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CARDIOTOXIC EFFECT OF LEAD ACETATE ON HEART MORPHOGENESIS BASED ON THE RESULTS OF ELECTRON MICROSCOPY

Shevchenko I.V. ✉, Nefedova O.O., Kushnarova K.A., Kuznetsova O.V., Rutgaizer V.G., Boyko O.V. Cardiotoxic effect of lead acetate on heart morphogenesis based on the results of electron microscopy.
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ABSTRACT. Background. In modern society, changes in the environment that arise under the influence of anthropogenic factors, an increase in the number of salts of heavy metals, which are teratogens and can provoke disorders in the development of organs, are the object of interest. One such compound is lead acetate. Lead acetate has a high polytropic toxicity. The heart and the vascular system are sensitive to the effects of lead compounds, during the prenatal and postnatal period. **Purpose.** To study the ultrastructural manifestations of the cardiotoxic effect of lead acetate on the morphogenesis of the heart. **Methods.** On the 1st and 7th day after birth, rat pups were dissected and hearts were taken for electron microscopic examination. The research was carried out using a transmission electron microscope. Electronograms were obtained by taking ultrathin sections on Agfa orthochromatic film. The diameter and bulk density of mitochondria, the length of sarcomeres of contractile myofibrils, and the bulk density of myofibrils were quantified. **Results.** The structure of the myocardium at the level of the right ventricle was investigated by the method of electron microscopy. A comparative analysis of the ultrastructure of cardiomyocytes and morphometric indicators, which may indicate dystrophic changes, was conducted. The decrease in the density of myofibrils in our own studies was detected already on the first day of the exposure of lead acetate. Changes in the density of myofibrils on day 7 were not detected, indicating a certain stability of these protein structures. At the same time, the length of sarcomeres was reduced. These changes should not be considered as a manifestation of the recovery process, since a significant number of myofibers have suffered damage. The general conclusion is an increase in the destructive changes in cardiomyocytes during the exposure of lead acetate. **Conclusion**. Cardiotoxic effects of lead acetate are manifested by swelling of cardiomyocytes and destructive changes in mitochondria and contractile myofibrils. The detected ultrastructural disorders are a consequence of the acute toxic effect of lead acetate.

Key words: lead acetate, heart, myocardium, matrix, myofibril, cardiomyocytes, sarcomere, kristi, cardiotoxic effect of lead acetate.

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Introduction

Pathological changes, anomalies and defects in the development of the organism, which arise as a result of various factors, including the negative impact of the environmental situation, occupy one of the central places in modern research. In modern society, changes in the environment caused by anthropogenic factors, an increase in the amount of heavy metal salts, which are teratogens and can provoke organ development disorders, are the object of interest. One such compound is lead acetate.

Lead acetate has high polytropic toxicity. The effect on physiology, organ morphogenesis, and metabolic parameters was studied in experimental studies and recorded in patients with acute intoxication [1]. The heart and vascular system are sensitive to the influence of lead compounds during the prenatal and postnatal period [2]. Many mechanisms have been proposed to explain lead-induced hypertension, including changes in calcium and sodium metabolism [3], disturbances in the functioning of the renin-angiotensin system [4], involvement of the sympathetic nervous system [5], effects on surface glycoconjugates of the heart [6] and increased sensitivity to other compounds [7]. At the biochemical level, toxicity is explained by a violation of redox balance, hyperproduction of free radicals, and damage to endogenous antioxidant systems [8]. The study of the impact on the development of this or that organ in the embryo after exposure to the mother's body by such teratogens as, for example, lead acetate is far from complete [9,10]. In this aspect, comparative embryology and its experimental research methods become important.

Purpose

To study the ultrastructural manifestations of the cardiotoxic effect of lead acetate on the morphogenesis of the heart.

