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ULTRASTRUCTURAL ORGANISATION OF THE SPLEEN OF OLD RATS IN PERSISTENT HYPERHOMOCYSTEINEMIA

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Annotation. *Homocysteine (Hz) is a naturally occurring amino acid formed during methionine metabolism, involving numerous cofactors and enzymes. While homocysteine plays a crucial role in keeping the essential amino acid methionine stable in the body, elevated homocysteine levels can have detrimental effects. An increase in the concentration of homocysteine in the blood serum is considered an independent marker of risk for cardiovascular diseases and pathologies of the respiratory system. This study aims to study ultrastructural organisation in old rats with persistent hyperhomocysteinemia. The experiment involved 22 male white rats aged 24-26 months, divided into control and experimental groups of 11 individuals. To simulate persistent hyperhomocysteinemia, the experimental group was administered D, L-thiolactone homocysteine hydrochloride at a dose of 200 mg/kg body weight intragastrically in a 1% starch gel solution once a day for eight weeks. At the end of the experimental period, the animals were humanely removed from the experiment by decapitation under thiopental anaesthesia for further analysis. Pieces of spleen 0.5-1 mm were fixed in a 2.5% glutaraldehyde solution on a phosphate buffer pH of 7.2-7.4. Subsequently, they were introduced into an epon-araldite mixture according to the generally accepted technique. Sections were made from the resulting blocks and stained with toluidine blue and Hayat. After precision microscopy of thin sections, ultrathin sections were made, which were contrasted with a 2% solution of uranyl acetate and lead citrate. The sections were examined and photographed under an electron microscope PEM125K with a magnification of 6-20 thousands of times. In the modelling of hyperhomocysteinemia in old rats, the stroma of the organ was characterised by the growth of connective tissue elements. Foci of lymphocyte apoptosis was noted in the spleen's white pulp. In the macrophages of the white pulp, lysis and fragmentation of nuclei, as well as accumulation of large amounts of residue in the cytoplasm, were observed. Plasma cells were numerous, and some of them showed signs of destruction. In the red pulp of the spleen, due to hemolysis of erythrocytes, the cytoplasm of macrophages was overflowing with hemosiderin and lipofuscin granules. The latter's presence is also evidence of active lipid peroxidation processes.*

Keywords: *hyperhomocysteinemia, spleen, macrophages, lipofuscin, plasmocytes, rats.*

Introduction

Homocysteine (Hz) is an amino acid formed during the metabolism of methionine, which occurs with the participation of many enzymes and cofactors [6, 9]. During the biochemical transformations of methionine, two essential intermediates are formed: S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) [7]. Usually, the ratio of SAM to SAH should be balanced. This balance is disturbed under certain circumstances (deficiency of vitamins B6, B9, B12, bad habits, the action of certain drugs). The imbalance can be associated with some diseases, such as inflammatory bowel disease, type II diabetes mellitus, coronary heart disease, stroke, etc. [5, 8].

In plasma, homocysteine exists in four forms: protein-bound homocysteine, the disulfide form of homocysteine, free homocysteine and the thiol form of this compound. Its average level in the body ranges from 5 to 15 μmol/L. Homocysteine plays a vital role in keeping the content of the essential amino acid methionine at a constant level. However, a significant increase in its concentration can have negative consequences [14]. With a Hz content above 15 μmol/L, hyperhomocysteinemia (GHz) is diagnosed. Scientists distinguish the following degrees: mild, in which Hz ranges from 15-30 μmol/l, moderate - 30-100 μmol/l and severe - 100 μmol/l or more. Moderate GHz before the age of 40 is usually asymptomatic. However, the results of studies conducted in recent years have shown that even the level of Hz of 10-12 μmol/l, which is the norm for people over 50 years of age, in the presence of concomitant diseases (cardiovascular pathology, kidney disease) and some other risk factors, should be considered as moderate GHz [13, 17].

