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HYPERHOMOCYSTEINEMIA AS A RISK FACTOR FOR THE NEURONAL SYSTEM DISORDERS

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Elevated concentration of the homocysteine (Hcy) in human tissues, resulting either from mutations in genes encoding Hcy-metabolizing enzymes, or from deficiencies of folic acid has recognized cytotoxic effect. Even a mild Hcy level increase is a risk factor for cardiovascular diseases and stroke in humans and also a risk factor for neurodegenerative disorders, such as dementia, or Alzheimer's disease. However, it is not yet clear whether homocysteine is a marker, or a causative agent. We present here an overview of recent data on the homocysteine metabolism and on the genetic and the metabolic causes of hyperhomocysteinemia-related pathologies in humans. In context of our results which detected an increased oxidative stress in hyperhomocysteinemic rats we discuss here the role of free radicals in this disorder. Imbalance between homocysteine auto-oxidation, production of reactive metabolites and cellular antioxidant defence induced by hyperhomocysteinemia results to cytotoxicity by oxidizing membrane lipids and proteins. Consequently, protein thiolation and homocysteinylolation results in the structural and functional modifications of cells, including neuronal. It is our hope that identification of prophylacting factors effective in the prevention of toxic effect of Hcy would lead to improved therapeutics, especially the brain tissue.

Key words: *hyperhomocysteinemia, oxidative stress, brain, antioxidant enzymes*

INTRODUCTION

Homocysteine (Hcy) is an intermediate sulfhydryl-containing amino acid derived from methionine. It belongs to essential amino acids derived from a dietary protein through S-adenosyl methionine conversion (1). Association between homocysteine and vascular diseases has been recognized since 1962 when Carson *et al.* (2) identified metabolic abnormalities which caused mental retardation in patients who excreted large amounts of Hcy in the urine. Subsequently, they uncovered a new disorder of methionine metabolism referred to as homocystinuria. In 1964, Gibson and co-workers reported that patients with homocystinuria expressed coronary vascular abnormalities and arterial thrombosis (3). In 1969, McCully found a connection between elevated Hcy level in the blood and atherosclerosis. Various deleterious manifestations of hyperhomocysteinemia (hHcy) are caused due to increased oxidative stress, protein thiolation and protein homocysteinylolation. Patients with severe hHcy exhibit a wide range of clinical manifestations including neurological abnormalities, such as mental retardation, cerebral atrophy, dementia and seizures (4).

Homocysteine is metabolized from the methionine by three independent alternative pathways: remethylation, transmethylation to methionine, or transsulfuration to cysteine. Elevated levels of the Hcy, called hyperhomocysteinemia

(hHcy), are associated with higher risk of neurovascular diseases. They represent a risk factor for neurotoxicity and lead to brain damage in humans (5). Though mutations or polymorphisms in the key genes of Hcy metabolism pathway have been well elucidated in stroke, emerging evidences suggested that epigenetic mechanisms, such as DNA methylation, chromatin remodeling, RNA editing, noncoding RNAs (ncRNAs), and microRNAs (miRNAs) might equally play an important role in the stroke development. Genetic regulation of enzymes involved in the Hcy metabolism and levels of the vitamin cofactors (folate, B6 and B12) determine the level of Hcy. Clinical studies suggest that genetic variations of genes involved in these pathways, such as methylentetrahydrofolate reductase (MTHFR), cystathionine- γ -synthase (CBS), DNA methyltransferase (DNMT) and nicotinamide N-methyl-transferase (NNMT) might increase the risk of stroke during hHcy. Nutritional supplements, *e.g.* folic acid (a cofactor in one-carbon metabolism), regulate epigenetic alterations and may play an important role in the maintenance of neuronal integrity.

