experiment plan is approved by the Bioethics Commission of State Institution "Kundiiev Institute of Occupational Health of the National Academy of Medical Sciences of Ukraine" (Minutes № 5, session of bioethics commission from 23.11.2017).

Results of the study and their discussion. In all tested heart samples of the control and experimental groups, the overall morphological structure of the heart was preserved. The endocardium, myocardium, and epicardium were recorded in the ventricles and atria. In the control group, the myocardium is represented by densely oriented fibers of cardiomyocytes. Cardiomyocyte nuclei and intercalated discs are recorded. There is almost no intercellular space between the fibers. Morphological differences were found in myocardium of the atria and ventricles: in the ventricles the fibers are oriented more straightforwardly, whereas in the atria they are often tortuous (nuclei of cardiomyocytes in the atria are larger). There are microvessels (arterioles, venules, capillaries), in the myocardium, their density is higher in the ventricles.

Morphological features of structural disorders of the heart were established in the experimental groups (Fig. 1). In the endocardium of the hearts of all 3 groups after 30 and 60 injections, no structural changes were detected; endotheliocytes and structurally preserved valves (including the aortic valve) were recorded. The epicardium is morphologically represented by a thin layer of connective tissue with elastic elements, as in the control groups, without structural changes. The ventricular and atrial myocardium was heterogeneous, like in the control, had a greater thickness and density in the ventricles, represented by bundles of cardiomyocytes, between which thin layers of collagen fibers were detected (when stained by the Picro-Mallory method). The density of collagen fibers was higher around medium- and large-caliber myocardial vessels, but without a clear difference in control groups. Some morphofunctional differences were found between the comparison groups. Thus, after 30 injections of $Pb(NO_3)_2$, less collagen fibers were detected in the ventricular myocardium, whereas no qualitative changes were observed in the atria compared to the control. In all 3 experimental groups, a lower density of cardiomyocyte nuclei was observed in the investigated areas of the ventricles, as well as a decrease in the tinctorial properties of the cardiomyocytes cytoplasm in the atria and dystrophic changes at the cellular level, in particular destructive contractile myocyte processes, this is an evidence of toxic effects both PbS NP and $Pb(NO_3)_2$.

After 60 injections, structural changes in the myocardium of experimental group animals were more pronounced. Increased interstitial space between cardiomyocyte fibers, stasis in ventricular myocardial microvessels, atrial damage, and dystrophic cardiomyocyte changes were observed. In the group with expose $PbS_{50-80,}$ local dystrophic myocardial changes (destructive changes of cardiomyocytes with loss of histological structure of the myocardium) were established between the ventricle and the atria. In the atrial epicardium of group with expose $Pb(NO_3)_2$, focal accumulation of mast cells was detected, which could indicate their infiltration / migration, but no other types of leukocytes (neutrophils,

lymphocytes, etc.) were detected. In general, in all experimental groups progressive damage to the myocardial cardiomyocytes, to a greater extent in the atria, was revealed.