The concentration of serum Hz is considered an independent marker of the risk of cardiovascular diseases and pathologies of the respiratory system. It is known that elevated levels of Hz in the blood can cause endothelial dysfunction, increase vascular production of reactive oxygen species (ROS), and decrease the bioavailability of endothelial nitric oxide (NO). As a rule, these processes become triggers for atherosclerosis development. In addition, the activation of the immune system may also play a vital role in the onset and progression of cardiovascular disease. Numerous immune defence cells, such as macrophages, dendritic cells, T lymphocytes, and B lymphocytes, are activated in this process. At the same time, the secretion of complement proteins, interleukins, tumour necrosis factor, and pro-inflammatory mediators is induced [3, 11]. In addition, recent experimental studies also confirm that certain factors, such as C-reactive protein, adhesion molecules, and metalloproteinases, are

positively correlated with Hz concentration [10]. However, the association between serum Hz levels and inflammatory/immune factors in healthy populations has not been systematically investigated. The sources of scientific literature also do not contain data on ultrastructural changes in the immune defence organs, particularly the spleen, at GHz, which actualises the chosen topic of study.

The study aimed to investigate the ultrastructural organisation of the spleen in rats aged 24-26 months with chronic hyperhomocysteinemia. The potential implications of our findings could significantly advance our understanding of the effects of hyperhomocysteinemia on the immune system and potentially lead to new strategies for managing related health conditions.

Materials and methods

We studied chronic hyperhomocysteinemia by experimenting on white male rats according to international guidelines for biomedical studies involving animals. The study adhered to the "General Principles of Work with Animals" approved by the First National Congress on Bioethics (Kyiv, Ukraine, 2001). It complied with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, France, 1986) [2]. The Committee approved it on Bioethics of VNMU. The scientific research complied with ethical principles (Protocol No. 3, dated 10/17/2019).

The experiment involved 22 white male rats aged 24-26 months, selected from the vivarium of National Pirogov Memorial Medical University, Vinnytsya. Laboratory rats were kept under standard vivarium conditions with a 12-hour night/day cycle and provided, by generally accepted norms, with water and balanced granulated feed ad libitum. The animals were carefully divided into control and experimental groups of 11 individuals during the experiment. Chronic hyperhomocysteinemia was induced by administering to experimental group D L-thiolactone homocysteine hydrochloride (Acros Organics, Italy) at a dose of 200 mg/kg body weight intragastrically using a 1% starch gel solution (1 ml/100 g rat weight), once daily for eight weeks. Upon completion of experimental simulations of chronic hyperhomocysteinemia, the animals were subjected to humane euthanasia using decapitation under thiopental anaesthesia (thiopental sodium 100 mg/kg).

Pieces of spleen 0.5-1 mm in size were fixed in a 2.5% glutaraldehyde solution on phosphate buffer pH 7.2-7.4. Subsequently, they were introduced into the epone-araldite mixture according to the generally accepted method [1, 4]. Sections were made from the resulting blocks and dyed with toluidine blue and Hayat. After precision microscopy of thin sections, ultrathin sections were made using ultramicrotomes LKB III (Sweden) and Reihart (Austria), which were contrasted with a 2% solution of uranyl acetate and lead citrate. The sections were examined and photographed under an electron microscope PEM125K with a magnification of 6-20 thousand times.

Results. Discussion

Administration of homocysteine to old rats was accompanied by the most pronounced changes in the ultrastructural components of the spleen. Compared to young and adult rats, significant destructive manifestations of hyperhomocysteinemia were observed in old animals. The spleen's stroma noted connective tissue components, particularly reticular cells. They had an elongated shape and hyperchromic nuclei. They were characterised by perinuclear oedema. The cytoplasm has a granular endoplasmic reticulum and the Golgi complex. In the white pulp of the spleen, under these conditions, as in previous groups of experimental animals, areas of death of T-lymphocytes were determined. This picture was especially pronounced in periarterial lymphoid sheaths. Lymphocytes that underwent apoptosis had dark, bizarre-shaped pycnotic nuclei. Their cytoplasm was pronouncedly enlightened, containing mainly mitochondria. The latter increased significantly in size due to oedema. Their cristas underwent lysis. According to Q. Zhang et al. (2002), GHz significantly potentiates the proliferation of T lymphocytes in the spleen of mice by partially inhibiting their apoptosis [16]. Increased IL-2 levels and ROS production accompanied t-cell proliferation. It was also established to increase B-lymphocyte proliferation by activating the signalling pathways - protein kinase C, p38 MARC and NF-kB transcription factor [14, 15].

