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# CARDIOPROTECTION FEATURES IN MODELING ANTHRACYCLINE CARDIOMYOPATHY IN RATS

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Currently actual problem of Clinical and Experimental Medicine is the study and pharmacological correction of cardiotoxic effects of anthracycline antibiotics, which are a part of the protocol chemotherapy most of the hematologic diseases. Experimental study was done to examine the cardioprotective effects of trimetazidine and meldony phosphate in rats with simulated doxorubicin cardiomyopathy. It was discovered that trimetazidine and meldony phosphate metabolic therapy had a protective effect on the myocardium of rats. Under the influence of metabolic therapy all the changes that occur in the myocardium under the influence of doxorubicin were subjected to reverse development. It was experimentally demonstrated the cytoprotective and anti-ischemic effect of the trimetazidine and meldony phosphate complex therapy on myocardium of rats with anthracycline cardiomyopathy.
**Keywords:** cardiotoxicity, cardiomyopathy, doxorubicin, cardioprotectors, rats.

**Statement of the problem.** As you know, in the treatment of pediatric oncohematological diseases an arsenal of anticancer chemotherapeutic drugs is used. To date, the actual problem in pediatrics is dysfunction in children with oncohematological pathology [6, p. 42]. It is known that almost all children with acute leukemia occurs lesions of the cardiovascular system [6, p. 43].

**Analysis of recent research and publications.** On the present day it was proved that antitumor therapy causes a number of side effects [3, p. 67]. Most types of toxicity of cytostatic therapy associated with rapidly proliferating cellular systems which are spontaneously regress with a minimum duration of toxicity. However, cardiomyocytes (CMC) have limited regenerative capacity and at the same time are the most susceptible to chemotherapeutic agents [3, p. 67]. Special attention deserve anthracycline antibiotics (AA) and cytotoxic agents (5- fluorouracil, cyclophosphamide). Of all the AA drugs the most intensively studied doxorubicin agent as a cardiotoxic model [7, p. 168; 8, p. 196; 10, p. 202]. It is noted that the AA cardiotoxicity grows with increasing its cumulative dose and occurs mainly when the total dose of doxorubicin is more than 550,0 mg/m$^2$ [9, p. 461]. However, in children the risk of heart damage is significantly increased when taking anthracyclines, even in small doses. With such therapy the incidence of cardiac complications ranged from 1,6% to 45,0% [6, p. 43].

**Tagging is not resolved before the general problem.** In the pathogenesis of secondary injury of myocardium dismetabolic changes plays a significant role, that occurs on a background of hypoxia with the accumulation of glycolysis end substrates, activation reactions of lipid peroxidation, violation of the integrity of plasma membranes, the release of lysosomal enzymes and the development of CMC energy imbalance. This metabolic stress can cause the reduction in working heart muscles, myocardial contractility and development of heart failure [2, p. 143]. Therefore, in clinical practice should take place optimal pharmacotherapy protect of the heart muscle from the damaging effect of AA.

**The purpose of the article.** The aim of the research was to evaluate the trimetazidine cardioprotective action of morphological substrate and meldony phosphate on injured myocardium in rats with simulated anthracycline cardiomyopathy.

**The main material.** An experimental research was conducted on 80 Wistar white mature male rats weighing (200±20) grams, which were on a vivarium normal diet. Simulation of anthracycline cardiomyopathy in rats was performed by intraperitoneal administration of doxorubicin for four weeks (Ukraine, JSC «Kyivmedpreparat») at a dose of 5,0 mg/kg body weight every week [5, p. 18]. During doxorubicin treatment 40 individuals (main group) were additionally injected with drugs for metabolic cardioprotective actions by scheme: 1 hour before each weekly administration of doxorubicin was intraperitoneally administered the meldonium phosphate solution («Grindex», Latvia) at a dose of 100,0 mg/kg body weight [4, p. 100]; intragastrically administered the meldonium phosphate («Grindex», Latvia) in powder form at a dose of 100,0 mg/kg body weight and the trimetazidine («Ratiopharm», Germany) in powder form at a dose of 6,0 mg/kg body weight daily for 42 days [1, p. 6]. Observations on animals were carried out for 28 days (simulation cardiomyopathy period), and for the next 14 days after the experimental part. Withdrawal of rats from the experiment was carried out by decapitation under ketamine anesthesia with dose of 100,0 mg/kg body weight.