Materials and methods

The research material was the heart of rats on the 1st and 7th day after birth. The control group consisted of pregnant females who received distilled water. The experimental group consisted of pregnant females who received a 2.5% aqueous solution of lead acetate at the rate of 50 mg/kg of rat body weight per day. The solutions were administered daily throughout the pregnancy.

On the 1st and 7th day after birth, rat pups were dissected and hearts were taken for electron microscopic examination. Heart ventricle samples were fixed at a temperature of +2°C in a 2.5% solution of glutaraldehyde in 0.1M phosphate buffer (pH 7.3) followed by postfixation for 1 hour in a 1% buffered solution of osmium tetroxide ("SPI", USA). After dehydration in alcohols of increasing concentration and in propylene oxide, the materials were embedded in Epon-812 ("SPI-PonTM 812 Epoxy Embedding Kit", USA), and ultrathin sections were made from the epoxy blocks on an УМТП-6М ultramicrotome ("SELMI", Ukraine) placed on support grids (Mesh Regular Grid 200). Double contrast was performed using the Reynolds method for 30 minutes. The research was carried out using a transmission electron microscope ПЭМ-100-01 ("SELMI", Ukraine) at an acceleration voltage of 75-90 kV and primary magnifications from 8000 to 80000 according to the scheme described [11]. Electronograms were obtained by taking ultra-thin sections on Agfa orthochromatic film at magnifications of ×3000 and ×5000, followed by scanning with a high-resolution Canon CanoScan 9000F scanner to obtain digital images [Fig.1]. The diameter and bulk density of mitochondria, the length of sarcomeres of contractile myofibrils, and the bulk density of myofibrils were quantified. Morphometric analysis was performed using Carl Zeiss software (AxioVision SE64 Rel.4.9.1) and Origin 8.0. Before the statistical analysis, the hypothesis of normal distribution of data in groups was tested using the Shapiro-Wilk or Kolmogorov-Smirnov test. The intergroup difference was assessed by the Kruskel-Wallis test. The data are presented in the form of the arithmetic mean (M) and the error of the mean deviation (m) [Table. 1].

Results and discussion

The structure of the myocardium at the level of the right ventricle was investigated by the method of electron microscopy. A comparative analysis of the ultrastructure of cardiomyocytes and morphometric indicators, which may indicate dystrophic changes, was conducted. At the ultrastructural level, structurally intact cardiomyocytes containing contractile myofibrils, mitochondria and cisterns of the sarcoplasmic reticulum were recorded in the control groups. The cytoplasm is granular, without signs of hydropic edema. The nuclei occupied the central position of the cell and contained chromatin of various levels of organization, with the dominance of euchromatin, which is a manifestation of active protein-synthetic processes. Cardiomyocytes were in close contact with each other. No significant difference was found between the period of observation on the 1st and 7th day of the experiment.

Pronounced swelling of the cytoplasm of cardiomyocytes and reduction of organelles were found in the experimental groups. The main manifestations of the cardiotoxic effect of lead acetate were the destruction of myofibrils and mitochondria. Some dependence on the period of exposure to lead acetate was revealed. Thus, on the 1st day of observation, mitochondrial fragmentation and stretching of sarcomeres dominated, on the 7th day of the experiment, sharp swelling and destruction of mitochondria and a lesser degree of damage to myofibrils were detected [Fig. 1].

The cardiotoxic effect of lead acetate was quantified by the morphometric method. A sharp decrease in the volume density of contractile myofibrils in the cytoplasm of cardiomyocytes and an increase in the length of sarcomeres for 1 day of exposure were established. The volume density of mito-

chondria did not differ from the control values, but their diameter was smaller, which is the result of fragmentation of the studied organelles. On the 7th day, the increase in the diameter of mitochondria and volume density is a consequence of matrix swelling and destruction of organelle crystals [Table. 1]. That is the results of morphometry confirmed the ultrastructural manifestations of the toxic effect of lead acetate on cardiomyocytes.

