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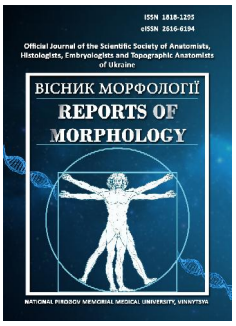

Submicroscopic changes in the heart of adult rats under conditions of persistent hyperhomocystemia

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Cardiovascular diseases are the leading cause of death and disability worldwide. It has been established that in recent years there has been a significant increase in the number of patients with this pathology, forcing researchers, scientists and physicians to look for risk factors of cardiovascular diseases, one of which is hyperhomocysteinemia (HHCys). The aim of the research is to study the features of submicroscopic changes in the heart of adult rats under conditions of HHCys. Experimental studies were performed on 22 white nonlinear adult (6-8 months) male rats in accordance with the principles of bioethics (Strasbourg, 1986; Kyiv, 2001). During the experiment, the animals were divided into two groups - control and experimental. Simulation of persistent HHCys was achieved by administering to rats the experimental group thiolactone homocysteine (HCys) at a dose of 200 mg/kg body weight intragastrally for 60 days. Ultrathin sections were studied in the PEM - 125K electron microscope. It was found that the introduction of thiolactone HCys to adult rats at a dose of 200 mg/kg causes the development of dystrophic and destructive changes in the heart of animals. Significant connective tissue edema was observed in the endocardium, and disturbances in the components of the microcirculatory tract were detected in the myocardium. Local enlightenment, cytoplasmic edema and local condensation of heterochromatin in hypertrophied nuclei were detected in hemocapillary endothelial cells. In cardiomyocytes, myofibrils are thickened, mitochondria are swollen with partial destruction of the cristae, tubules of smooth endoplasmic reticulum and T-tubules are dilated. These findings indicate that in adult rats HHCys caused the development of pathological changes in the endocardium, myocardium of experimental animals and in the microcirculatory tract.

Keywords: *hyperhomocysteinemia, heart, muscle fibers, cardiomyocytes, mitochondria, sarcomeres, rats.*

Introduction

Cardiovascular diseases are the leading cause of death and disability worldwide. It has been established that in recent years there has been a significant increase in the number of patients with this pathology, forcing researchers, scientists and doctors to look for risk factors of cardiovascular diseases in order to diagnose them in the early stages of development and prevention [18, 19].

Over the last decade, scientists' interest in the problem of HHCys has increased significantly, as an increase in plasma of this sulfur-containing amino acid leads to the emergence, progression and development of complications of cardiovascular diseases [2, 7, 17].

A significant amount of scientific works is devoted to the study of the main mechanisms of pathological action of elevated HCys levels on the human body, but the most studied today is hypomethylation and homocysteinylation of proteins. Hypomethylation causes a violation of the expression of a number of genes, which in turn is reflected in severe endothelial dysfunction, inhibition of its regeneration, vascular damage, excessive accumulation of lipids in the vascular wall, increased thrombosis. Homocysteinylation of proteins under conditions of HHCys is characterized by changes in their structure and functions. By homocysteinylation, important protein molecules lose their activity, for example, proaccelerin, fibrinogen, endothelial cell apoptosis factors, and so on [3, 4, 5, 13].

The above pathological changes that occur under

HHCys conditions contribute to the development of endothelial dysfunction. Prolonged negative effects of HCys on the vascular wall lead to the release of cytokines, chemokines (MCP-1, IL-8), the expression of adhesion molecules (VCAM-1), the initiation of platelet and coagulation hemostasis, activation of thrombin synthesis, and inhibition of anticoagulation and fibrinolysis. In addition, HCys affects all pathological processes leading to the formation of atherosclerotic plaques [6, 9, 11, 21].

Thus, increasing the concentration of HCys in blood plasma is a predictor of the development of pathologies of the cardiovascular system, and the study of its impact on structural changes in the heart and blood vessels is relevant today.

The aim of the research is to study the features of submicroscopic changes in the heart of adult rats under conditions of HHCys.

