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REVIEWS

Mechanisms of Toxic Effects of Homocysteine on the Nervous System

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The review describes the metabolism of homocystein, causes of hyperhomocysteinemia, mechanisms underlying the respective negative effects on the nervous system, and main principles of correction of such disorders.

Keywords: homocysteine, hyperhomocysteinemia, toxic effects, pathogenesis, nervous system.

INTRODUCTION

The role of homocysteine in neurochemical and neurophysiological processes in mammalians, including humans, is difficult to overestimate. The metabolism of homocysteine is mostly based on its re-methylation and trans-sulfonation occurring at sufficient levels of vitamins B_6, B_{12}, and that of folic acid (vitamin B_9). The balance between the above-mentioned processes crucially determines the level of homocysteine in the organism. The effects of this compound on nerve tissues in the case of its excessive amount may be due to direct neurotoxic effects or are mediated by vascular mechanisms. Homocysteine affects many physiological processes in the human organism. A high level of homocysteine causes a direct cytotoxic effect on the endothelium, which simultaneously increases the consumption of nitric oxide and initiates the release of cytokines, cyclins, and other mediators of inflammation. Also, it causes excessive proliferation of smooth muscle cells of the vascular wall and of endotheliocytes, acts as a procoagulant, increases the concentrations of low-density and very-low-density lipoproteins, and reduces the production of endothelium-derived relaxing factor and sulfated glycosaminoglycans. Thus, the role of hyperhomocysteinemia in pathological events in the vascular system is twofold. First, it damages the endothelium, which is associated with early atherogenesis, and second, it increases the probability of development of thrombosis in veins and arteries, including cerebral ones.

Metabolism of Homocysteine. Homocysteine is a low-molecular weight thiol-containing essential amino acid. It is synthesized during a multistage process of metabolism of another essential amino acid, methionine [1, 2]. The human body is not able to synthesize the latter by its own; therefore, animal food products (meat, eggs, cottage cheese) are the necessary sources of methionine [3, 4]. In the case of sufficient levels of folic acid (B_9), B_6, and B_{12} vitamins, homocysteine can be used for methionine synthesis again [5–7].

As was mentioned above, homocysteine metabolism is mainly based on two biochemical processes, re-methylation and trans-sulfonation. The balance between these processes practically determines the level of homocysteine [8]. Vitamins B_1, B_6, folic acid (B_9), and B_{12} act as coenzymes in re-methylation and trans-sulfonation [9–12]; therefore, sufficient concentrations of the above agents are necessary for the functioning of both pathways. In the first step of homocysteine biosynthesis, the adenosine group from ATP attaches to the methionine molecule, which results in the

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synthesis of S-adenosylmethionine (SAM) [13]. This reaction is catalyzed by S-adenosylmethionine synthase. Then, the methyl group is transferred to the acceptor molecule. This leads to the synthesis of adenosine, while adenosylmethionine is then hydrolyzed to L-homocysteine. Such trans-methylation occurs in almost all cells. L-homocysteine may be included in two main metabolic pathways, back-conversion to methionine with tetrahydrofolate (with cobalamin, B_{12}, as a co-factor) or conversion to L-cysteine. Cystathionine synthase may turn homocysteine and serine into cystathionine; pyridoxine (vitamin B_6) functions as a cofactor in this reaction [14, 15]. Then, cystathionine can be broken down into cysteine and ketobutyrate with cystathionine lyase. Ketobutyrate may be metabolized to succinyl-CoA. This conversion of homocysteine into cysteine occurs in the small intestine, liver, kidneys, and pancreas [16–18]. Thus, the biochemical role of homocysteine is to maintain the endogenous stock of methionine (which is rapidly consumed during methylation) and to provide the possibility to synthesize cysteine and ketobutyrate (the latter is the precursor of succinyl-CoA) [19]. Cysteine, due to its high reactivity, is involved in many biochemical processes. It is a powerful antioxidant and is essential for the formation of disulfide bonds in proteins; it is also one of the main sources of sulfides for the metabolism of metal ions [20]. Succinyl-CoA is involved in the synthesis of acetyl-CoA, the main substrate for the tricarboxylic acid cycle [21]. A decrease in the amount of vitamin B_{12} or functional deficiency of the above-mentioned enzyme may cause irreversible conversion of homocysteine into cysteine. This reaction is catalyzed by cystathionine-β-synthase, which requires the presence of vitamin B_6 and goes through an intermediate product, cystathionine [22, 23]. In the case of deficiency of both reactions, homocysteine is eliminated into the extracellular space and then to the bloodstream. This is a kind of protective reaction against the toxic effect of excessive homocysteine on the cells [24].