HOMOCYSTEINE METABOLISM

Hcy is a non-essential, sulphur-containing, nonproteinogenic amino acid synthesized from the methionine in methylation

pathways, (the transfer of activated methyl groups from tetrahydrofolate to S-adenosylmethionine) (6), or remethylation pathway. Remethylation is catalyzed by methionine synthase (MS), which requires vitamin B₁₂ in the form of methylcobalamin as a cofactor, or N-5-methyltetrahydrofolate-homocysteine methyltransferase (7, 8). Dysregulation of Hcy metabolism is implicated in a number of adverse clinical outcomes. Transsulfuration converts Hcy to the cystathionine, and subsequently, to cysteine. Formed cysteine can then enter glutathione (GSH) synthesis, or taurine synthesis pathways (7). Transsulfuration of Hcy depends on the vitamin B₆. The enzyme cystathionine- β -synthase (CBS) represents the first step in cysteine formation, and it catalyses the condensation of Hcy with serine to form cystathionine. Alternatively, Hcy can be remethylated to methionine by an addition of a methyl group from 5-methyltetrahydrofolate (5-MTHF), which is synthesized by 5,10-methylenetetrahydrofolate reductase (5,10-MTHFR). In the brain, the Hcy metabolism (*Fig. 1*) differs from other organs. The trans-sulfuration pathway is not active and the remethylation pathway using betaine is absent (8). Thus, the capacity for Hcy metabolism is largely dependent on the supplies of folate and cobalamin. The glial cells possess very low stores of vitamin B₁₂ that are quickly depleted during the negative balance. Mechanism of the Hcy action in the development of neuronal diseases appears to be complex and not clearly understood. Although a toxicity of the homocysteine to CNS neurons has been recognized (9), effects of the

homocysteine on Purkinje neurons of the cerebellum (that play a vital role in motor function) remain unexplored yet. Oldreive and Doherty (9) established primary cultures of the embryonic cerebellar Purkinje neurons and exposed them to a concentration range of the homocysteine and determined the neuronal survival rate. These experiments revealed that all tested concentrations of the homocysteine (from 50 to 500 μ M) caused a significant decrease in a number of cerebellar Purkinje neurons. Also, exposure to homocysteine may have a detrimental effect on the ability of neurons to transmit signal and thus to form functional neural networks. It is known that during development, cerebellar granular neurons switch from a state, in which they are resistant, to very high concentrations of homocysteine, becoming postnatally vulnerable to this agent. Taken together, these data reveal that homocysteine is toxic to cerebellar Purkinje neurons *in vitro*, inhibiting both their survival and the outgrowth of neurites. As suggested by Oldreive and Doherty (9), homocysteine decreases both the magnitude and complexity of the neurite arbor extended by cerebellar Purkinje neurons demonstrating that it has effects on these cells that go beyond neuronal survival.

CAUSES OF HYPERHOMOCYSTEINEMIA

The reference total plasma Hcy range in humans is 5-10 μ M. Under normal conditions, plasma Hcy concentrations do not

