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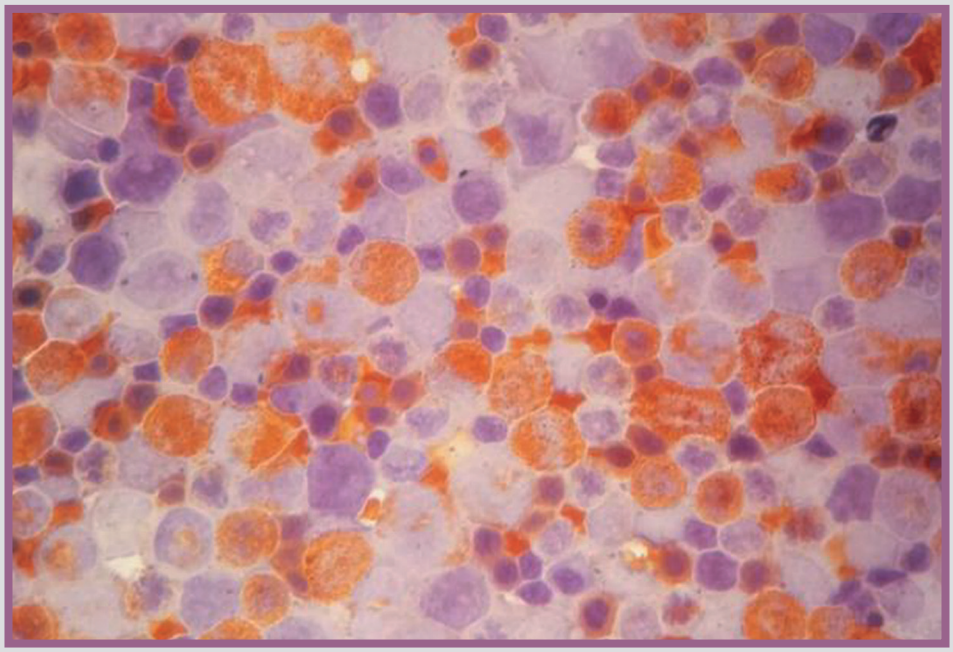

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REVIEW

Hyperhomocysteinemia in the pathogenesis of cardiovascular and endocrine diseases: translational messages

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ABSTRACT

For about half a century, researchers have been particularly interested in the amino acid homocysteine (Hcys), a product of methionine dimethylation. Hcys metabolism is based on two biochemical constants: remethylation and trans-sulfuration. The balance between these two mechanisms determines Hcys level in the body. For the functioning of both pathways, a sufficient concentration of vitamins B1, B6, B12 and folic acid, which act as cofactors in remethylation and transulfuration reactions, is required. Under normal conditions, Hcys is present in the human body in the range of 5-15 μmol/L and plays an important role in maintaining normal levels of the essential amino acid methionine. According to modern hypotheses, in addition to the physiological function Hcys has a pathogenetic effect. It damages the tissue structures of blood vessels, initiating the release of cytokines. The accumulation of Hcys in the blood leads to loosening of the walls of the arteries, the formation of local defects in the endothelium, increasing the risk of thrombosis. In addition, it is a potential procoagulant due to its ability to inhibit antithrombin III, protein C and activate factors V and XII, which play a particularly important role in the development of atherothrombotic and cardiogenic strokes. One of the mechanisms through which the toxic effect of Hcys is realized is through its ability to generate reactive oxygen species (ROS). In fact, possessing an active thiol group, Hcys is easily oxidized, causing the production of powerful acid radicals. Increased levels of Hcys lead to the development of ROS by inhibiting the transcription, translation and catalytic activity of major antioxidant enzymes. Since Hcys is an independent risk factor for many diseases, including cardiovascular and endocrine pathology, the study of the peculiarities of its metabolism is relevant.

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Diseases of the cardiovascular (CV) and endocrine systems occupy a significant place in the morbidity burden of the world's population.[1-3] Therefore, their prevention, based on identification and elimination of risk factors is of paramount relevance.[4-8] One of the recently identified risk factors is the increased plasma homocysteine (Hcys) level.[9]