Causes of Hyperhomocysteinemia. Hyperhomocysteinemia is a state when the homocysteine level in the blood exceeds 15 μM (15 to 30 μM, mild, 30 to 100 μM, moderate, and more than 100 μM, severe) [25]. Hyperhomocysteinemia is a multifactorial phenomenon; genetic and non-genetic mechanisms are responsible for its formation. Hyperhomocysteinemia can be both inherited and acquired [26].

First, we will describe hyperhomocysteinemia as a result of genetically determined defects in the enzymes involved in re-methylation and transsulfonation. One of the main methionine metabolism-related genes is *MTHFR* that codes the structure of methylenetetrahydrofolate reductase enzyme [27, 28]. Several allelic variations of this gene are known to cause the enzyme deficiency. Among those, the C677T point mutation is the most common. Cytosine at position 677 is replaced by thymine, which leads to the replacement of valine by alanine. This makes the MTHFR enzyme thermolabile and reduces its activity by approximately 35% [29]. Heterozygous carriers of this mutation are characterized by moderately increased homocysteine levels in the blood; the homozygous state is associated with more prominent hyperhomocysteinemia [30]. Another known point mutation is the replacement of adenine at position 1298 by cytosine (A1298C). Heterozygous carriers of this mutation do not possess an increased homocysteine blood level. However, the combination of C677T and A1298C heterozygous states leads to a significant decrease in MTHFR enzyme activity and a corresponding increase in the homocysteine level, which is commensurated with that in homozygous C677T carriers [31, 32].

Another factor is the alimentary deficiency of pyridoxine (B_6), cyanocobalamin (B_{12}), or folic acid (B_9), which can cause hyperhomocysteinemia even in people with the normal *MTHFR* gene structure [33, 34].

Many drugs affect the level of homocysteine [35]. Their effects may be mediated by the influence on the metabolism of vitamins, synthesis of homocysteine, kidney functions, or levels of a few hormones [36]. Of particular importance are methotrexate (a folic acid antagonist used for psoriasis treatment), some anticonvulsants (e.g., phenytoin that decreases the deposition of folic acid in the liver), nitrous oxide (an agent used for anesthesia; it inactivates vitamin B_{12}), metformin (a drug used to treat *diabetes mellitus* and the polycystic ovary syndrome), H_2-receptor antagonists (that reduce the absorption of vitamin B_{12}), and aminophylline (that inhibits vitamin B_6 activity) [37, 38]. Homocysteine levels may also be increased due to the intake of hormonal contraceptives.

Other factors contributing to increases in the homocysteine level are some comorbid nosologies. Among those, vitamin deficiency states and kidney failure are the most important [39].

Also, thyroid diseases, diabetes, psoriasis, and leukemia can trigger a significant increase in the blood homocysteine level. The most common reason for vitamin deficiency conditions leading to hyperhomocysteinemia is impaired absorption of vitamins due to gastrointestinal tract (GIT) diseases (e.g., the malabsorption syndrome) [40]. This explains (at least partly) a greater probability of vascular complications in the presence of chronic GIT diseases and the fact that strokes and infarctions, and not anemia, are frequent immediate reasons of death under conditions of vitamin B_{12} deficiency [41].