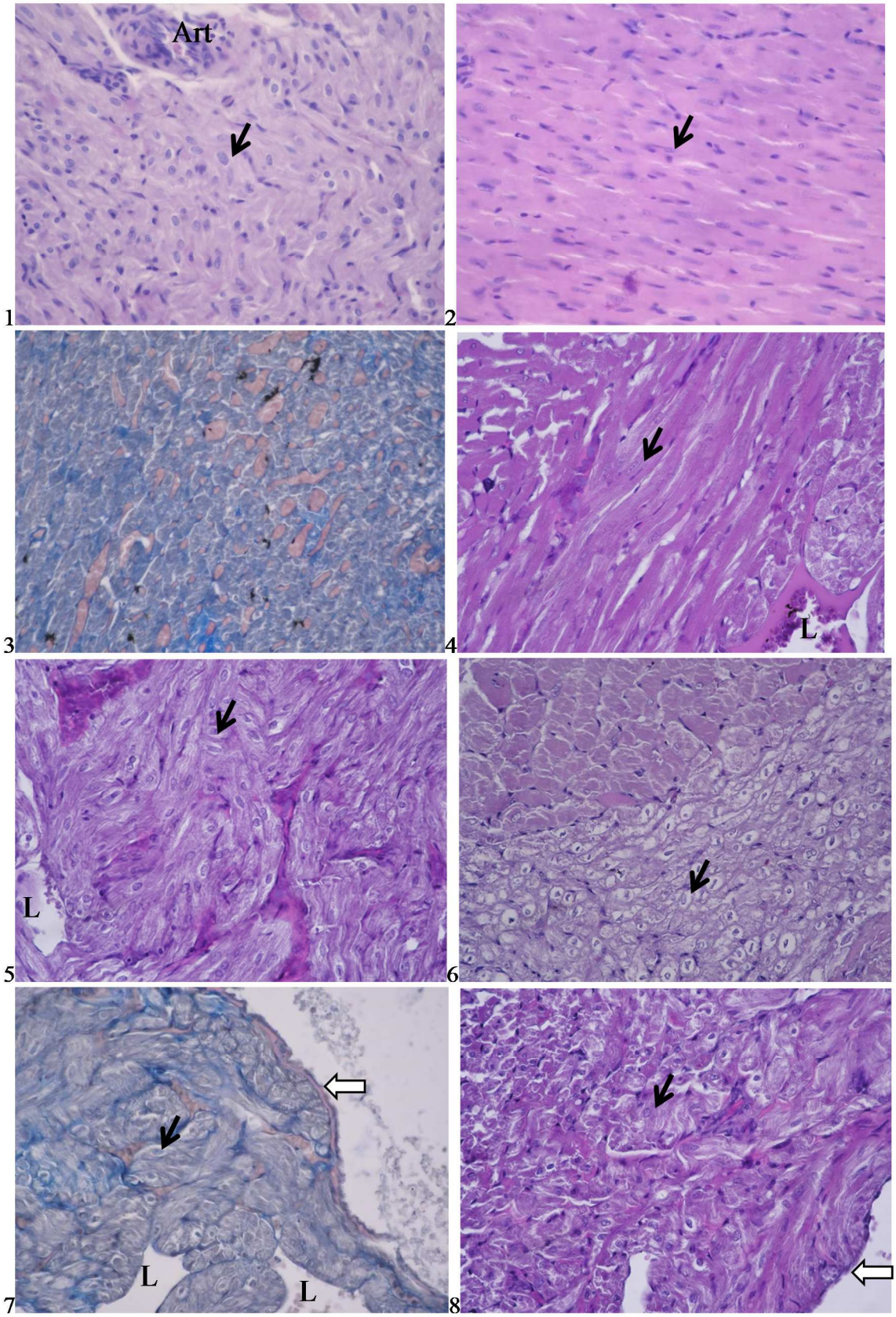

Fig. 1. Damage to the myocardium of the rat's heart against the background of exposure to Pb compounds. Significant structural changes in the atrial myocardium and partial damage to the fibers of the cardiomyocytes in the ventricle, blood filling, erythrocytic stasis of the microvessels. Increasing interstitial space between cardiomyocytes. Note: 1, 2 - atria and ventricle of the control group; 3, 4 - ventricle of $PbS_{26\text{-}34nm}$ NP group after 30 and 60 injections; 5, 6 - atria of the $PbS_{50\text{-}80nm}$ NP group after 30 and 60 injections; 7, 8 - atrium of $Pb(NO_3)_2$ group after 30 and 60 injections: cardiomyocyte nuclei; ⇦ the epicardium; Art - artery; L - atrial / ventricular lumen. Hematoxylin-eosin (1, 2, 4, 5, 6, 8), Picro-Mallory (3, 7): obj. 40, oc. 10.

In the control and experimental groups, the histological structure of the aortic wall had similar morphological features. All three tunicae were registered – inner (intima), middle (media) and outer (adventitia). In the control group, the elastic membranes of the tunica media were tightly oriented, with nuclei of smooth muscle cells registered between them (fig. 2). The adventitia was represented by a fibrous connective tissue; adipose tissue was often detected around the aorta. The results of aortic wall morphometry are shown in table 1.