Hcys is a thiol-containing amino acid formed during the metabolism of methionine.[10] It is metabolized by complex biochemical transformations, including remethylation and trans-sulfation. A significant amount of Hcys undergoes remethylation in the liver by methionine synthetase, a coenzyme requiring vitamin B12 as cofactor, which transports the methyl group from 5-methyltetrahydrofolate to Hcys. In turn, the last is formed by the reduction of 5, 10-methylenetetrahydrofolate with the participation of the enzyme 5, 10-methylenetetrahydrofolate reductase (MTHFR). The synthesis of 5, 10-methylenetetrahydrofolate occurs by transferring the hydroxymethyl group from serine to tetrahydrofolate. The source of the last is vitamin B9.[11-13] The reduction of Hcys to methionine is extremely important, as it is involved in the synthesis of proteins, nucleic acids and creatinine. An alternative remethylation pathway is also known. This is implicated in the transport of the

methyl group from betaine to Hcys by the enzyme betaine-homocysteine methyltransferase (BHMT) and is carried out in both the liver and the kidneys. The process of trans-sulfation takes place under the action of cystathionine-β-synthase (CBS), the coenzyme of which is vitamin B6 and is characterized by the formation of such end products as cysteine, ammonia, α-ketobutyrate. Cysteine, in turn, by oxidation, can convert to taurine and inorganic sulfates, which are necessary for the synthesis of heparin, heparan sulfate and chondroitin sulfate.[14-16] Thus, the presence of sufficient amounts of vitamins B6, B9 and B12 is important for the implementation of metabolic pathways of Hcys transformations. It has been shown that S-adenosylmethionine (AdoMet) plays a major role in the regulation of Hcys metabolism, being an inhibitor of MTHFR and BHMT and an activator of CBS, thus ensuring the coordination of re-methylation and trans-sulfation processes.[17, 18]

In this narrative review, we assessed the role of abnormal Hcys levels in the pathogenesis of cardiovascular and endocrine diseases, two systems deeply correlated.[19]

Data and hypotheses on pathogenetic involvement of hyperhomocysteinemia

Hcys accumulates in cells or enters directly into the blood. In the latter, it can be present in several forms, namely protein-bound (70-80%), disulfide and in compounds with cysteine (20-30%), reduced (thiol) form (1-2%). The level of Hcys in blood plasma in the range of 5-15 μmol/L is considered normal for adults. Today, it is known that its concentration can vary depending on age, sex and numerous exogenous and endogenous factors.[20] In particular, men have higher Hcys levels than women, but with the onset of menopause there is a tendency to increase also among the latter. This is associated with a decrease in estrogen, which has a protective effect on endothelial cells. Besides, the number of people with hyperhomocysteinemia (HHcys) increases with age due to decreased liver[21] and kidney function. There are 3 stages of HHcys: mild, in which the level of Hcys is 15-30 μmol/L, moderate when this is -31-100 μmol/L and severe, in case of Hcys>100 μmol/L.[22]

Currently, several factors that cause the development of HHcys have been identified. Hcys metabolic processes are directly dependent on vitamins B6, B9 and B12, which act as cofactors and donors of methyl groups and folate. In case of their absence or insufficient amount in the body, Hcys is unable to be utilized and accumulates in cells and blood plasma. The main causes of deficient conditions are diseases of the gastrointestinal tract, in which there is a decrease in the absorption of vitamins. These include gastritis and peptic ulcer, frequently associated to *Helicobacter pylori* infection, celiac disease, enteritis, Crohn's disease, ulcerative colitis, liver diseases due to excessive alcohol intake.[23-26] It is also known that cancers such as those of the breast and pancreas, ovaries and lymphoblastic leukemia are accompanied by a state of HHcys, because malignant cells have high mitotic activity, requiring a significant number of methyl groups.[27-30]

A significant amount of scientific works has investigated the main pathogenetic mechanisms in which HHcys is involved. Among the most studied there are hypomethylation and homocysteinilation of proteins. The methylation process is important and integral in the regulation of protein and nucleic acid function, as well as gene expression. Under HHcys conditions, a significant decrease in its activity occurs due to the accumulation of S-adenosylhomocysteine (AdoHcys). The last has a strong inhibitory effect against methyltransferases. Normally, AdoHcys is rapidly destroyed by hydrolysis by the enzyme S-adenosylhomocysteine hydrolase (AdoHcyase). However, this process is reversible, and in the presence of high concentrations of Hcys and adenosine, on the contrary, promotes not the breakdown but the synthesis of AdoHcys, which significantly slows down the course of methyltransferase reactions. Hypomethylation is particularly unfavorable for DNA, as it causes a violation of the expression of several genes, which in turn is reflected in significant endothelial dysfunction, inhibition of its regeneration, vascular damage, excessive accumulation of lipids in arteries and increased thrombotic events. It was found that patients with an increased risk of arterial and venous thrombosis had a significant increase of AdoHcys level and a decrease of AdoMet in blood plasma and erythrocytes. In addition, it is known that HHcys disrupts the process of incorporation of thymidine into the DNA of aortic endothelial cells.[31, 32]