Ultrastructural changes in the myocardium were studied on electron microscope «PEM-125K» at accelerating voltage of 75 kV with followed photography at magnification from 2000 to 40000 times. Cuts with thickness 20-50 nm were performed on ultramicrotome «Tesla BS-490» and painted with uranyl acetate and Reynolds mixture.

The experiment was conducted in accordance to follow standards: The Law of Ukraine № 3447-IV «On the protection of animals from cruelty»; The Norms of European Convention for the protection of vertebrate animals which are used for experimental and scientific purposes (1985); EU Directive № 609(1986); Order of the Ministry of Health of Ukraine №281 (01.11.2000) «On measures to further improve organizational norms of using experimental animals»; The Protocol of SHEE «Ivano-Frankivsk National Medical University» Bioethics Committee № 63/12 (23.04.2012).

Electron microscope examination of the ultrastructure of left ventricular in animals which was administered with doxorubicin for anthracycline cardiomyopathy simulation revealed a number of typical changes related both to CMC and blood capillary. At the stage of cardiomyopathy development (28th day of experiment) the CMC nuclei had low electron optical density. In zone around nucleus secondary lysosomes were observed. In most of the CMC there were recorded the cristae destruction, fragmentation and destruction of the inner and outer membranes

of mitochondria. Most myofibrils were at the state of segmental contractions. In some areas there was observed their fragmentation and analysis (fig. 1).

At this stage of research the erythrocytic aggregates in lumen between individual blood capillaries were observed. The sail-shaped protrusions were detected on the cytolemma lumenal surface. In addition most of mitochondria were increased and contained reduced cristae and enlightened matrix. The elements of Golgi apparatus and the granular endoplasmic nets were greatly expanded. In the peripheral regions of endothelial cells a large number of vacuoles were determined. The perivascular space was greatly enlarged. The basement membrane was thickened and their contours were indistinct (fig. 2).

The myocardium submicroscopic research in rats with background of metabolic correction after 28 days (fig. 3) since the first administration of doxorubicin showed significantly lower changes expression as an intracellular edema with violation of the ultrastructure of organelles in cardiomyocytes: the chromatin granules are evenly distributed over the nucleoplasm; longitudinal orientation of myofibrils is largely preserved, between which are placed in a chain mitochondria of different size and shape. It should be noted that the applied metabolic therapy had potent cytoprotective effect (only isolated mitochondria were subjected to partial lysis; was dominated the moderate degree of injury; were met some cells with signs of increased functional activity along with degenerative and destructive changes).

Nevertheless, the use of trimetazidine and meldony phosphate resulted in significant improvement of blood capillary ultrastructure: it was noted the increased processes of transport activity through their wall; the blood capillary endothelials were in a state of high transport activity and the overhydration effects of them were less pronounced, it was recorded only a slight invagination of the nuclear membrane; the chromatin granules were evenly distributed on karyoplasm (fig. 4). Some of mitochondria were not much increased and had minimal disruption of cristae. In addition, in treated animals pathological process in the myocardium was noted significantly lower, not only by the severity but also by the prevalence. Introduction of trimetazidine and meldony phosphate also caused the improvement of blood rheology (platelet-leukocyte or erythrocytic aggregants in the lumen between individual blood capillaries were not detected).