After 30 injections of $PbS_{26\text{-}34nm}$ and $PbS_{50\text{-}80nm}$ NP group, a decrease in rats' aortic wall thickness as well as in connective tissue density in the adventitious layer was detected. After 30 injections of $Pb(NO_3)_2$, on the contrary, a local increase in the density of collagen fibers and fibroblasts was observed (the connective tissue density in the adventitia of the $PbS_{50\text{-}80nm}$ NP group was lower). The morphometric study confirmed the morphological changes: the thickness of the aortic wall statistically significantly decreased in both PbS NP groups, compared to control. In $PbS_{50\text{-}80nm}$ NP group, the thickness of the adventitial layer is less than that of $PbS_{26\text{-}34nm}$ NP group and $Pb(NO_3)_2$ (Table 1). In the $Pb(NO_3)_2$ group, quantitative and morphological changes in aorta were less pronounced (wall thickness decreased, but the relative content of adventitia remained at the level of statistical error). Slight reduction in the number of elastic membranes in tunica media of aorta is observed in all groups, especially with $PbS_{26\text{-}34nm}$ NP and $Pb(NO_3)_2$ expose.

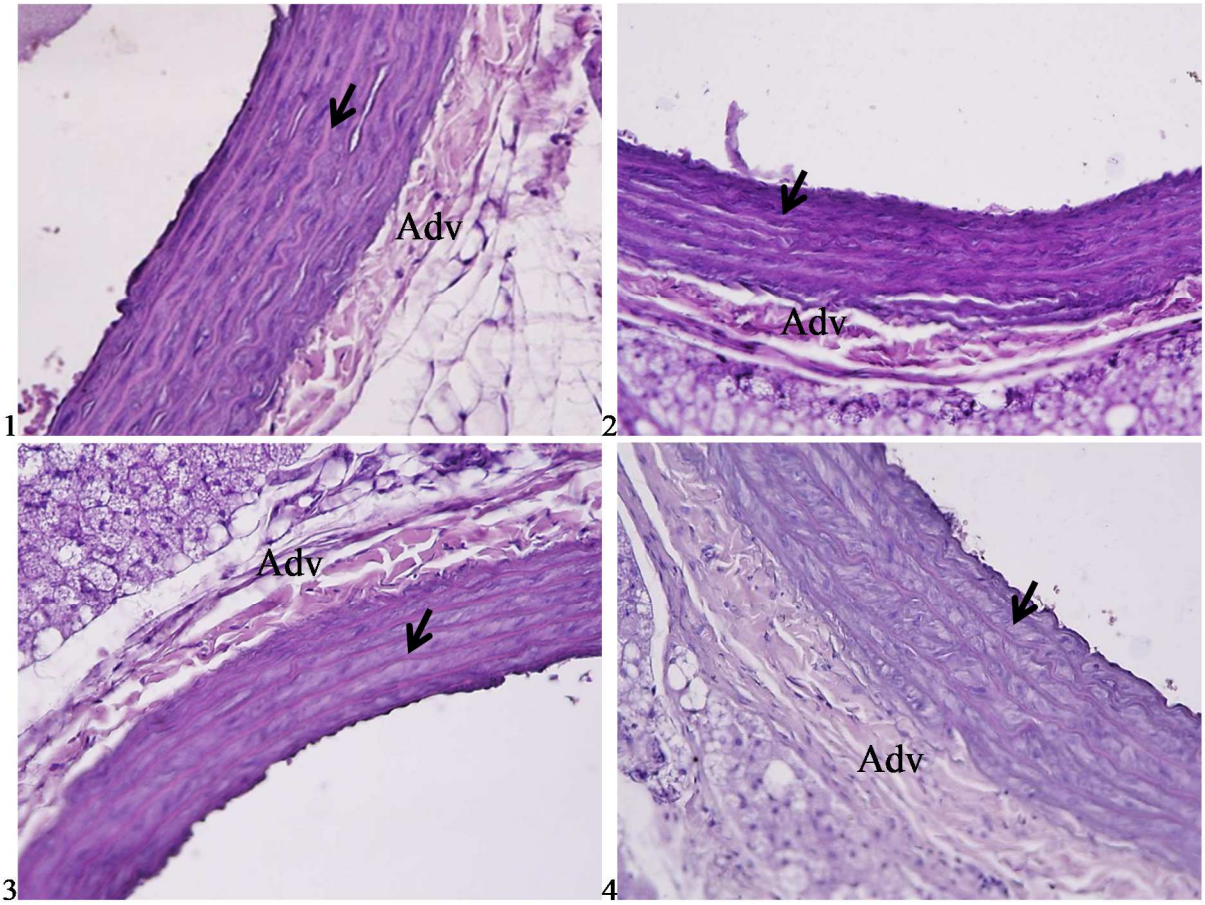

Fig. 2. Structural changes of the aorta after 60 injections of Pb compounds. Changes in the density of structural elements in the tunica media. Note: 1 - control group; 2 - $PbS_{26\text{-}34nm}$ NP group; 3 - $PbS_{50\text{-}80nm}$ NP group; 4 - $Pb(NO_3)_2$; ← elastic membranes of tunica media; Adv - adventitia. Hematoxylin-eosin, obj. 40, oc. 10.