Homocysteinilation of proteins at HHcys is characterized by changes in their structure and functions and occurs during translation or posttranslational modifications. This process has two mechanisms. The first is that under the conditions of Hcys interaction with nitric oxide (NO), a compound called S-nitrosohomocysteine (S-nitroso-Hcys) is formed. The last easily separates the nitroso group, and the released Hcys remains in the protein. This incorporation significantly changes the structure of the protein and, consequently, the last is unable to fully perform its function. The second mechanism is characterized by the binding of proteins by disulfide bonds with thiolactone dur-

ing posttranslational changes of them. Thiolactone Hcys is an extremely active metabolite and has a cytotoxic effect. Normally, it is destroyed by the enzyme thiolactonase (arylesterase), which is part of high-density lipoprotein (HDL), in the presence of calcium ions, but at HHcys there is a significant accumulation of thiolactone in the blood. Thiolactone Hcys reduces the catalytic activity of superoxide dismutase (SOD) and glutathione peroxidase (GP), which have antioxidant properties. By homocysteinilation, important proteins lose their activity, for example, proaccelerin, fibrinogen and endothelial cell apoptosis factors. In addition, HHcys causes stress of the endoplasmic reticulum, leading to the so-called misfolding, which is manifested by a violation of the synthesis and maturation of proteins (the formation of their three-dimensional structure). Ultimately, this triggers a cascade of apoptotic reactions due to the accumulation in the cell of a significant number of unformed proteins.[33-35]

It has been proved that for every 5 μmol/L of Hcys increase the risk of CV disease increased by 16%. More than 60% of patients with coronary heart disease are diagnosed with HHcys. Similar data have been reported for the risk of myocardial infarction, congestive heart failure, restenosis after coronary artery bypass grafting, and severity of CV diseases. Experimental studies have shown that the pathogenesis of lesions of the CV system under the conditions of HHcys was based on several mechanisms, namely endothelial dysfunction, atherosclerosis, thrombosis, fibrosis and oxidative stress.[36-42]

Endotheliocytes of the vascular wall play an important role in the regulation of their tone, hemostasis and cell migration. Thiolactone Hcys, formed in excessive amounts, has a direct cytotoxic effect on endothelial cells, ultimately leading to their apoptosis. Decreased synthesis of NO under these conditions and reduced tissue sensitivity to it, cause vascular tone disorders. Prolonged negative effects of Hcys on the vascular wall led to the release of cytokines, chemokines (Monocyte chemoattractant protein-1 [MCP-1], interleukin [IL]-8), the expression of vascular cell adhesion molecule 1 (VCAM-1), the initiation of platelet and coagulation hemostasis, activation of thrombin synthesis, and inhibition of the anticoagulant and fibrinolytic systems. An increase in the level of Von Willebrand factor causes an increase in platelet adhesion.[43, 44] The link between HHcys and increased synthesis of factor V (proacelerin) has been proven. The last triggers the coagulation mechanism by stabilizing the prothrombin complex and forming sites of factor Xa binding to prothrombin. As the Hcys level increases, the activity of protein C decreases, which is accompanied by a decrease in the affinity of thrombomodulin for thrombin. These changes are due to the direct effect of HHcys on disulfide bonds within the thrombomodulin and protein C domains. Inhibition of heparan sulfate expression in endothelial cells and disruption of the interaction of tissue activator plasminogen (t-PA) and plasminogen itself have also been shown. Furthermore, Hcys prevents the interaction of t-PA with annexin II on the surface of endothelial cells, which reduces the generation of plasmin.[45]

Hcys affects all pathological processes leading to the formation of atherosclerotic plaques. This mechanism is triggered by the production of a significant number of free radicals (ROS), reduced activity of the antioxidant system, increased levels of nicotinamide adenine dinucleotide phosphate (NADP) (H)-oxidase. ROS cause 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, subsequently turn into macrophages. The last can transform into so-called foam cells due to the absorption of oxidized low-density lipoprotein (LDL). In case 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. A gradual increase in the subendothelial layer of foam cells under these conditions creates a vicious circle, causing a progressive increase in the number of atherosclerotic plaques.[46]