Materials and methods

The studies were performed on 22 white nonlinear adult (6-8 months) male rats. During the experiment, the animals were divided into two groups - control and experimental (11 animals in each group). Simulation of the state of hyperhomocysteinemia was achieved by administering to rats of the experimental group thiolactone HCys at a dose of 200 mg/kg body weight intragastrally for 60 days. Animals were decontaminated by decapitation under thiopental anesthesia.

The provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes" (Strasbourg, 1985) and the "General Ethical Principles for Animal Experiments", approved by the First National Congress on Bioethics, were followed in keeping, caring for and manipulating all animals (Kyiv, 2001).

Small pieces of rat heart were selected for ultrastructural study. They were fixed in 2.5-3.0 % solution of glutaraldehyde and and postfixed in 1 % osmium tetraoxide solution on the pH 7.2-7.4 phosphate buffer, followed by dehydration in alcohol and propylene oxide and then embedded into mixture of epoxy resins. Ultrathin sections were contrasted with uraniacetate and lead citrate according to Reynolds and studied in the PEM - 125K electron microscope [8, 12].

Results

Submicroscopic studies of the heart of adult animals in experimental HHCys revealed damage to the organ wall in the form of dystrophic and destructive changes. Thickening of collagen fibers and pronounced edema of the main substance of the endocardium were revealed. Partial stratification of muscle fibers has been identified. In the intercalated discs, the integrity of intercellular contacts was violated (Fig. 1).

The nuclei of cardiomyocytes changed their shape due to increased invagination of the karyolemma. The amount

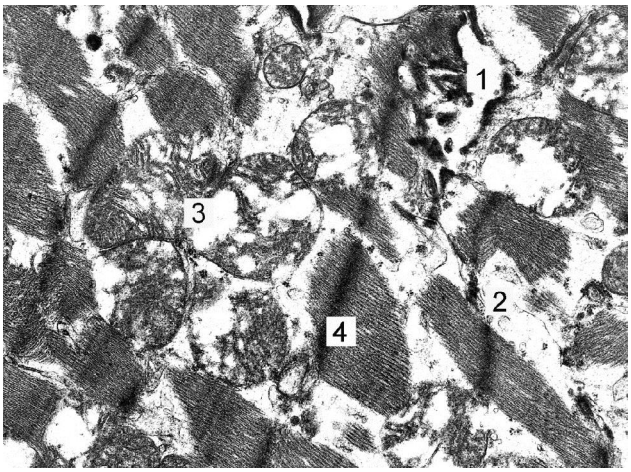

Fig. 1. Electronogram of cardiomyocytes of the myocardium of a mature rat with hyperhomocysteinemia: 1 - intercalated disc; 2 - cytoplasm of the cardiomyocyte; 3 - mitochondria; 4 - myofibrils. x14 000 magnification.

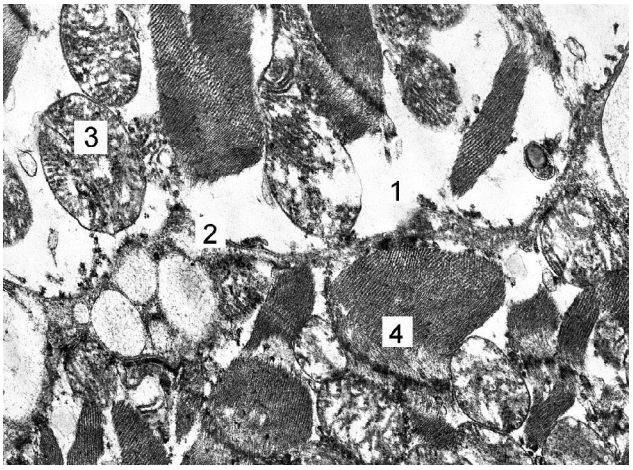

Fig. 2. Electrogram of myocardial cardiomyocyte of mature rat in hyperhomocysteinemia: 1 - cytoplasm of the cardiomyocyte; 2 - plasmalemma; 3 - mitochondria; 4 - myofibrils. x14 000 magnification.

of condensed chromatin increased compared to the intact group of adult animals. Karyoplasm is heterochromic, perinuclear spaces are partially expanded. Significant ultrastructural changes were found in the cytoplasmic organelles of cardiomyocytes. Thickening and partial loosening of myofibrils were detected. Thinning and partial lysis of myofilaments in myofibrils were determined. Locally, the sarcomeres were placed in a disordered manner, areas of myofibril overexpansion were detected. Mitochondria are swollen, the matrix is enlightened, cristae are destructured (Fig. 2). The expansion of the tubules of the smooth endoplasmic reticulum and T-tubes was revealed.