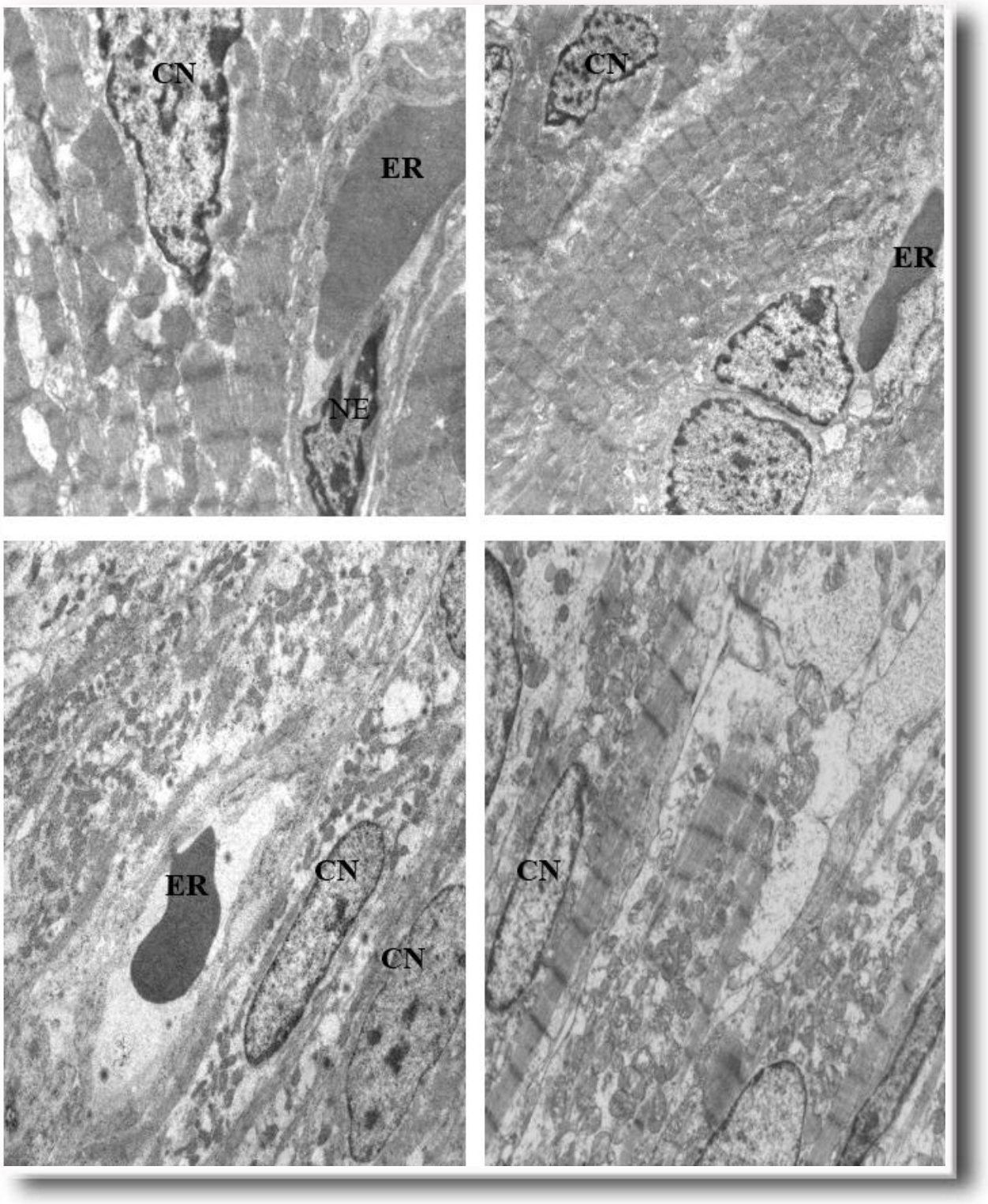

Fig. 1. Ultrastructure of the myocardium of animals of the control and experimental groups. Swelling and dystrophic changes of cardiomyocytes, reduction of organelles, destruction of contractile myofibrils, swelling of mitochondria. Note: 1 – control, 1 day (×5000); 2 – control, 7 days (×3000); 3 – experiment, 1 day (×3000); 4 – experiment, 7 days (×5000); CN – cardiomyocyte nucleus; NE - endotheliocyte nucleus; ER - erythrocyte. Electronograms.