Careful analysis of the scientific literature proves that HHcys leads to structural rearrangement of the myocardium. This fact has been explained by the increased expression of matrix metalloproteinases (MMP). The result is the active synthesis of collagen in the interstitial space of the heart muscle, the formation of foci of fibrosis, electrical heterogeneity and dilatation of the chambers of the organ. Activation of lipid peroxidation (LP) causes significant disturbances in the metabolism of cardiomyocytes of the conduction system and the formation of electrical instability of the myocardium. There are pronounced changes in the lipid composition of the sarcolemma and sarcoplasmic reticulum of heart muscle cells under the influence of LP products. The last have a negative effect on mitochondria, deepening adenosine triphosphate (ATP) deficiency.[47-49] In parallel, there is a decrease in the activity of enzymes involved in maintaining the transmembrane potential of cardiomyocytes. HHcys is also characterized by disruption of ion channels, which leads to an increase in Na + and Ca2 + inside myocardial cells. It is believed that glutamate N-methyl-D-aspartate (NMDA) receptors

are also involved in this process. The result is a dispersion of the refractory period and an increase in the excitability of the conductive cardiomyocytes and, accordingly, the arrhythmogenic readiness of the heart muscle. In addition, the increase in Na + and Ca2 + levels inside the cells further exacerbates mitochondrial dysfunction. This is associated with impaired electron transport in the respiratory chain and development of hypoxia. O2 deficiency causes the activation of Ca2 + sensitive receptors of the sarcoplasmic reticulum and the entry of Ca2 + ions into the mitochondria. Under these conditions, the mechanisms of cardiomyocyte apoptosis are activated, and focal fibrosis develops. There is also a compensatory increase in the expression of factors that activate neoangiogenesis – hypoxia-induced factors (HIF), vascular endothelial growth factor (VEGF).[50-52]

Focusing on endocrinology, thyroid hormones regulate the activity of the enzyme MTHFR, so a decrease in the functional activity of the thyroid gland leads to changes in methionine metabolism and the development of HHcys. However, the state of hypothyroidism is characterized by impaired absorption of folate and vitamin B12 and decreased activity of remethylation enzymes BHMT, AdoMet synthetase, AdoHcyase.[53-55]

Experimental hyperglucocorticoidemia is accompanied by inhibition of liver activity of the enzymes CBS, cystathionine-γ-lyase (CSE) and cysteine aminotransferase (CAT). The result is an increase in the level of Hcys with a simultaneous decrease in hydrogen sulfide (H2S) activity.[56]

HHcys causes a violation of catecholaminergic regulation of the reproductive function of female rats by affecting the noradrenergic system of the medial preoptic region of the hypothalamus and the dopaminergic system in the middle elevation with arcuate nuclei. This supports the negative impact of HHcys on the synthesis and secretion of gonadotropins and impaired hypothalamic regulation of reproductive function.[57, 58]

The problem of HHcys in the modern world and the COVID-19 pandemic, that is inducing wherever significant damages are relevant.[56] Recent studies showed that high plasma Hcys level in patients with COVID-19 is a risk factor for severe disease, complications, and even death. It has been reported that men with chronic diseases and HHcys have an increased vulnerability to poor prognosis during COVID-19. Therefore, in this context, early risk stratification by measuring plasma Hcys levels and screening for MTHFR polymorphism is advisable.[59]

HHcys and the state of antioxidant system

Among the numerous mechanisms of human adaptation, the leading role is occupied by biochemical processes, providing an increase in the body's resistance to the adverse effects of environmental factors.[60] An important place, among these bioregulators, belongs to the system of antioxidant protection (AOP) because it is known that the action of factors of different genesis activates the processes of free radical oxidation.[61-64] During the normal functioning of the body, free radical processes and the activity of the AOP system form an oxidative metabolism. The development of various pathological conditions is characterized by excessive production of ROS, accompanied by depletion of reserves of the antioxidant system (AS). This state is called oxidative stress (OS) and acts on the structure and function of cells by changing the properties of their membranes due to increased processes of lipid oxidation.[65, 66] Also, biological macromolecules such as DNA and proteins are oxidized by suppressing the endogenous mechanisms of the AOP system. These changes are accompanied by increased synthesis of hydrophobic radicals, which, interacting with polyunsaturated fatty acids, increase the production of lipid radicals. The last react with O2 and iron complexes, supporting lipoperoxidation processes and creating a vicious circle, as they provide the formation of more and more ROS. Formed lipid radicals and their oxidation products such as malonic dialdehyde (MDA), diene and triene conjugates induce oxidative modification of proteins and nucleic acids.[67, 68] Lipoperoxidation threatens the stability of membrane structures in general and this is reflected in the functional state of associated proteins, including those with catalytic activity. In such a way inhibits the activity of glucose-6-phosphatase and Na+ / K+-ATPase. Although there is a violation of cellular organelles, the greatest impact is on mitochondria. In particular, the damage is due to the inhibition of the activity of matrix enzymes and the imbalance in the work of the electron transport chain.[69, 70]

The AS is extremely complex and multicomponent and contains as main structural elements enzymatic antioxidants. Among them, there are SOD, GP, catalase, paraoxonase (PON).[71, 72] SOD is a metalloprotein, an enzyme of the oxidoreductase class, consisting of two identical subunits, each of which contains Cu and Zn ions. Its main function is the dismutation of oxygen radicals, namely superoxide anions into hydrogen peroxide and molecular oxygen. There are varieties of SOD, such as cytosolic (SOD1), mitochondrial (SOD2) and extracellular (SOD3). SOD 1 and SOD2 are dimers, while SOD3 is a tetramer.