Macrophages were numerous and located near plasma cells in the white spleen pulp of old rats. In macrophages, the nucleus shifted mainly towards the poles of cells due to the accumulation of excessive detritus in the cytoplasm. In some cells, nuclear lysis and fragmentation were clearly defined (Figs. 1, 2).

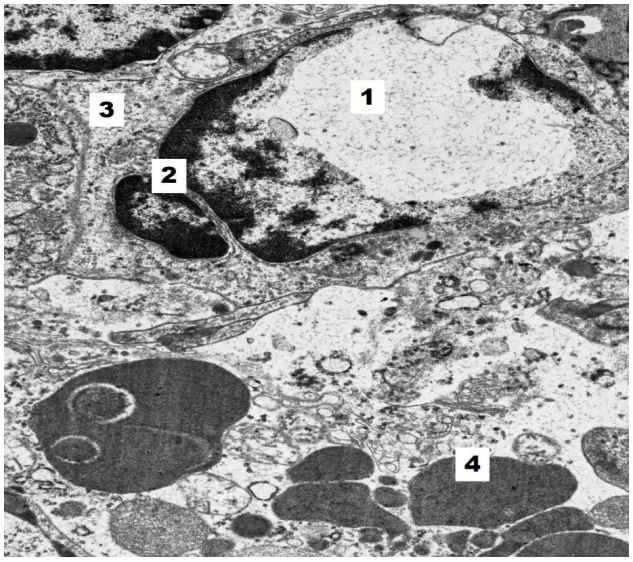

Fig. 1. Ultrastructure of the spleen of an old rat with hyperhomocysteinemia. Macrophage nucleus lysis (1), nuclear fragmentation (2), macrophage cytoplasm (3), and erythrocyte (4). Magnification: x 9000.

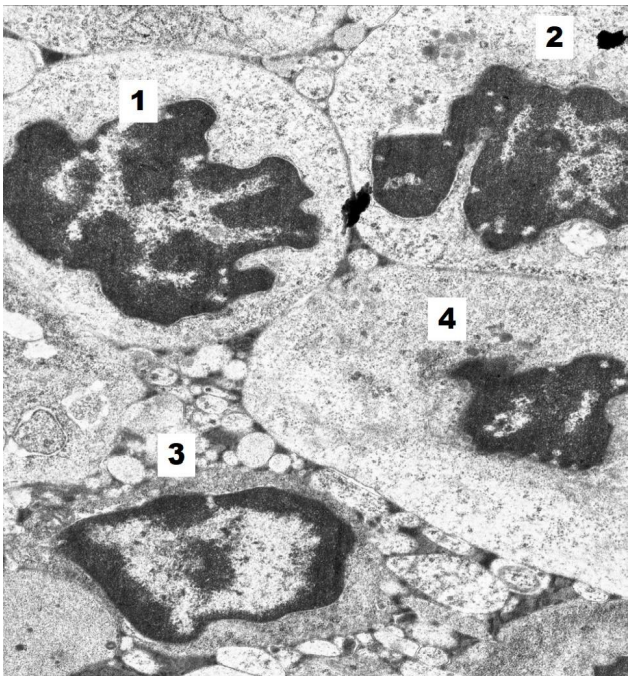

Fig. 2. Ultrastructural changes in the spleen of an old rat with hyperhomocysteinemia. Pycnosis of the lymphocyte nucleus (1), macrophage (2), lymphoblast (3), and plasma cell (4). Magnification: x 9000.