**Conclusions and recommendations.**

1. In animals with cardiomyopathy, induced by doxorubicin the pathomorphological changes of cardiomyocytes, appear in the mitochondria disruption, the swelling and the severe vacuolation of endothelial cells. All these mentioned facts together

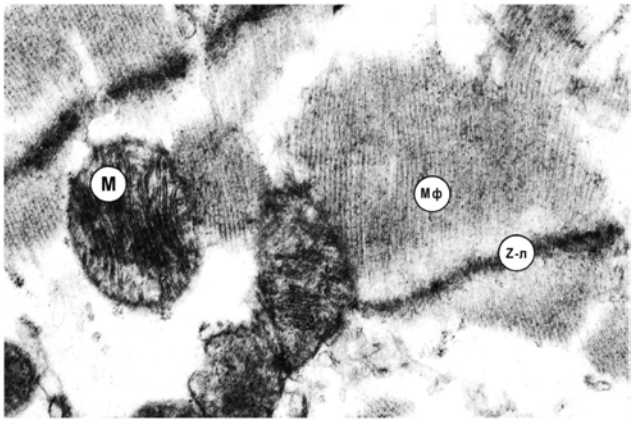


**Fig. 1. Submicroscopic organization of the cardiomyocytes of left ventricular in 28 days after doxorubicin administration. М – mitochondria; Мф – myofibrils; Z-л – Z-line. Electron microscope photograph. Zoom 20000.**

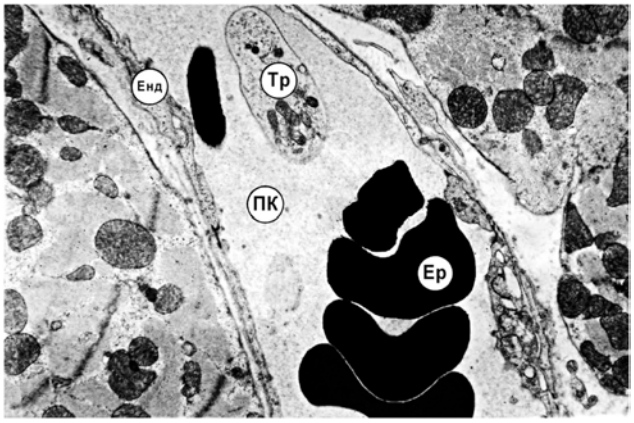


**Fig. 2. Ultrastructural state of the blood capillary of left ventricle in 28 days after doxorubicin administration. Енд – endothelial cells; Ер – erythrocytes; Тр – platelet; ПК – capillary lumen. Electron microscope photograph. Zoom 10000.**

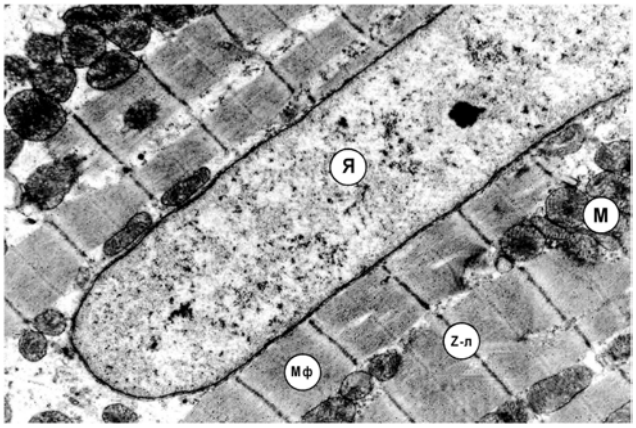


**Fig. 3. Ultrastructural organization of the cardiomyocytes of left ventricular in 28 days after administration of doxorubicin on the background of trimetazidine and mildronat correction. Я – the core; М – mitochondria; Мф – myofibrils; Z-л – Z-line. Electron microscope photograph. Zoom 8000.**