Cu ions are present in the active site of SOD1 and SOD3, and Zn serves only as a structural component. SOD2, in contrast to the last, contains Mn in the active site. SOD1 shows about 80-90% of all SOD activity. The enzyme is localized mainly in the cytoplasm of the cell but may be present in lysosomes, peroxisomes, nucleus, and intermembrane space of mitochondria. SOD2 performs approximately 10% of antioxidant activity and is expressed in the mitochondrial matrix. SOD3 is localized on the surface of cell membranes or in the extracellular matrix. In blood vessels, it is connected to the surface of endothelial cells.[73, 74]

Catalase belongs to the class of oxidoreductases and catalyzes the heterolytic cleavage of the O-O bond in H2O2. The activity of this enzyme is a synergistic activity of SOD. Catalase is an important component of systems where electrons are transported by cytochromes. The main site of localization is peroxisomes. The enzyme is detected in erythrocytes, hepatocytes, kidney cells. The structure of catalase is a tetramer consisting of four subunits, each of which contains protoporphyrin IX and chelated iron atoms. In the coordination sphere of hemin iron four places belong to porphyrin cycles, the fifth belongs to imidazole histidine residues, and in the sixth, there are processes of catalytic cleavage of H2O2.[75, 76]

GP catalyzes the reduction of lipid peroxides and hydrogen peroxide. The enzyme is a selenium-containing tetrameric glycoprotein. There are several isoforms of GP encoded by different genes. Isoenzymes differ in location and substrate specificity. The most common is GP1, which is located in the cytoplasm of liver and intestinal cells. The gene encoding this enzyme is located on chromosome 3. A characteristic feature of GP1 is the presence of a polymorphism of the polyalanine sequence at the N-terminal end. In addition to this function, it takes part in protecting cells from CD95-induced apoptosis.[77, 78]

Paraoxonase is an enzyme of the hydrolase family. There are three isoforms, namely PON1, PON2 and PON3. PON1 is a glycoprotein consisting of 55 amino acid residues. It has a wide substrate specificity, but normally the primary substrates are oxidized lipids. It is expressed in the liver and is in the HDL-bound state by the N-terminal hydrophobic signal peptide. Antioxidant properties of PON1 are realized by the destruction of specific cholesterol esters and phospholipids in oxidized lipoproteins. PON2 is an intracellular enzyme not found in the blood. The predominant site of localization is mitochondria, where it prevents the development of OS. The highest concentrations of PON2 are in dopaminergic neurons and astrocytes. PON3, as well as PON1, are associated with HDL, protecting them from oxidative modification. Also, it can participate in the breakdown of lactones. PON3 inhibits the formation of atherosclerotic plaques and reduces the level of the hormone leptin in the blood. This isoform is found in the liver and kidneys.[79, 80]

The analysis of scientific sources makes it possible to identify several other components of AS. It is known that AS includes glutathione-S-transferase, peroxiredoxins and ceruloplasmin. Glutathione-S-transferase provides the interaction of lipid peroxides with reduced glutathione. Peroxiredoxins control the level of cytokine-induced peroxides. Ceruloplasmin participates in the oxidation of Fe2 + ions to Fe3 + without the formation of hydroxyl radicals, preventing the initiation of free radical processes.[81, 82]

The main list of ROS includes superoxide anion, singlet oxygen, hydrogen peroxide and hydroxyl radical. Under the conditions of OS, these compounds are generated in excessive amounts, causing a modification of the main macromolecules of the organism. It is known that the dysfunction of the AS leads to the oxidation of lysine, proline, arginine, which are structural components of the main proteins of the human body.[83, 84] ROS affect all levels of organization of the proteins, creating conditions for their aggregation or fragmentation. The ability of free radicals to cause modification of enzymes that contain metals with variable valence has been proven. Under these conditions, OS leads to the peroxide-dependent synthesis of hydroxyl radical. The last, interacting with amino acids that are part of the active center of the enzyme, causes inhibition of its activity or complete inactivation. In some cases of absence or defects of AS enzymes, severe biochemical and metabolic changes in cell activity develop. In particular, SOD mutation is associated to progressive dysfunction of the first complex of the respiratory chain, inhibition of succinate dehydrogenase activity and the Krebs cycle. Furthermore, the carboxyl groups of protein molecules are subject to oxidative modification, and the process itself is accompanied by the formation of both Schiff bases and numerous crosslinks of proteins and, accordingly, changes in their activity.[85, 86]