An increase in the relative volume of myocardial connective tissue was found. The thickness of collagen fibers increased, they often formed thick bundles. The blood vessels were full-blooded. Violation of the structural organization of the hemocapillary wall was revealed. Local

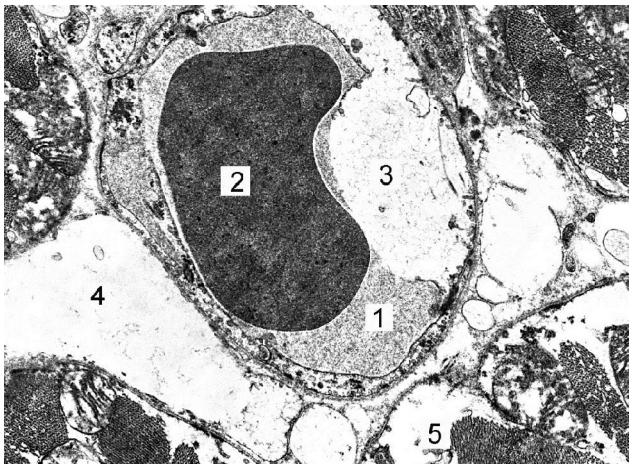

Fig. 3. Electronogram of hemocapillaries of the myocardium of a mature rat with hyperhomocysteinemia: 1 - hemocapillary lumen; 2 - erythrocyte; 3 - edema of the cytoplasm of the endothelial cell; 4 - perivascular space; 5 - cytoplasm of the cardiomyocyte. x8 000 magnification.

edema and cytoplasmic enlightenment were found in endotheliocytes. Endothelial cell nuclei were enlarged, heterochromatin became more condensed, and localized to the inner membrane of the karyolemma. The basement membrane is characterized by compaction, in some areas it was swollen, homogeneous (Fig. 3).

Discussion

According to research in recent years, HHCys causes the development of a wide range of disorders of the cardiovascular system. In particular, it is known that HHCys is a potential initiator of the development of an imbalance of prooxidant and antioxidant enzymes in the myocardium of rats, especially in males. It is also proved that under the conditions of administration of thiolactone HCys to rats, the activity of lipid peroxidation and oxidative modification of myocardial proteins increases. This fact is confirmed by the increase in the content of malonic dialdehyde and carbonyl groups of proteins in the myocardium. At the same time, the levels of these compounds were directly related to the concentration of HCys in the plasma of experimental animals. According to biochemical studies, HHCys also causes the development of cardiomyocyte cytolysis, as evidenced by the probable increase in AST and CPK activity in experimental rats [15].

The results of clinical studies show that in patients after coronary artery bypass grafting an increase in the concentration of plasma HCys has a negative impact on the state of the structural components of the aortic walls. Thus, histological examination of aortic fragments in this category of patients with a HCys plasma level of 15.72± 6.03 μmol/l in 29.69 % of individuals noted thickening of the tunica media with diffuse lymphocytic infiltration. Signs of tunica media hypertrophy and sclerosis, lymphocytic infiltration were observed in 51.85% of patients. In the subendothelial layer there was a decrease in the number of elastic fibers. Elastic membranes showed signs of partial destruction. The inner elastic membrane had areas of single ruptures [16].

It is a well-known fact that elevated HCys level is associated with a risk of cardiovascular disease. A large number of experimental studies show that HHCys causes the development of oxidative stress and ER stress, which are the causes of endothelial dysfunction. In addition, this amino acid under certain conditions causes a violation of the stability of atherosclerotic plaque and increases the degree of thrombotic complications. W. K. C. Lai and M. Y. Kan note that HCys disrupts the transport of nitric oxide (NO) precursor - L-arginine to endothelial cells, enhances the production of reactive oxygen species (ROS) with NADPH oxidase, which causes disorders of NO synthesis and its bioavailability. HCys also destroys the enzyme dimethylaminohydrolase and leads to the accumulation of NO synthase inhibitor - asymmetric dimethylarginine. It is also known that thiolactone HCys is able to interact with lysine-rich proteins, act as a trigger of ER stress and apoptosis of vascular wall endothelial cells [14].