Table 1

Results of morphometric assessment of cardiomyocytes under lead acetate intoxication

Group	Sarcomere length, mkm	Mitochondrial diameter, mkm	Volume density of myofibrils, %	Volumetric density of mitochondria, %
Control, 1 day	1,65±0,04	0,69±0,05	36,08±3,58	5,04±1,29
Control, 7 day	1,51±0,10	0,62±0,04	32,87±5,12	5,91±1,06
Study, 1 day	1,85±0,04*	0,26±0,02*	11,55±4,41*	4,91±0,48
Study, 7 доба	1,47±0,03*#	0,66±0,03#	16,33±2,23*^	12,67±3,95*#^

Note: * - significantly compared to control for 1 day (P<0.05); ^ - significantly compared to the control on the 7th day (P<0.05); # - reliable up to 1 day of the experiment (P<0.05)

The cardiotoxicity of lead acetate on the morphogenesis of the heart was fragmentarily described in scientific publications [12]. Thus, a decrease in the thickness of the myocardium of the atria and ventricles of the heart, thinning of the interventricular septum and a negative effect on the morphogenesis of the aortic valve were found, but the structural basis of the delay in the morphogenesis of the myocardium remained unclear. Biochemical methods and in vitro studies can help to understand the causes of delay and disorders of heart development. An increase in the level of O2 and the subsequent accumulation of H2O2 have been shown in the culture of human endothelial cells [13]. These results prove the endogenous development of oxidative stress and explain the mechanisms of endothelial dysfunction [14]. Dystrophic changes in the endothelium of blood capillaries of the myocardium have been confirmed in previous studies [15,16]. Our results do not contradict the studies of other authors and further expand the understanding of the structural basis of the cytotoxic action of lead acetate at the stage of morphogenesis of blood vessels of the heart.

Identifying primary signs of cardiotoxicity is a difficult task. Thus it is known from literary sources that the earliest ultrastructural manifestations of damage to cardiomyocytes are changes in mitochondria. At the same time, the morphology of organelles can have significant variability, and some of the forms are related to the mechanism of damage. Thus a decrease in the electron density of the matrix is first recorded, then the swelling of the matrix occurs. Swelling of the cristae ends with their destruction, which explains the cause of energy deficit in cardiomyopathy. Such changes were found in our own research on the 7th day of exposure to lead acetate. The destruction of mitochondria was most pronounced in cardiomyocytes with a sharp reduction of organelles. These changes have a causal relationship. Primary mitochondrial fragmentation appears to be a response to acute lead acetate intoxication, and subsequent mitochondrial impairment is caused by an energy deficit that progresses with duration of lead acetate exposure. Hyperproduction of free radicals causes lipid peroxidation of organelle membranes [17], which was recorded as destruction of mitochondrial cristae. Membrane damage is not the only consequence of energy deficiency. Proteolysis, protein degradation is also a manifestation of destructive changes. In cardiomyocytes, these changes are most strikingly manifested in the destruction of myofibrils. Myofibrils in intact cardiomyocytes are complexly organized into sarcomeres. Changes in the length and density of contractile proteins in sarcomeres are a manifestation of their destruction. A decrease in the density of myofibrils in our own research was detected already on the 1st day of exposure to lead acetate, no changes in the density of myofibrils were detected on the 7th day, which indicates a certain stability of these protein structures [Fig. 1]. At the same time, a decrease in the length of sarcomeres was noted. These changes should not be considered as a manifestation of the recovery process, since a significant number of myofibrils were damaged

Conclusion

The general conclusion is an increase in destructive changes in cardiomyocytes upon exposure to lead acetate. Thus cardiotoxic effects of lead acetate are manifested by swelling of cardiomyocytes and destructive changes in mitochondria and contractile myofibrils. The detected ultrastructural disorders are a consequence of the acute toxic effect of lead acetate.