The mechanisms underlying the pathogenesis of cell membrane damage due to the destruction of their bilipid layers are important. This process consists in the production of polar groups, namely ketones, aldehydes, lipoperoxides in the hydrophobic part of the plasmalemma and causes fragmentation of phospholipids. These changes are accompanied by autooxidation of carbohydrate determinants of the glycocalyx and transmembrane proteins, such

as Na + / K + -ATPase and Ca2 + -ATPase, which impairs their functional ability. Under these conditions, the influx of Ca2 + into the cell increases, which activates phospholipase A2, triggers a cascade of arachidonic acid, and therefore, causes cell death by necrosis. The hydroperoxides of the formed lipids are unstable compounds and can be converted very quickly into such oxidation products as diene conjugates due to the separation of hydrogen atoms from polyunsaturated fatty acid molecules, peroxide radicals, and malonic dialdehyde.[87]

Under the conditions of HHCys there is an increase in the blood of dinitrophenylhydrazones (DNFG), which are markers of OS and evidence of the accumulation of fragmented proteins in the heart muscle tissue. Ketone DNFG is an indicator of the growth of aggregated oxidized molecules. Ultimately, Hcys has a cytotoxic effect on cardiomyocytes and causes dysfunction of their organelles. Mitochondrial damage is accompanied by activation of free radical processes, damage of heart tissue in the form of oxidation of myoglobin, impaired binding of troponin to Ca2 + and decreased myocardial contractility. Characteristic is the start of the processes of necrosis and apoptosis of cardiomyocytes and the accumulation of protein degradation products in cell compartments. Mitochondrial dysfunction in experimental HHCys is manifested by an increase in lactate levels in the cytoplasm of cardiomyocytes and carbonylation of mitochondrial proteins. NO deficiency under these conditions inhibits the processes of aerobic oxidation, as evidenced by a decrease in the activity of succinate dehydrogenase, lactate dehydrogenase, and impaired oxidative phosphorylation.[88]

The generation of oxygen radicals in the cells of the cardiovascular system organs is carried out by such cytoplasmic enzymes as xanthine oxidase, NO synthase, and enzyme complexes cytochrome P450, NADPH-oxidoreductase, I and III complexes of the respiratory chain of mitochondria. NADPH oxidase is synthesized by neutrophils, blood monocytes and cardiomyocyte mitochondria. The last use of oxygen in redox processes is associated with the synthesis of ATP molecules. In the mitochondrial respiratory chain, electron transport is accompanied by the release of energy required for translocation of protons across the inner mitochondrial membrane from the matrix into the intermembrane space and the formation of the difference of electric potentials and concentration gradients. About 0.5-1.5% of oxygen entering the mitochondria is used to restore molecular oxygen in the I and III complexes of the respiratory chain or is converted into a superoxide anion, which acts as an intracellular messenger, affecting the radical-sensitive enzyme signaling systems. Under the conditions of HHCys, the generation of these free radicals increases significantly, develops OS, and leads to peroxidation of biomolecules of cell membranes of cardiomyocytes, damage of the DNA structure of mitochondria, and consequently, apoptosis of heart cells.[89, 90]

It was found that the introduction of methionine in rats for three weeks caused a violation of the normal functioning of the mitochondria of heart cells, which was manifested by an increase in lactate levels in the cytoplasm, as well as the development of OS and carbonylation of mitochondrial proteins. Activation of the AOS system somewhat compensated for the manifestations of OS but was not able to eliminate the negative impact of HHCys on the body. The authors also noted that under these conditions, the modeling of NO deficiency, by introducing N(ω)-nitro-L-arginine methyl ester (L-NAME), caused inhibition of aerobic oxidation processes in mitochondria. The last manifests in the form of a significant decrease in the activity of the enzymes' succinate dehydrogenase, lactate dehydrogenase and the accumulation of lactate in the cytoplasm of cardiomyocytes. Besides, inhibition of oxidative phosphorylation processes was registered in organelles.[91]

ROS are the cause of vascular atherosclerosis. In the presence of a significant number of them due to the HHCys-induced OS, LDL oxidation processes are activated. Oxidized lipids are captured by macrophages, causing them to turn into foam cells. This process is accompanied by increased activity of myeloperoxidase in macrophages, damage of the endothelial lining of the vascular wall, decreased NO production, hypercoagulation and proliferation of smooth muscle cells.[92]