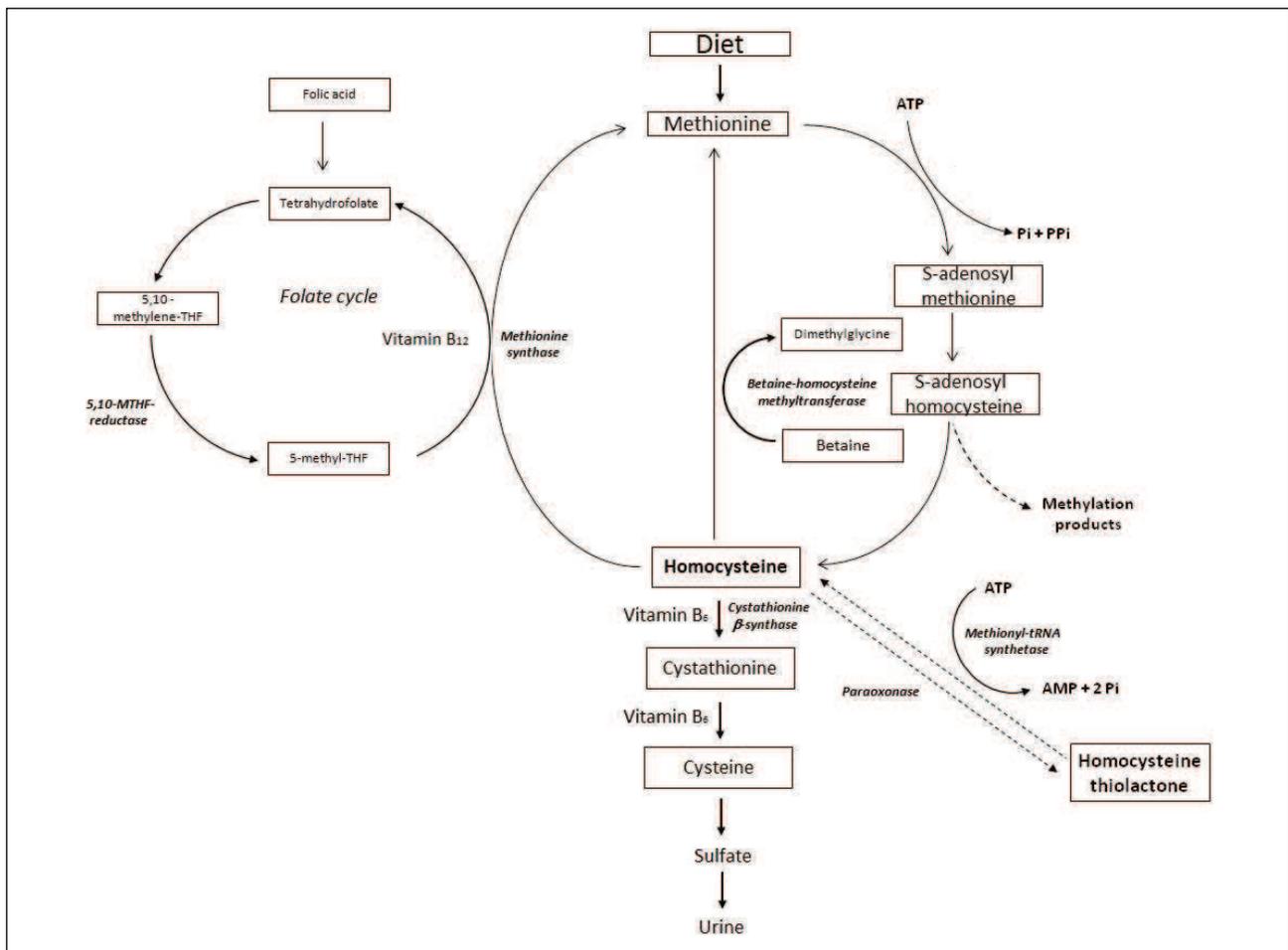


Fig. 1. Schematic representation of mammalian homocysteine metabolism.

ATP, adenosine triphosphate; Pi, orthophosphate; Ppi, pyrophosphate; 5,10-methylene-THF, 5,10-methylene-tetrahydrofolate; 5,10-MTHF-reductase, 5,10-methylenetetrahydrofolate reductase; 5-methyl-THF, 5-methyl-tetrahydrofolate; AMP, adenosine monophosphate.

exceed 15 μM (6). Elevation of plasma Hcy is known as hyperhomocysteinemia (hHcy). Several types of hHcy are classified in relation to the total plasma Hcy concentration such as: moderate (for concentrations between 16 and 30 μM), intermediate (for concentrations of 31-100 μM), and severe (for concentrations higher than 100 μM) (10). Severe hHcy occurs in homocystinuria, an innate metabolic disorder characterized by a deficiency of CBS enzyme activity. Affected patients exhibit plasma concentrations of Hcy that can reach up to 500 μM (11). Hyperhomocysteinemia is the result of perturbed Hcy metabolism where regulating enzyme activities are disturbed, in condition such as dietary deficiencies in folic acid, vitamin B₆, and/or vitamin B₁₂ (12).

Increased Hcy levels are associated with several disorders, like cardio- and cerebrovascular diseases and neurodegenerative diseases (13) that affect the central nervous system (CNS), such as epilepsy (14), stroke (15), Alzheimer's disease (16), dementia (17), as well as with classical homocystinuria (11) (*Table 1*).

Hyperhomocysteinemia can be caused by genetic deficiencies in methionine and homocysteine metabolism, including cystathione β -synthase, methionine synthase and methylenetetrahydrofolate reductase (MTHFR) deficiencies (18). While homocysteine is formed in all tissues, its detoxification occurs only in the liver/kidney through the transsulfuration pathway. So in other tissues such as the blood vessels and the brain, remethylation is the only alternative available. With significant reduction in MTHFR activity homocysteine cannot be remethylated to methionine, hence accumulates within the nervous system. To study the consequences of MTHFR deficiency, MTHFR knockout mouse has been generated through targeted deletion of MTHFR gene with resulting hyperhomocysteinemia (19). Homozygotes generated are poor survivors with 10 times higher elevations in Hcy levels (33.1 μM) compared to wild type mice (3.2 μM), with tremor, motor and gait abnormalities and a 25% mortality by postnatal week 5. Moreover, in humans two common polymorphisms (C677T and A1298C) in the gene encoding MTHFR have been described up to now (20, 21). The role of the MTHFR C677T polymorphism has been studied by different laboratories and its role as a risk factor for ischemic stroke was established. Individuals homozygous for a common MTHFR thermolabile variant, 677C>T, are at a risk of thrombotic disease. This molecular variant is associated with the elevated plasma Hcy levels and increased stroke risk. (21, 22). The significant association of the C677T polymorphism with the