Prospects for further research

The next stage of work is planned to investigate the expression levels of immunohistochemical markers on the morphogenesis of the heart under the influence of lead acetate.

Information about conflicts of interest

Potential or obvious conflicts of interest, related to this manuscript, at the time of publication does not exist and is not expected.

Connection of publications with planned research works. The research was carried out according to the theme of the departmental scientific work of the department of human anatomy of the Dnipro State Medical University, "Morphogenesis of organs and systems of the human body and experimental animals in ontogenesis in the norm and under the influence of external factors" (state registration number 01170006976).

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Шевченко І.В., Нефьодова О.О., Кушнарьова К.А., Кузнецова О.В., Рутгайзер В.Г., Бойко О.В. Кардіотоксична дія ацетату свинцю на морфогенез серця на основі електронної мікроскопії.

РЕФЕРАТ. Актуальність. В сучасному суспільстві об'єктом інтересу стають зміни в навколишньому середовищі, що виникають під впливом антропогенних факторів, збільшення кількості солей важких металів, які є тератогенами та можуть провокувати порушення розвитку органів. Ацетат свинцю має високу політропну токсичність. Серце та судинна система чутливі до впливу сполук свинцю, протягом пренатального та постнатального періоду. **Мета.** Дослідити ультраструктурні прояви кардіотоксичного впливу ацетату свинцю на морфогенез серця. **Методи.** Дослідження проводили за допомогою трансмісійного електронного мікроскопа ПЭМ-100-01 («SELMI», Україна). Електронограми отримували, знімаючи ультра тонкі зрізи із подальшим скануванням за допомогою сканера з високою роздільною здатністю. Кількісно оцінювали діаметр і об'ємну щільність мітохондрій, довжину саркомерів скоротливих міофібрил і об'ємну щільність міофібрил. **Результати.** Методом електронної мікроскопії досліджено будову міокарду на рівні правого шлуночка. На ультраструктурному рівні у контрольних групах реєстрували структурно неушкоджені кардіоміоцити, які містять скоротливі міофібрили, мітохондрії та цистерни саркоплазматичної сітки. У дослідних групах встановлено виражений набряк цитоплазми кардіоміоцитів та редукцію органел. Головними проявами кардіотоксичної дії ацетату свинцю були деструкція міофібрил і

мітохондрій. На 1 добу спостереження домінували ознаки фрагментація мітохондрій та розтяг саркомерів, на 7 добу експерименту виявлено різкий набряк і деструкцію мітохондрій та менший ступінь пошкодження міофібрил. Зменшення щільності міофібрил у власних дослідженнях виявлено вже на 1 добу експозиції ацетату свинцю, змін щільності міофібрил на 7 добу не виявлено, що вказує на певну стійкість цих білкових структур. Найбільш ранніми ультраструктуриними проявати пошкодження кардіоміоцитів є зміни мітохондрій. Проявом кардіотоксичної дії ацетату свинцю є набряк кардіоміоцитів та деструктивні зміни мітохондрій і скоротливих міофібрил. **Підсумок.** Загальним висновком є збільшення деструктивних змін кардіоміоцитів при експозиції ацетату свинцю. Проявом кардіотоксичної дії ацетату свинцю є набряк кардіоміоцитів та деструктивні зміни мітохондрій і скоротливих міофібрил. Виявлені ультраструктурні порушення є наслідком гострої токсичної дії ацетату свинцю.

Ключові слова: ацетат свинцю, серце, міокард, матрикс, міофібрили, кардіоміоцити, саркомери, кристи, кардіотоксична дія ацетату свинцю.