Focusing on endocrine disorders, currently, the dysfunction of the thyroid gland is associated with an imbalance in the functioning of the body as a whole. In addition to regulating the basic metabolism, thyroid hormones affect the state of adaptation mechanisms and immune-antioxidant status. Therefore, it is likely that a change in their concentration may cause dysfunction of the AS. In patients with thyroid pathology, there is an imbalance in the pro-/antioxidant system with manifestations of OS. In case of hypothyroidism there are a decrease in catalase activity and an increase in SOD, *i.e.*, the diversity of enzymes. These changes are often accompanied by excess H2O2 production against the background of accelerated dismutation of the superoxide anion radical with increased SOD activity. H2O2 can become the most aggressive of ROS (HO-) in the reactions of Haber-Weiss, Fenton. Hydroxyl radical, having high reactivity and aggressiveness, leads to severe

metabolic disorders. Under these conditions, there is a decrease in the level of reduced glutathione and an increase in markers of endogenous intoxication - molecules of medium weight.[93]

Proteolytic profile of tissues in cardiovascular and endocrine diseases

Currently, proteolysis is considered as a special form of biological control, which occupies a central place in the implementation of various biochemical processes and the rapid physiological response of the organism to changing conditions. Proteolytic enzymes, which are active participants in the proteolysis system, control virtually all aspects of biological processes that occur at the molecular, cellular, tissue and organ levels. Proteolysis reactions play a special role not only in the regulation of intracellular protein metabolism but also in the translocation of the last inside and outside the cell, the formation of enzymes, hormones and other biologically active substances. All proteinases are divided by the reaction mechanism they catalyze, in which the name of the main groups of enzymes comes from amino acid residues in the active site, which provides the features of the catalytic mechanism. There are such families as serine, cysteine (thiol, sulfhydryl), aspartate (carboxyl), and metalloproteinases. These enzymes are now actively used to diagnose many diseases, including those of the cardiovascular and endocrine systems.[94]

According to the literature, there is a relationship between HHCys and activation of the proteolytic system. As mentioned earlier, Hcys causes the development of OS and currently, the most significant manifestation of the last is considered an oxidative modification of biomolecules, especially proteins, as this process is accompanied not only by the formation of stable and easy to study and interpret markers but significantly changes their functions. The mechanisms of irreversible oxidation of proteins with the formation of carbonyl derivatives are important. These proteins lose their native functions, forming aggregates that are subject to degradation. Numerous types of cellular and extracellular proteases act as agents of such degradation.[95]

Several studies showed that cysteine proteases are involved in the development of CV diseases. This fact is due to their action on the elements of connective tissue. In fact, it is known that cathepsins B and L can cause damage to collagen types II, IX, XI, as well as they have elastolytic and proteoglycanolytic effects.[96] It has been proved that the violation of NO synthesis, which occurred due to the increase in the level of Hcys, caused disorders of compartmentalization of lysosomes. For a long time, scientists have held a clear position on these organelles. It is known that they are stable compartments of the cell and the release of hydrolytic enzymes beyond them is possible only under conditions of apoptosis or necrosis. However, recent studies have shown high sensitivity of lysosomes to destabilizing factors. Also, the increased permeabilization of lysosomal membranes in the absence of a violation of its structure caused by HHCys, accompanied by the release of cathepsins into the cytosol, has been found.[97]

Another type of proteolytic enzymes associated with CV disorders are MMPs. The MMP family is a group of zinc-dependent endopeptidases actively involved in the degradation of the structural components of the basement membrane and extracellular matrix. They also have a role in the processes of apoptosis and angiogenesis. MMPs are the only enzymes that can destroy fibrillar collagen. This family includes collagenases (MMP-1, MMP-8, MMP-13), stromelysins (MMP-3, MMP-10), matrilisins (MMP-7, MMP-26), membrane type (MI-MMP) and gelatinase (MMP-2, MMP-10).[98, 99] These proteases have several domains. Due to the N-terminal signal domain, protein molecules are exported across the plasma membrane into the intercellular space. The propeptide domain of the enzyme ensures that the MMP remains in an inactive form and can cleave only when it is proteolytically activated. The catalytic domain, which contains a conserved amino acid sequence with three histidine residues bound to zinc ions, has endopeptidase activity. MMP-2 and MMP-9 contain fibronectin in their active sites, which allows them to bind with denatured collagen-gelatin. The MMP family has a variety of substrate specificity in the extracellular matrix. MMP-1 has the highest activity against collagen. They cleave the last into two fragments, which are further decomposed by the action of gelatinases MMP-2 and MMP-9. Extracellular targets of MMP-2 are also some matrix proteins such as laminin, elastin, type IV collagen, fibronectin. Stromelysins destroy proteoglycans, fibronectin, laminin, gelatin.[100, 101]