ischemic stroke development was proved also by Li and Qin (23) by meta-analysis, when nineteen case-control studies associated with MTHFR gene C667T involving 2223 cases and 2936 controls were included. A significant synergistic interaction was also found with the double heterozygote MTHFR C677T/A1298C (24). Homocysteine levels were found significantly higher in ischemic stroke in Tunisian patients with MTHFR C677T (CT and TT genotypes), however, the difference was not significant with the MTHFR A1298C variant (AC and genotypes CC) (24).

Increased Hcy levels are associated with several disorders that affect CNS, however, another molecular variant of the MTHFR, G1793A was found to be associated with a different tumorigenesis in men (25-27).

Migraine is a chronic disabling neurovascular condition that may be in part caused by endothelial and cerebrovascular disruption induced by hHcy (28). These authors provided an evidence that vitamin supplementation is effective in reducing migraine and also that genotypes MTHFR C677T and MTRR A66G (methionine synthase reductase) gene variants are acting independently to influence treatment response in female migraineurs. Another disease that hHcy might be involved in is coeliac disease (29). These authors believe that Hcy in coeliac disease might, by damaging the blood brain barrier expose neuronal tissue to neuro-irritative metabolites, including Hcy.

HYPERHOMOCYSTEINEMIA-RELATED PATHOGENESIS

Several hypotheses concerning the toxicity of Hcy neurotoxicity were proposed. The *three* main pathways of Hcy biotoxicity are obviously discussed in the literature (5).

Hcy-dependent oxidative stress

At first, dysregulation in redox equilibrium and oxidative stress have been suggested as a primary biochemical mechanism responsible for hHcy-related pathogenesis. Oxidative stress is defined as a serious imbalance between the production of reactive species and antioxidant defenses, and can result from diminished levels of antioxidant and/or increased production of reactive species (30, 31). Studies showed that redox reactions may be a key factor in the development of vascular hypertrophy, thrombosis and atherosclerosis in hyperhomocysteinemic animals (32). Oxidative stress is generated during oxidation of the free thiol group of Hcy, when Hcy binds *via* a disulphide bridge with plasma proteins - mainly albumin - or with other low-molecular plasma thiols, or with a second Hcy molecule. The increased production of reactive species caused by Hcy may induce the subsequent oxidation of proteins, lipids and nucleic acids (33) and can lead to the endothelial dysfunction and damage to the vessel wall, followed by platelet activation and thrombus formation (34). Accumulation of oxidized biomolecules alters the biological functions of many cellular pathways. Hcy acts as a potent oxidizing agent of -SH groups by reactive species production, such as superoxide anion (O₂⁻) and hydrogen peroxide (H₂O₂), mainly during its auto-oxidation.

Five mechanisms have been proposed for Hcy-induced oxidative stress. They include: 1) inhibition of the activity of cellular antioxidant enzymes; 2) Hcy auto-oxidation; 3) nitric oxide synthase (NOS)-dependent generation of superoxide anion *via* uncoupling of endothelial NOS (eNOS); 4) disruption of extracellular superoxide dismutase from endothelial surfaces; and 5) activation of NADPH oxidases. ROS and oxidative stress promote the formation of nitrotyrosine, an indicator of NO and superoxide radical reaction, resulting in the formation of strong oxidant peroxynitrite. Peroxynitrite leads to tyrosine nitration

Table 1. Causes of elevated homocysteine (34-36).

Mild (16-30 μM)
Mild-moderate renal disease
Hypothyroidism
Psoriasis
MTHFR 677C>T variant
Mild moderate folate or vitamin B ₁₂ deficiency
Increasing age
High protein intake
Low consumption of vegetables or fruits
Moderate (31-100 μM)
End-stage renal disease
Moderate vitamin B ₁₂ deficiency
Severe folate deficiency
MTHFR 677C>T variant combined with low folic acid levels
Severe (>100 μM)
Severe vitamin B ₁₂ deficiency
CBS deficiency

which causes the alteration in protein function and induces cellular dysfunction (35).