After 60 injections, all structural samples showed similar structural changes as in the previous term, as well as the tendency of decreasing density of the structural elements of the adventitia after the introduction of Pb. Additionally, focal disorganization of the tunica media (delamination, edema, reduction of cell nuclei between elastic membranes) and areas of reduced density of elastic membranes were revealed in all experimental group. Especially these manifestations are significant in $PbS_{26\text{-}34nm}$ NP and $Pb(NO_3)_2$. experimental animal groups. After 60 injections of $PbS_{50\text{-}80nm}$ NP, the tendency of the aortic wall thickness to increase due to the development of structural changes (loosening of collagen fibers) of the adventitial layer and elastic membranes in the tunica media was established.

Currently, there is insufficient data on the toxic effects of lead NPs on the cardiovascular system, so it is important to study the features of cardiovascular toxicity in order to prevent possible negative effects

of lead nanoparticles on the health of the population. Ferreira de Mattos G. et al. explain the morphofunctional disorders of the heart in people affected by lead poisoning, disorders of cardiac excitability, the appearance of a negative inotropic effect, and increased diastolic tension [5]. But debatable is the question of the dependence of lead NP toxicity on their size. Thus, Qingzhao et al. express a view of the dependence of a greater degree of lung lesion by NP of smaller size, in particular 30 nm [11]. Luhovsky S.P. et al. found that 12.5 nm PbS NP compared to 100 nm NP have a greater area of interaction with the structural elements of the skin and thus increases the permeability through the skin and toxic effects on the liver, kidneys and heart [9]. It is already established that Pb NP accumulate in tissues, crystallize, are phagocytozed by siderophages, causing hydropic dystrophy of organ cells having dose-dependent toxicity [3]. Lebedová J. et al. found that lungs and kidneys are more sensitive to Pb NP than liver and brain, and Pb NP toxicity was correlated with oxidation and decreased glutathione [8]. The latter is explained by the development of oxidative stress and a decrease in the activity of antioxidant enzymes, which leads to dystrophic and proteolytic processes [11]. In our own experiments, we found damage to the myocardium and the aorta wall due to the action of Pb compounds in nanoscale and ionic form, and observed a tendency for progressive morphological changes after 60 injections, which was manifested by a decrease in the number of nuclei of cells in the walls of some of medium caliber vessels in myocardium and blood filling of ventricle capillaries. The decreasing of the density of cardiomyocyte cytoplasm (in the inner layers of the myocardium, while the outer layers remained more compact) may indicate a damage of the cardiomyocytes contractile elements and their destruction against the background of NP Pb exposure.

Table 1

Results of morphometric investigation of the aortic wall in control and experimental groups (Me [Q1-Q3])

Group	Aorta wall thickness, mkm	T.adv. thickness, mkm	Comparative amount of collagen fibers in t.adv., %	Number of elastic membranes in t.media, conv. un.
Control group	230.1 [220.7-240.7]	97.3 [85.3-110.7]	42.2 [38.3-46.1]	11 [10.2-11]
$PbS_{26-34nm}$ NP – 30 injections	199.0 [185.5-220.0] *	70.2 [63.8-71.9] *	35.3 [29.2-38.8] *	8 [8-9]
$PbS_{50-80nm}$ NP – 30 injections	193.4 [171.9-200.4] *	57.1 [42.1-60.8] * P<0.05 до Pb1. Pb3	29.3 [24.5-30.4] * P<0.05 до Pb1	9 [9-9]
$Pb(NO_3)_2$ – 30 injections	193.2 [171.6-205.4] *	85.9 [67.0-99.6]	44.6 [38.0-47.7]	8 [8-9]
$PbS_{26-34nm}$ NP– 60 injections	183.2 [176.2-201.8] *	51.1 [46.1-62.6] *	28.4 [25.0-31.8] *	9[9-9]
$PbS_{50-80nm}$ NP – 60 injections	215.5 [200.1-223.3]	82.8 [60.6-96.3] P<0.05 до Pb1	38.3 [30.2-43.1]	9 [8-10]
$Pb(NO_3)_2$ – 60 injections	197.0 [192.1-198.2] *	68.2 [56.4-72.1] *	34.6 [29.3-36.3] *	8[8-8]