Cigarette smoking and consumption of large amounts of coffee can also contribute to the development of hyperhomocysteinemia [42].

Historical Reference. Homocysteine began to be studied in 1932 when an American biochemist, Nobel Prize winner Vincent Du Vigneaud, synthesized a new, previously unknown, amino acid by exposing methionine to sulfuric acid. The resulting substance differed from cysteine by one carbon atom and was called homocysteine. Du Vigneaud investigated the possibility to substitute methionine as an important nutritional component with choline and homocysteine. However, later on, until the 1950s, further experiments to study the role of homocysteine were practically not carried out [43, 44]. In 1933, a clinical case of combined dementia, lens dystopia, and skeletal malformations in an 8-year-old boy was described; this child died from an ischemic stroke. Autopsy performed by a pathologist, Tracey Mallory, revealed a significant narrowing of the lumens of the carotid arteries due to the presence of numerous atherosclerotic plaques, a situation commonly found in elderly people. It is noteworthy that, hyperhomocysteinuria that could not be corrected by pyridoxine was diagnosed in 1965 in a nephew of this child [45]. In 1968, a case of homocysteinuria in a 2-month-old baby caused by a methionine synthase defect was described. The autopsy revealed atherosclerotic lesions of all major arteries. These cases were of interest to Kilmer S. McCully, an employee of the National Institutes of Health, who in 1969 first suggested the role of high homocysteine in the development of cerebrovascular diseases [46, 47].

Pathogenetic Mechanisms Responsible for the Clinical Symptoms of Hyperhomocysteinemia. The increased level of homocysteine initiates damage to the vascular tissue, disrupting the coagulant balance. It can exert a direct cytotoxic effect on the endothelium and can also damage the latter through other molecules [48], while the consumption of nitric oxide used to neutralize homocysteine increases [49]. The excess homocysteine undergoes autoxidation with the formation of H_2O_2, superoxide, and hydroxyl radicals which then can damage the endothelium [50, 51]. Besides, homocysteine initiates the excessive proliferation of smooth muscle cells in blood vessels [52].

Platelet hyperaggregation is the other effect of hyperhomocysteinemia [53]. An increase of thromboxane A_2, a platelet aggregation agonist and vasoconstrictor, is typical of this process. Homocysteine itself is a procoagulant due to its capability of activating of FXII and tissue factor [54]. Other possible mechanisms of platelet hyperaggregation in the case of hyperhomocysteinemia are the following. The activities of antithrombin III and endogenous heparin decrease in both blood and endothelium, and the content of thrombomodulin on the surface of the inner lining of the blood vessels also drops [55].

Hyperhomocysteinemia is accompanied by increase in the amount of low-density and very-low-density lipoproteins, decrease in the production of endothelial relaxing factor and sulfated glycosaminoglycans (heparinoids), and also activation of serine proteases [56]. The above processes damage endotheliocytes and the elastic membrane. At the same time, the synthesis of prostacyclin is reduced, while growth of vascular smooth muscle cells and proliferation of endothelium are activated [57]. An increase in the homocysteine concentration also leads to inhibition of the synthesis of thrombomodulin. This endothelial protein is involved in thrombin activation by natural anticoagulants (proteins C and S). In turn, these proteins normally exert a pressor effect on the activity of factors Va and VIIIa [58]. At the same time, coagulation factor V is modified and becames insensitive to protein C [59]. The described processes lead to hypercoagulation [60]. It should also be noted that homocysteine damages the arterial tissues, which initiates the release of cytokines, cyclins, and other inflammatory mediators [61]. Accumulation of homocysteine leads to loosening of the arterial walls and to the formation of local endothelial defects. The latter processes, in turn, lead to the deposition of cholesterol and calcium compounds on the vascular wall surface and initiate the development of hypercholesterolemia and atherosclerosis [62]. The effects of homocysteine on the process of tissue respiration and accumulation

of low-density lipoproteins and other components intensify the oxidation of the atherosclerotic plaques, and this provokes oxidative stress in endothelial cells. Also, these events lead to blocking of the synthesis of nitric oxide, a powerful endogenous vasodilator, by inhibiting the NO synthase enzyme [63, 64].