One of the effects of hHcy is an increased lipid peroxidation and protein oxidation. Therefore, in our laboratory we have investigated the effect of chronic hHcy on some parameters of lipid oxidation and oxidative damage of proteins (Table 2). These results are in correlation with previously published studies (36, 37). Our findings contribute to better knowledge in a brain dysfunction, since Hcy can interact with lipoproteins, initiating the process of lipid peroxidation and proteins. The accumulated reactive species may join to form hydroxyl radicals as the most potent, powerful free radical with the ability to remove electrons from other molecules rapidly and have harmful effects for most cellular components including lipids, proteins, carbohydrates, and DNA (36, 37).

Homocysteine-induced protein structure modifications, named homocysteinylation

At second, two main types of homocysteinylation have been detected: S-homocysteinylation and N-homocysteinylation, both of which are considered as posttranslational protein modifications. S-homocysteinylation occurs when Hcy reacts, by its free thiol group, with another free thiol derived from a cysteine residue in a protein molecule. These changes can alter the thiol-dependent redox status of functional proteins (38). N-homocysteinylation takes place after acylation of the free ϵ -amino (e.g., lysine) groups of different proteins to form adducts under physiological conditions. Degree of the protein homocysteinylation increases with increased plasma Hcy. (39). It appears that the conversion of Hcy to Hcy-thiolactone followed by protein N-homocysteinylation largely contributes to manifestations of Hcy toxicity. Homocysteinylation causes immune activation, autoimmune inflammatory response, cellular toxicity, cell death and enhanced protein degradation (40).

Free radical-scavenging enzymes, such as superoxid dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), are in the first line of cellular defense against oxidative injury, decomposing O_2^- and H_2O_2 to prevent formation more reactive hydroxyl radical (HO). These enzymes protect the red blood cells against O_2^- and H_2O_2 -mediated lipid peroxidation and their lower activities could be related to inactivation of the enzymes by cross-linking or to exhaustion of the enzymes by increased peroxidation (41). In our laboratory we observed a 57.9 % decrease of MnSOD activity in the hHcy group (9.34 ± 1.901 U/mg proteins) compared to the control group (22.186 ± 4.017 U/mg proteins). On the other hand, we have determined the 13.6% increase in the protein level in hHcy group compared to the control group using Western blot analysis (Fig. 2). The results might indicate increased post-translational modifications of MnSOD, probably due to higher level of Hcy. In addition, its metabolites contribute to the inactivation of this enzyme by homocysteinylation and thiolation. Our results are also in correlation with the immunohistochemical analysis (Fig. 3). Further, we have detected 12.46 % increase of catalase (CAT) activity in the hHcy group (180.068 ± 3.57 nM/min/mg proteins) compared to the control group (157.62 ± 1.14 nM/min/mg proteins). Also, these changes may be a consequence of an increased level of ROS due to the presence of Hcy. Imbalance detected among antioxidant enzymes caused by Hcy can possibly alter reactive species elimination, and thus leads to the increasing amount of free radicals (37).

Chemical pathology of homocysteine, excitotoxicity

Moreover, also other pathways of Hcy biotoxicity have been reported, including Hcy induced NMDA receptor and group I metabotropic glutamate receptor (mGluR) mediated neurotoxicity (37). Glutamatergic excitotoxicity appears to be associated with brain damage caused by Hcy. This amino acid induces neurodegeneration *via* N-methyl-D-aspartate (NMDA)

Table 2. Effect of hHcy on the parameters of lipid and oxidative damage.

Sample	Conjugated dienes (A_{233}/A_{215})	TBARs (nM/mg proteins)	Fluorescence intensity of dityrosine (arbitrary units)	-SH group content ($\square\square$ /mg proteins)
Control	0.255 ± 0.005899	5.105 ± 1.815	51.999 ± 6.075	0.05 ± 0.01
hHcy group	$0.3716 \pm 0.05349^{***}$	$16.125 \pm 3.343^{***}$	$124.79 \pm 13.588^{***}$	$0.092 \pm 0.012^{***}$

Values are expressed as means \pm S.D. for 6 animals in each group. $^{***}p < 0.001$; significantly different when compared to the control. (hHcy group = group with induced hyperhomocysteinemia, TBARs = thiobarbituric acid reactive substances).