Significant research has been devoted to the role of MMPs in the occurrence or progression of CV diseases. For a long time, these enzymes were considered as belonging to the extracellular matrix. Currently, there is evidence that in the case of ischemia and cardiac perfusion, activation of MMPs within cardiomyocytes is possible. Under these conditions, MMPs cause the destruction of

sarcomeres, leading to damage of heart cells. Increased expression of MMP-2 in the heart of transgenic mice leads to the decreased functional activity of the heart muscle and pronounced morphological changes, namely ventricular remodeling, hypertrophy of contractile cardiomyocytes, lysis of myofilaments, destruction of sarcomeres, troponin I, light chains of myosin, α-actinin and fibroblasts proliferation. Besides, it is known that OS causes the activation of the intracellular isoform MMP-2, which due to significant structural changes in the myocardium leads to the development of heart failure. In the extracellular matrix, this enzyme is activated by peroxynitrite, which increases under the conditions of HHCys and OS. The presence of products of oxidative modification and glutathione affect the catalytic center of MMP-2, activating it.[102]

It has been established that MMPs play a significant role in the processes of destabilization of atherosclerotic plaques. Collagen types II and III are the main components of the plaque, they provide strength to their fibrous roof. MMP-1 degrades collagen, leading to loss of resistance to mechanical stress and sensitivity to rupture of the plaque cap, which causes myocardial infarction and ischemic stroke.[103, 104]

The effect of hormones on the proteolytic system of the body is not sufficiently studied, but there are some data on the probable role of thyroid hormones in its activation. According to researchers, iodine-containing thyroid hormones can increase the activity of LP, causing dysfunction in the protease/antiprotease system. Products of LP cause damage of the integrity of lysosomal membranes and the release of proteases into the bloodstream stimulates damage of proteins. Hyperthyroidism changes the intensity of the specified processes ambiguously. It has been noted the growth of diene conjugates of MDA, the basis of Schiff. At the same time, there is a decrease in the activity of the antioxidant system (SOD, GP, glutathione reductase) in the heart and kidneys of rats. In the case of hypothyroidism opposite changes are noted. The effect of thyroid hormones on the activity of MMPs was found in an experimental model of primary hypothyroidism in rats. The use of propyluracil (inhibitor of the processes of peripheral conversion of T4 to T3) cause a fivefold increase in the activity of MMP-2, MMP-4 and a decrease in the content of collagen types I and III, levels of TIMP1. Decreased T3 activity led to increased degradation of the extracellular matrix of the ovaries of rats by MMP, which disrupted the normal architecture and function of the tissue, and hence folliculogenesis.[105]

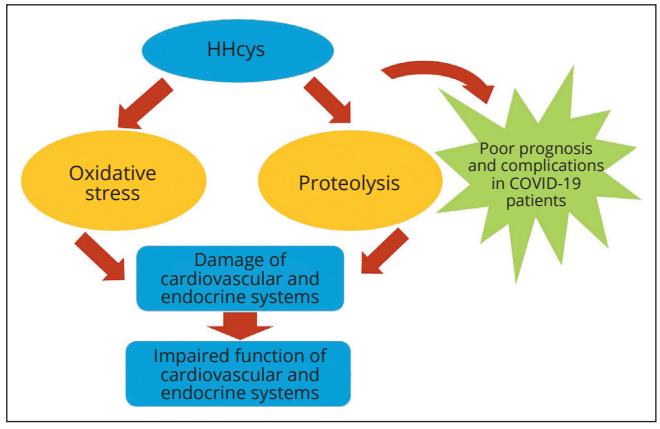

Figure 1.—Interaction between hyperhomocysteinemia, oxidative stress development, and damage to the cardiovascular and endocrine systems.

Conclusions

Analysis of the literature demonstrates the presence of a direct relationship between HHCys, OS development and damage to the CV and endocrine systems (Figure 1). The complex and multifactorial pathogenetic mechanisms do not allow to fully explore all links in the pathogenesis but attracts the attention of scientists to one of the new and important risk factors. Detection of OS markers at an elevated level of Hcys opens opportunities for timely elimination its negative impact and reduction of the degree of severity, and, consequently, prevention of irreversible changes in the structure of these systems. The normal functioning of the proteolytic system plays an important role in protecting the body from the accumulation of degradation products of protein molecules, as well as defective and oxidized-modified compounds. However, under the conditions of HHCys, OS there is an imbalance in the components of this system, which can cause excessive or uncontrolled proteolysis, cell death, remodeling of organ structure and, accordingly, changes in their functional capacity, which underlies the development of pathological conditions.

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