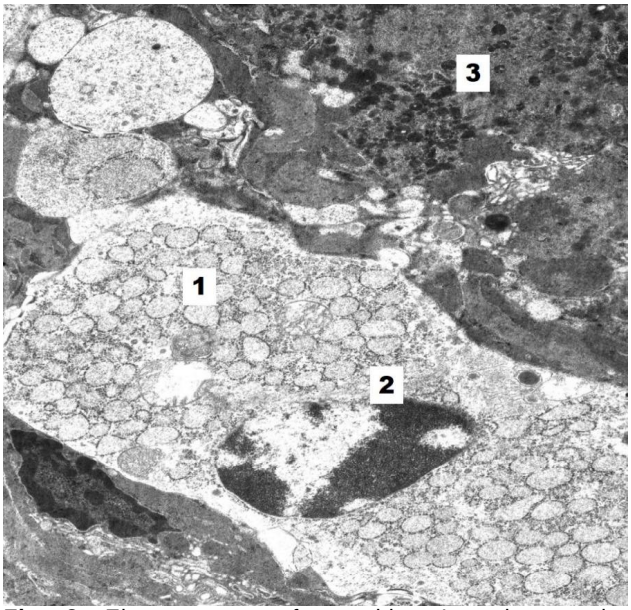

Fig. 3. Electronogram of an old rat's spleen under hyperhomocysteinemia conditions. Plasma cytoplasm (1), plasmocyte nucleus (2), macrophage fragment (3). Magnification. x 9000.

Plasma cells, in turn, increased quantitatively due to increased proliferation of B-lymphocytes. Plasma cells varied considerably since those with a preserved morphological structure and signs of pronounced submicroscopic shifts in organisation were observed. The preserved macrophages had a large nucleus with invaginations of the karyolemma. Heterochromatin was located under the karyolemma, predominating over the euchromatin. The nucleolar was continuous, and the nuclear pores were pronounced. The cytoplasm of such plasma cells contained dilated tubules of the granular endoplasmic reticulum, mitochondria, and free ribosomes. A significant part of the cytoplasm was occupied by swollen, dilated tubules of the granular endoplasmic reticulum (Fig. 3).

Studies of the effect of elevated homocysteine levels on the respiratory system of old rats have found that under such conditions, there is an increase in the number of active forms of plasma cells surrounded by cellular detritus, destroyed erythrocytes and collagen fibres in the interalveolar space. It was found that the cytoplasm of plasma cells is filled with dilated tubules of the granular endoplasmic reticulum containing gamma globulins. In addition, macrophages containing clusters of cytoplasmic lysosomes, auto- and phagosomes were found in the alveolar wall [12].

The sinusoidal vessels of the spleen of old rats were dilated, and erythrocytes, leukocytes, and platelets were found in them. In the red pulp of the spleen of rats of this group with hyperhomocysteinemia, numerous platelets and their fragments were observed. Erythrocytes underwent hemolysis. Granular leukocytes, mainly neutrophils, were also noted. The defining feature of this group of experimental animals was the significant number of macrophages in the red pulp. They had hyperchromic nuclei, sometimes elongated in shape. Large phagosomes, remnants of destructively altered erythrocytes, granulocytes and platelets were found in their cytoplasm. In addition, they had accumulations of hemosiderin and lipofuscin granules. The latter facts are characteristic not only of old

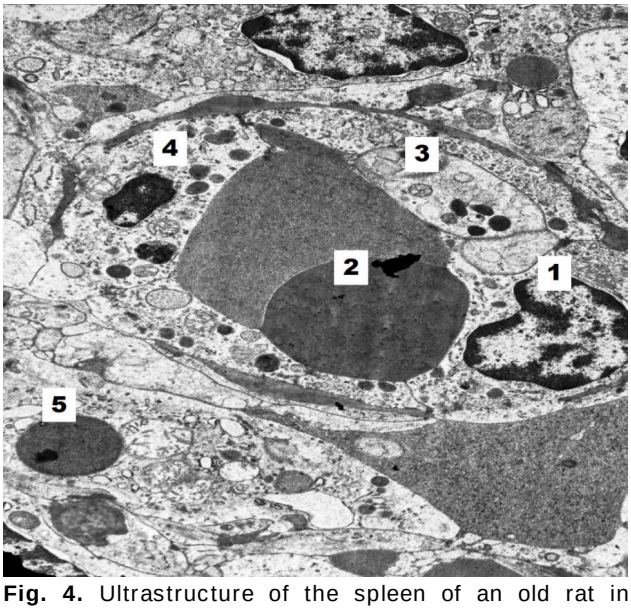

Fig. 4. Ultrastructure of the spleen of an old rat in hyperhomocysteinemia. Macrophage nucleus (1), phagocytosed erythrocyte (2), phagocytosed platelet (3), macrophage cytoplasm (4), lymphocyte apoptosis (5). Magnification. x 9000.