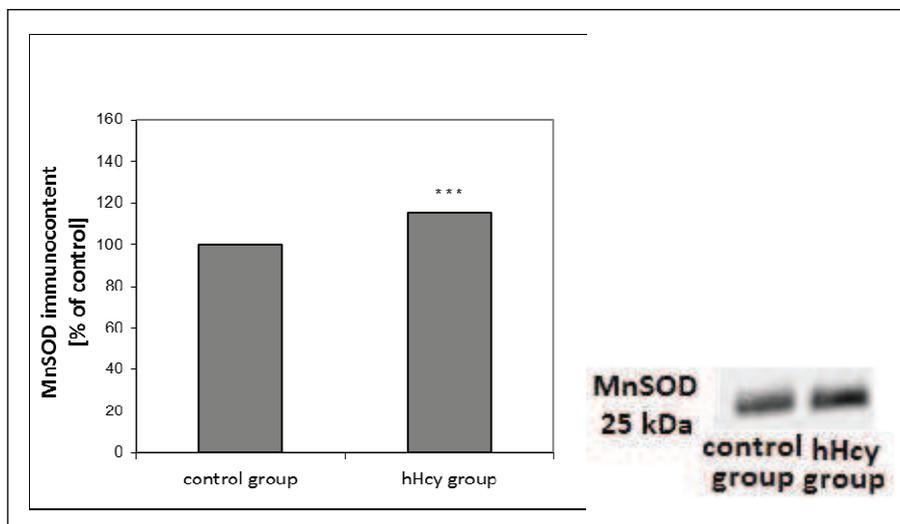


Fig. 2. The MnSOD protein production. Data are means \pm S.D. for 6 animals in each group. Results are expressed in % of control as mean \pm S.D. $^{***}p < 0.001$ compared to the control group.

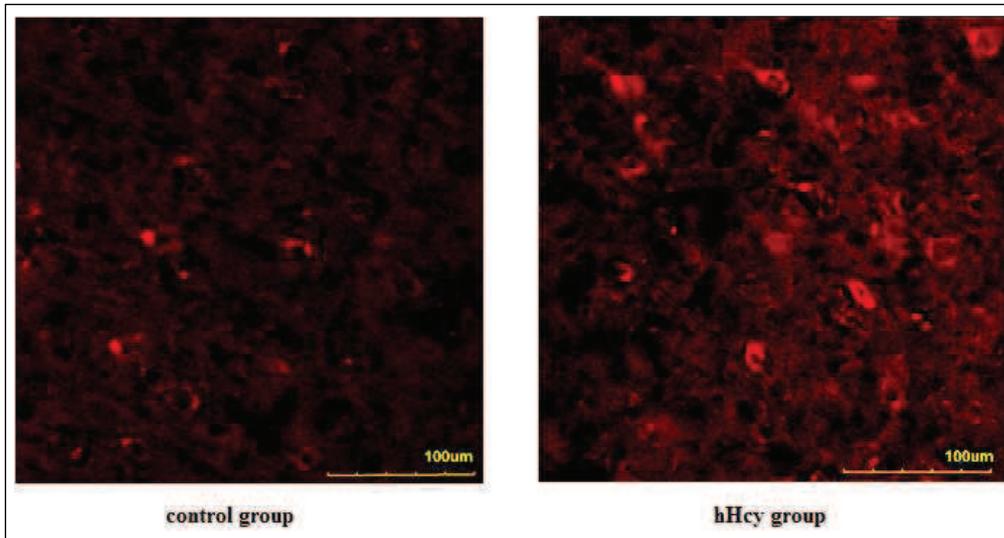


Fig. 3. Immunoreactivity of MnSOD in the control group and the hHcy group of the rat brain cortex. Fluorescent counterstaining with Texas Red. (Scale bars 100 μ m).

receptor overstimulation and/or by reducing glutamate uptake, as detected in the parietal cortex of rats (37, 42) and in the hippocampus of young rats (43, 44). In addition, Hcy was shown as an inductor of caspase-dependent apoptosis in human dopaminergic cells, rat hippocampal and mouse cortical neurons (45, 46). However, caspase-independent cell death has been reported in murine cerebellar granule cells in the presence of high levels of Hcy (47). Hcy has also been reported to alter hippocampal plasticity and synaptic transmission (48).