Homocysteine can go through the placenta, which may induce teratogenic and fetotoxic effects [65]. It has been proven that hyperhomocysteinemia is one of the causes of anencephaly and failure of spine closure. Anencephaly is a lethal phenomenon, while *spina bifida* leads to the development of severe neurological defects in newborns, including paralysis, lifelong disability, and premature death. A direct toxic effect of excessive homocysteine on the fetal nervous system also cannot be ruled out. Hyperhomocysteinemia is frequently combined with an increase in the level of antibodies with respect to phospholipids (cardiolipin) [66, 67].

Thus, the pathogenetic role of hyperhomocysteinemia is twofold. First, it damages the endothelium and induces associated early atherogenesis, and second, it increases the tendency toward development of venous and arterial thrombosis [68–70].

Hyperhomocysteinemia and injury of the nervous system.

The data of MONICA, one of the largest WHO studies carried out in the 1980s and covering 38 populations of 21 countries, showed that classical risk factors (smoking, high systolic blood pressure, overweight, and hypercholesterolemia) cannot fully explain the growth of cardiovascular diseases since their prevalence reaches 15% in women and 40% in men [71–73]. The Framingham Heart Study revealed a significant increase in the frequency of carotid artery stenosis with hyperhomocysteinemia exceeding 14.4 μM, while the concentrations of folic acid and pyridoxine phosphate were simultaneously reduced [74, 75]. Thus, hyperhomocysteinemia may be a considerable factor underlying the development of thrombotic cerebrovascular diseases [76]. The level of homocysteine correlates with the severity of damage to the brain white matter, and this is an independent risk factor for the development of dementia and Alzheimer's disease [77]. Decreased glucose metabolism in brain cells can be noticed long before clear manifestations of these diseases [77]. This is associated with a decrease in transketolase activity during hyperhomocysteinemia and the consequent inability of glucose breakdown in the pentose phosphate pathway [78]. The decreased glucose metabolism in brain cells and decreased intensity of cerebral blood flow in patients with Alzheimer's disease were demonstrated by positron emission spectroscopy. With normal and pathological aging, the mitochondria in cerebral tissues gradually become dysfunctional due to a reduced intensity of oxidative phosphorylation, decreased expression of genes associated with glucose transportation, and suppression of Na^+/K^+-ATPase. This can lead to decreases in energy production and oxygen consumption in the affected brain areas [79, 80]. All these events lead to a decrease of the mitochondrial membrane potential and to intensification of oxidation of proteins, DNA, and phospholipids [81, 82]. An increased level of neurodegeneration due to oxidative stress associated with hyperhomocysteinemia has also been described. Increases in the cytosolic calcium level, that of reactive oxygen species (ROSs), and apoptosis were observed in cultured embryonic cortical neurons and differentiated SH-SY-5Y human neuroblastoma cells grown in a folate-free substrate. Inhibition of homocysteine synthesis prevented an increase in the ROS levels [83–85]. High amounts of ROSs due to hyperhomocysteinemia also change the functioning of smooth muscles and promote proliferation of vascular smooth muscle cells [38]. It was also shown that homocysteine inhibits the activity of endothelial nitric oxide synthase (eNOS) in cultured aortic endothelial cells of adult mice and humans. Decreased eNOS activity determines inhibition of endothelium-dependent vasodilation. Together with changes in the vascular smooth muscle cells, these data provide additional insight into question why high homocysteine is a risk factor for the development of dementia and other cognitive impairments [86]. Also, homocysteine was proved to be a damaging factor with respect to the blood-brain barrier (BBB) [87–89]. This situation may be due to several different processes. First, high homocysteine causes an imbalance between the activity of matrix metallopeptidase 9 (MMP9) and a tissue inhibitor of metallopeptidase 4 (TIMP4) by increasing the MMP9 level and decreasing the activity of TIMP4. Subsequently, MMP9 interacts with various BBB components and damages this structure [15, 90]. Second, homocysteine can competitively bind to γ-aminobutyric acid (GABA) receptors; high homocysteine leads to increase in the vascular permeability and also affects NMDA