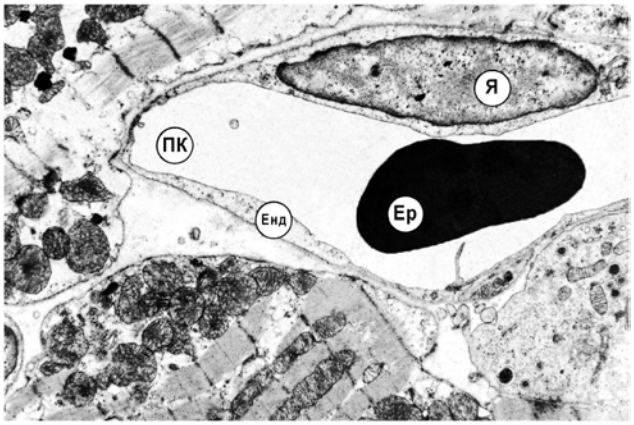


**Fig. 4. Submicroscopic changes of the blood capillaries of left ventricle in 28 days after doxorubicin administration on the background of trimetazidine and mildronat correction. Я – the core; Енд – endothelial cells; Ер – erythrocyte; ПК – capillary lumen. Electron microscope photograph. Zoom 10000.**

with the background of blood capillary thrombosis lead to the disorders of myocardium microvasculature.

2. In the application of trimetazidine and meldony phosphate in animals with simulated doxorubicin cardiomyopathy the minimal signs of damage with reversible and shown as moderate edema of cardiomyocyte organelles were noted, especially mitochondria.

3. Under the influence of the cardioprotective drugs use improvement of transport processes in the microvasculature vessels was noted. It should be considered as the activation of myocardium compensatory mechanisms which are aimed at the elimination of doxorubicin caused changes.

4. Effectiveness of trimetazidine and meldonium phosphate in the experiment opens the new perspectives for their use in clinical practice during protocol chemotherapy for the prevention and treatment of doxorubicin cardiomyopathy.

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## МОЖЛИВОСТІ КАРДІОПРОТЕКЦІЇ ПРИ МОДЕЛЮВАННІ АНТРАЦИКЛІНОВОЇ КАРДІОМІОПАТІЇ У ЩУРІВ

**Анотація**
На сьогоднішній день актуальною проблемою клінічної та експериментальної медицини є вивчення та медикаментозна корекція кардіотоксичних ефектів антрациклінових антибіотиків, які входять в склад протокольної хіміотерапії більшості онкогематологічних захворювань. Експериментальна частина дослідження полягала у вивченні кардіопротекторних ефектів триметазидину та мельдонію фосфату у щурів із модельованою доксорубіциновою кардіоміопатією. Встановлено, що метаболічна терапія триметазидином та мельдонієм фосфатом мала протективний ефект щодо міокарда щурів. Під впливом метаболічної терапії всі зміни, що виникали в міокарді під впливом доксорубіцину, зазнавали зворотнього розвитку. Експериментально продемонстровано цитопротекторну та антиішемічну дію комплексної терапії триметазидином та мельдонієм фосфатом щодо міокарду щурів із антрациклінової кардіоміопатією.
**Ключові слова:** кардіотоксичність, кардіоміопатія, доксорубіцин, кардіопротектори, щурі.

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## ВОЗМОЖНОСТИ КАРДИОПРОТЕКЦИИ ПРИ МОДЕЛИРОВАНИИ АНТРАЦИКЛИНОВОЙ КАРДИОМИОПАТИИ У КРЫС

**Аннотация**
На сегодняшний день актуальной проблемой клинической и экспериментальной медицины является изучение и медикаментозная коррекция кардиотоксических эффектов антрациклиновых антибиотиков, входящих в состав протокольной химиотерапии большинстве онкогематологических заболеваний. Экспериментальная часть исследования заключалась в изучении кардиопротекторных эффектов триметазидина и мельдонию фосфата у крыс с моделируемой доксорубициновой кардиомиопатией. Установлено, что метаболическая терапия триметазидина и мельдонием фосфатом имела протективный эффект в отношении миокарда крыс. Под влиянием метаболической терапии все изменения, возникавшие в миокарде под влиянием доксорубицина, подвергались обратному развитию. Экспериментально продемонстрировано цитопротекторное и антиишемическое действие комплексной терапии триметазидина и мельдонием фосфатом относительно миокарда крыс с антрациклиновой кардиомиопатией.
**Ключевые слова:** кардиотоксичность, кардиомиопатия, доксорубицин, кардиопротекторы, крысы.