Scientists note that HCys affects all pathological processes leading to the formation of atherosclerotic plaque. According to them, the mechanism is triggered by the production of significant amounts of ROS, reduced activity of the antioxidant system, increased levels of NADPH oxidase. ROS causes not only damage of endothelial cells, but also a decrease in the number of endothelial progenitors. Blood monocytes, replacing endothelial cells of the vascular wall, later turn into macrophages. The last are able to transform into so-called foam cells due to the absorption of oxidized VLDL. Under conditions of HHCys, their content increases due to inhibition of the synthesis of alipoprotein A-1 and disruption of the reverse transport of cholesterol to the liver. The gradual increase in the subendothelial space of foam cells under these conditions creates a vicious circle, causing a progressive increase in the number of atherosclerotic plaques [1, 10, 20].

Conclusion

Studies of the heart of adult animals in experimental HHCys have established dystrophic and destructive changes in the wall of the organ. Connective tissue edema was found in the endocardium. Violation of the components of the microcirculatory tract was detected in the myocardium. Local enlightenment, cytoplasmic edema and local condensation of heterochromatin in hypertrophied nuclei were detected in hemocapillary endothelial cells. In cardiomyocytes, myofibrils are thickened, mitochondria are swollen with partial destruction of the cristae, tubules of smooth endoplasmic reticulum and T-tubules are dilated. These findings indicate that in adult rats HHCys caused the development of pathological changes in the endocardium, myocardium of experimental animals and in the microcirculatory channel.

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СУБМІКРОСКОПІЧНІ ЗМІНИ В СЕРЦІ ДОРОСЛИХ ЩУРІВ ЗА УМОВ СТІЙКОЇ ГІПЕРГОМОЦИСТЕЇНЕМІЇ

Камінський Р. Ф., Дзевульська І. В., Янчишин А. Я., Матківська Р. М., Самборська І. А.

Захворювання серцево-судинної системи є провідною причиною смертності та інвалідності населення у всьому світі. Встановлено, що протягом останніх років реєструють значне зростання чисельності хворих з даною патологією, що змушує дослідників, науковців та лікарів до пошуку факторів ризику хвороб серцево-судинної системи, одним з яких є гіпергомоцистеїнемія (ГГц). Метою дослідження є вивчення особливостей субмікроскопічних змін в серці дорослих щурів за умов ГГц. Експериментальні дослідження проведені на 22 білих нелінійних дорослих (6-8 місяців) щурах-самцях з дотриманням принципів біоетики (Страсбург, 1986; Київ, 2001). Протягом дослідження тварин поділено на дві групи - контрольну та дослідну. Моделювання стану стійкої ГГц досягали шляхом введення щурам дослідної групи тіолактону гомоцистеїну (Гц) в дозі 200 мг/кг маси тіла інтрагастрально протягом 60 днів. Ультратонкі зрізи досліджували за допомогою електронного мікроскопу РЕМ - 125К. Встановлено, що введення дорослим щурам тіолактону Гц в дозі 200 мг/кг спричиняє розвиток дистрофічних та деструктивних змін в серці тварин. В ендокарді виявлений значний набряк сполучної тканини. У міокарді визначалося порушення компонентів мікроциркуляторного русла. В ендотеліоцитах гемокапілярів виявлено локальне просвітлення та набряк цитоплазми та локальна конденсація гетерохроматину в гіпертрофованих ядрах. У кардіоміоцитах міофібрили потовщені, мітохондрії набряклі з частковою деструкцією крист,

канальці гладкої ендоплазматичної сітки та Т-трубочки розширені. Дані знахідки свідчать, що в дорослих щурів ГГц зумовлювала розвиток патологічних змін в ендокарді, міокарді дослідних тварин та в мікроциркуляторному руслі.
Ключові слова: *гіпергомоцистеїнемія, серце, м'язові волокна, кардіоміоцити, мітохондрії, саркомери, щури.*