Note: * - statistically significant differences compared to control (P <0.05)

Our results are confirmed by other authors. Thus, dystonic changes of arteries and capillaries (paretic enlargements and spasmodically constricted vessels), blood stasis in capillaries, red blood cells aggregation and development of cardiomyocyte dystrophy in the form of appearance of cells with signs of their contractural damage were revealed [9]. One of the long-term effects of lead toxicity on cardiovascular system is the restructuring of the connective tissue of the aorta, capillaries, and myocardium, which is identical to changes in accelerated aging of these organs. As a result of the damage to an elastic carcass of vessels there was development of aortic delamination and its aneurysm [4]. Disorganization of the aortic tunica media was detected in the $PbS_{50-80nm}$ and $Pb(NO_3)_2$ groups, as well as changes in connective tissue in all study groups. According to the results of the morphometric study after the introduction of Pb NP there was found statistically significant decrease of the thickness of its wall, after 30 and 60 injections (an average of 15.6% and 14.3%), which is associated with a decrease in the thickness of the adventitious layer and a slight reduction in the number of elastic membranes in its tunica media. This may indicate a suppression of the development (morphogenesis) of the connective tissue against the background of Pb NP exposure.

Conclusion

Prolonged administration of $PbS_{26-34nm}$ NP and $PbS_{50-80nm}$ NP groups to rats caused damage to the myocardium and structural changes of the aorta wall, which consist in cardiomyocyte dystrophy,

delamination of the elastic membranes of tunica media in aorta, and a decrease in the content of connective tissue in tunica adventitia.

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Реферати

КАРДІОВАЗОТОКСИЧНИЙ ЕФЕКТ ВВЕДЕННЯ НАНОЧАСТНОК СВИНЦЮ РІЗНИХ РОЗМІРІВ

Губар І.В., Лавриненко В.Є., Чухрай С.М., Савосько С.І., Сокуренко Л.М., Апихтіна О.Л., Яворовський О.П.

У експериментах досліджували кардіовазотоксичну дію НЧ (наночастинок) свинцю розміром 26-34 нм та 50-80 нм. Морфологічні та морфометричні методи були використані для виявлення структурних змін серця та аорти через 30 і 60 введень НЧ Pb. Результати експериментів вказують на пошкодження міокарду серця, які полягають у збільшенні інтерстиційного простору між волокнами кардіоміоцитів, дистрофії кардіоміоцитів, стазу крові у мікросудинах шлуночків серця. Виявлено більшу чутливість міокарду передсердь до токсичної дії НЧ Pb. Структурні зміни аорти полягали у розшаруванні еластичних мембран, зменшенні щільності сполучної тканини адвентиційної оболонки та мали тенденцію до прогресуючих змін.

Ключові слова: свинець, Pb, наночастинки, морфологічні зміни, аорта, міокард.

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КАРДИОВАЗОТОКСИЧЕСКИЙ ЭФФЕКТ ВВЕДЕНИЯ НАНОЧАСТИЧЕК СВИНЦА РАЗЛИЧНЫХ РАЗМЕРОВ

Губарь И.В., Лавриненко В.Е., Чухрай С.Н., Савосько С.И., Сокуренко Л.М., Апыхтина Е.Л., Яворовский А.П.

В экспериментах исследовали кардиовазотоксическое действие НЧ (наночастиц) свинца размером 26-34 нм и 50-80 нм. Морфологические и морфометрические методы были использованы для обнаружения структурных изменений сердца и аорты через 30 и 60 введений НЧ Pb. Результаты экспериментов указывают на повреждения миокарда сердца, которые заключаются в увеличении интерстициального пространства между волокнами кардиомиоцитов, дистрофии кардиомиоцитов, стаза крови в микрососудах желудочков сердца. Выявлено большую чувствительность миокарда предсердий к токсическому действию НЧ Pb. Структурные изменения аорты заключались в расслоении эластических мембран, уменьшении плотности соединительной ткани адвентициальной оболочки и имели тенденцию к прогрессирующим изменениям.

Ключевые слова: свинец, Pb, наночастицы, морфологические изменения, аорта, миокард.

Рецензент Єрошенко Г.А.