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age but also of the development of oxidative stress and peroxidation of lipids (Fig. 4).

Conclusions and prospects for further development

1. In modelling hyperhomocysteinemia in old rats, the organ's stroma was characterised by the growth of connective tissue elements.

2. In the white pulp of the spleen, foci of lymphocyte apoptosis were noted. In the macrophages of the white pulp, lysis and fragmentation of nuclei and the accumulation of large amounts of detritus in the cytoplasm were observed. Plasma cells were numerous; some of them showed signs of destruction.

3. In the red pulp of the spleen, due to hemolysis of erythrocytes, the cytoplasm of macrophages was overflowing with granules of hemosiderin and lipofuscin. The latter's presence is also evidence of active lipid peroxidation processes.

A promising direction for further research is the expansion and deepening of the intensification of research on various levels of the structural organisation of the spleen. This approach can significantly enhance our understanding of the progression of pathological processes in the spleen of rats during long-term chronic hyperhomocysteinemia.

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УЛЬТРАСТРУКТУРНА ОРГАНІЗАЦІЯ СЕЛЕЗІНКИ СТАРИХ ЩУРІВ ПРИ СТІЙКІЙ ГІПЕРГОМОЦИСТЕЇНЕМІЇ

Гриценко А. С.

Анотація. *Гомоцистеїн (Гц) - це природна амінокислота, яка утворюється під час метаболізму метіоніну, процесу за участю численних кофакторів та ферментів. У той час як гомоцистеїн відіграє вирішальну роль у підтримці незамінної амінокислоти метіоніну в організмі на стабільному рівні, підвищений рівень гомоцистеїну може мати згубний вплив. Підвищення концентрації гомоцистеїну в сироватці крові вважається незалежним маркером ризику серцево-судинних захворювань і патологій дихальної системи. Метою дослідження є вивчення ультраструктурної організації селезінки у старих*

щурів із стійкою гіпергомоцистеїнемією. В експерименті брали участь 22 білих щури-самці віком 24-26 місяців, розділених на контрольну та дослідну групи по 11 особин у кожній. Для моделювання стійкої гіпергомоцистеїнемії щурам дослідної групи вводили D,L-тіолактону гомоцистеїну гідрохлорид у дозі 200 мг/кг маси тіла внутрішньошлунково на 1% розчині крохмального гелю 1 раз на добу протягом 8 тижнів. По закінченню експериментального періоду тварин гуманно виводили з експерименту шляхом декапітації під тіопенталовою анестезією для подальшого аналізу. Шматочки селезінки розміром 0,5-1 мм фіксували в 2,5% розчині глутаральдегіду на фосфатному буфері pH 7,2-7,4. Згодом їх вводили в суміш епон-аральдиту за загальноприйнятою методикою. З отриманих блоків виготовляли зрізи, які фарбували толуїдиновим синім і Hayat. Після прицільної мікроскопії тонких зрізів, виготовляли ультратонкі зрізи, які контрастували 2% розчином уранілацетату та цитрату свинцю. Зрізи досліджували та фотографували під електронним мікроскопом PEM125K зі збільшенням в 6-20 тисяч разів. При моделюванні гіпергомоцистеїнемії у старих щурів строма органу характеризувалась зростанням елементів сполучної тканини. У білій пульпі селезінки відмічали вогнища апоптозу лімфоцитів. У макрофагах білої пульпи спостерігали лізис та фрагментацію ядер, накопичення великої кількості детриту в цитоплазмі. Плазматичні клітини були чисельними, окремі з них мали ознаки деструкції. В червоній пульпі селезінки, внаслідок гемолізу еритроцитів, цитоплазма макрофагів була переповнена гранулами гемосидерину та ліпофусцину. Наявність останнього є також свідченням активних процесів перекисного окислення ліпідів.

Ключові слова: *гіпергомоцистеїнемія, селезінка, макрофаги, ліпофусцин, плазмоцити, щури.*