receptors [4, 91]. Free radicals can activate subunit NR1 of the NMDA receptors, which potentiates the effects of excitatory amino acids [92, 93]. Some pro-inflammatory agents (i.e., endotoxins, cytokines, or oxidative stress factors) exert excitatory effects on MMP9 in astrocytes *in vitro*. In any case, accumulation of toxic free radicals plays a key role in BBB damage [11, 35, 45]. Persons with a high blood level of homocysteine usually have more prominent signs of cerebral atrophy, mainly in the hippocampus [94, 95]. At the same time, the intake of folic acid, cobalamin, and pyridoxine leads to limitation of atrophy of the cerebral gray matter areas vulnerable to Alzheimer's disease with cognitive impairment in elderly people [80]. An increase in the serum homocysteine level leads to increased risk of developing both vascular dementia and Alzheimer's disease, which may be indicative of the existence of common pathogenetic mechanisms [2]. The interaction of homocysteine with NMDA receptors is considered one of such mechanisms. It should, however, be noted that activation of NMDA receptors depends on the glycine concentration [78]. At a normal concentration of glycine (about 10 μM), homocysteine acts as a partial antagonist with respect to the glycine site of NMDA receptors and indirectly inhibits their activity acting as a neuroprotective factor [57, 77]. Therefore, the toxic effect of homocysteine can in this case be induced by severe hyperhomocysteinemia *per se*. In contrast, if the glycine level is higher than 10 μM, even a low concentration of homocysteine (~10 μM) can activate the NMDA receptors initiating excitation and intensification of the calcium influx [69, 30]. Notably, an increase in the glycine level occurs in cerebral ischemia, head injuries, or even migraine.

More recent data revealed a novel possible mechanism of the action of high homocysteine. This is direct activation of metabotropic glutamate receptors of group I via competition of homocysteine with inhibitory neurotransmitters, such as GABA. This process induces an increase in the inflow of calcium ions and ROSs and leads to intensification of apoptosis and the respective CNS injuries [97]. Activation of NMDA receptors, i.e., an increase in the level of excitation, is also affected by homocysteic acid, which is a product of oxidation of homocysteine [43]. Such combined activation leads to an increase in the intracellular calcium level, which in turn results in activation of different kinases [98]. Excessive activation of these receptors due to hyperhomocysteinemia can then lead to an increase in the level of free radicals and abnormal activity of caspases, which in turn initiates intense apoptosis and neurodegeneration [96]. The use of NMDA receptor antagonists can, to some extent, block negative neurotoxic effects of homocysteic acid in the brain [28].

A number of "clinical" papers are focused on possible direct effects of homocysteine on the intensity of the neurodegeneration processes [76]. It seems probable that homocysteine can significantly induce and enhance intracellular and extracellular accumulation of β-amyloids, which produce well-known toxic effects on the CNS [46]. Homocysteine also increases the toxicity of β-amyloids with respect to smooth muscle cells of the small cerebral arteries. The homocysteine-induced increase in the amount of endoplasmic reticulum protein (HERP) can enhance the activity of c-secretase; this contributes to more intense accumulation of β-amyloids in the brain [69]. Soluble β-amyloid oligomers can significantly modify the redox processes during DNA methylation; this affects the process of gene transcription, inhibits cysteine uptake, and intensifies hyperhomocysteinemia [8, 41]. The latter state (hyperhomocysteinemia) resulting from DNA hypomethylation can lead to increased activity of the presenilin genes, in particular, those of presenilin 1 (*PS-1*). The latter gene is closely related to the process of methylation in the brain; first of all, it contributes to the synthesis of amyloid precursor protein (APP). Hyperhomocysteinemia activates the *PS-1* gene and, therefore, increases the production of APP, contributing to intensified amyloid synthesis [71, 88]. Tau protein is another molecule directly involved in many neurodegenerative pathologies. It coordinates the assemblage of microtubules, i.e., subcellular structures necessary for axonal transport [13, 90]. Protein phosphatase methyl transferase 1 (PPM1) regulates the activity of protein phosphatase methyl transferase 2A (PP2A), which dephosphorylates tau protein [74]. In patients with Alzheimer's disease the stability of the above enzyme (PP2A) changes, implying the increase of phosphorylated tau protein with the subsequent deposition of neurotubules [43]. Homocysteine induces higher expression of C-reactive protein; thus, it may affect the pro-inflammatory processes in smooth muscle cells of small cerebral arteries [22].

