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

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ARTICLE INFO
Received: 20 May 2022
Accepted: 24 June 2022
UDC: 616-001.17:615.451.3
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CONFLICT OF INTEREST
The authors have no conflicts of interest to declare.
FUNDING
Not applicable.

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, 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.

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HHCys conditions contribute to the development of endothelial dysfunction. Prolonged negative effects of HHCys 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, HHCys affects all pathological processes leading to the formation of atherosclerotic plaques [6, 9, 11, 21].

Thus, increasing the concentration of HHCys 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 HHCys at a dose of 200 mg/kg body weight intragastrically 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 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 myofilaments 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...
category of patients with a HCys plasma level of 15.72±
Thus, histological examination of aortic fragments in this
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concentration of plasma HCys has a negative impact on
coronary artery bypass grafting an increase in the
carbonic anhydrase activity noted in the serum of patients
causes the development of cardiomyocyte cytolysis, as
animals. According to biochemical studies, HHCys also
time, the levels of these compounds were directly related
to the concentration of HCys in the plasma of experimental
animals. According to research in recent years, HHCys causes
the development of a wide range of disorders of the
nervous 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 HCs 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. HCs also destroys the enzyme
dimethylaminohydrolase and leads to the accumulation of
NO synthase inhibitor - asymmetric dimethylarginine. It is
also known that thiolactone HCs is able to interact with
lysine-rich proteins, acts as a trigger of ER stress and
apoptosis of vascular wall endothelial cells [14].

Scientists note that HCs 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 LDL. Under conditions of HCs,
their content increases due to inhibition of the synthesis of
apolipoprotein 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].

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
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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 a wide range of disorders of the
endothelial and myocardial layers, as well as changes in
the microcirculatory channel.
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канальці гладкої ендоплазматичної сітки та Т-трубочки розширені. Дані знахідки свідчать, що в дорослих щурів ГГц зумовлювало розвиток патологічних змін в ендокарді, міокарді досліджуваних тварин та в мікроциркуляторному русі.

Ключові слова: гіпергомоцистеїнемія, серце, м'язові волокна, кардіоміоцити, мітохондрії, саркомери, щури.