The increased level of homocysteine is associated with leukoaraiosis, a particular abnormal change in the appearance of the white matter in proximity to the lateral ventricles [48]. Hyperhomocysteinemia also represents an independent risk factor for the development of repeated strokes, as well as of focal and diffuse changes in cerebral white matter [72]. Microangiopathy, i.e., impairment of small vessels, is a significant factor for the development of circulatory-related encephalopathy in hyperhomocysteinemia [31].

Summarizing, we conclude that the development of cognitive impairments in hyperhomocysteinemia is based on several mechanisms, namely direct effects of homocysteine on the neuronal metabolism and neurotransmitter machinery and also on cerebral microangiopathy, endothelial dysfunction, oxidative stress, increased neurotoxicity of β-amyloid, and apoptosis [2, 17, 30, 58].

Correction of hyperhomocysteinemia.

Hyperhomocysteinemia is a permanent risk factor with respect to cognitive impairment, dementia, and Alzheimer's disease; fortunately, it can be relatively successfully detected [10]. The test for the homocysteine blood level should be obligatory for all young and young adult patients (according to the WHO classification, up to 45 years) with an acquired cerebrovascular accident, with ischemic strokes of unknown origin, with cognitive impairment of unknown genesis, and also with severe atherosclerosis of the carotid and peripheral arteries. It is also obligatory for patients (regardless of their age) who take anticonvulsants, especially for those who have demonstrated decreased cognitive abilities [71]. The treatment of hyperhomocysteinemia includes the intake of folic acid, 3–5 mg daily during 1–2 months; intramuscular injection of vitamins B_6 (up to 250 mg) and B_{12} (500 μg daily, 2–3 courses per year); smoking cessation, and alcohol abstinence. The efficiency of therapy should be controlled every 6–8 weeks by the measurement of blood homocysteine [59]. The target level is 10 μM. Some researchers recommend the use of betaine (or trimethylglycine; up to 6 g per day), i.e., a hepatoprotective agent that activates some metabolic processes in the liver. In the case of cerebrovascular complications during hyperhomocysteinemia, antiplatelet, vasoactive, and neurotrophic agents should be added to the treatment [86]. The diet should be corrected to ensure the availability of a sufficient quantity of folic acid and that of vitamins B_6 and B_{12}. Folic acid can be found in greens (lettuce, parsley, spinach), some vegetables (cabbage, carrots, tomatoes), rye bread, offal (liver, kidneys), beef, and eggs (yolk). Vitamin B_6 (pyridoxine) is present in the liver, rye bread, and legumes, while beef liver, kidneys, and fish liver are rich in vitamin B_{12}. In the case of early and quick development of atherosclerosis, it is necessary to limit the intake of animal fats (fatty meat and fish, processed meat, fatty cheese, butter, etc.) and of bakery products [64].

Thus, disorders in the metabolism of homocysteine leading to hyperhomocysteinemia may result in significant negative changes in blood vessels, in particular, in the cerebral ones. These changes may be a significant risk factor for the development of neurological diseases, first of all, of acute impairments (stroke) and neurodegenerative ones.

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