This sulphur containing amino acid can act as an agonist at the glutamate binding site of the NMDA receptors which are expressed in both neurons and astrocytes, the most abundant and important cells in the CNS (49, 50) and also critical in the glial-vascular interface as part of the blood-brain barrier (51). The importance of astrocytes in the regulation of brain metabolism and in particular in the brain energy metabolism has been documented (52). Maler *et al.* (53) reported that Hcy showed a dose dependent cytotoxic effect at doses of 2 mM and above in cortical astrocytes. Furthermore, it has been found that astrocytes regulate the expression of NMDA receptor subtypes, which increase neuronal sensitivity to glutamate toxicity. Another issue, that deserves comment concerns differences in density of NMDA receptor subunit 2B, which is critical determinant for synaptic plasticity. Lehner *et al.* (54) have shown that rats that are more anxious have altered pattern of GluN2B subunit expression in the frontal cortex and limbic structures, which control emotional behaviour. These results also indicate that GluN2B subunits are required for the initiation of neural changes in the prefrontal cortex, dentate gyrus and of the hippocampus and amygdala of rats and provide evidence in support of the preclinical use of NMDA(R) modulators (54). Therefore, increased level of the Hcy leads to an enhanced excitatory glutamatergic neurotransmission in different brain areas, whereby neuronal damage derives from excessive Ca^{2+} influx and reactive oxygen generation. Also, Hcy can induce neuronal apoptosis or apoptotic processes by a mechanism involving DNA damage, poly-ADP-ribose polymerase (PARP), and mitochondrial dysfunction by caspase-3 activation (50, 55).

Hcy, at the concentration found in the hHcy, acts on the endogenous IF-associated phosphorylation system. Such effects were dependent on glutamate receptors and Ca^{2+} channels activating different signaling pathways in slices of hippocampus and cerebral cortex of rats during development (56).

Homocysteine is the most reactive amino acid in biological systems. In addition to transmethylation to methionine or

transsulfuration to cysteine, Hcy can be converted to other metabolites, such as AdoHcy, Hcy-containing disulfides, homocysteic acid or S-nitro-Hcy. Homocysteine is also metabolized to the thioester Hcy-thiolactone in an error-editing reaction in protein biosynthesis when Hcy is erroneously selected in place of methionine by methionyl-tRNA-synthetase (57). Human, animals, and tissue culture studies have shown that Hcy-thiolactone contributes to Hcy pathobiology (37, 39, 58). Hcy-thiolactone is chemically reactive metabolite that causes protein N-homocysteinylation through the formation of amine bonds with protein lysine residues (59, 60) which impairs or alters the structure and function of proteins, causes protein damage by the thiol radical mechanism (61) and contributes to multiple human pathologies including atherosclerosis (62), thrombosis (63) and Alzheimer's disease (64). Plasma Hcy-thiolactone and N-linked protein Hcy (N-Hcy-protein), have been identified as constituents of blood, and are greatly elevated under conditions predisposing to atherothrombosis, such as hyperhomocysteinemia caused by mutation in CBS or MTHFR gene in human or a high-Met diet in mice (65, 66). Protein N-homocysteinylation induces pathophysiological responses, such as an autoimmune activation and increased susceptibility to thrombosis. Chronic activation of these processes can lead to vascular disease (58).

Interestingly, temporal lobe epilepsy as the most common type of epilepsy in adults is usually associated with a poor response to antiepileptic drugs (67). Several clinical studies have reported that patients treated with antiepileptic drugs have elevated plasma homocysteine levels (68). It has been found that an increase in plasma homocysteine levels may provoke seizures (69). In agreement with this finding it was suggested that systemic administration of homocysteine at high doses is able to induce convulsions in mice (70) and in immature rats (71). Pilocarpine is a parasympathomimetic alkaloid obtained from the leaves of tropical American shrubs from the genus *Pilocarpus*. It is a non-selective muscarinic receptor agonist (72) in the parasympathetic nervous system, which acts therapeutically at the muscarinic acetylcholine receptor M3. Pilocarpine administration is frequently used to mimic temporal lobe epilepsy in rodents. Increased homocysteine levels can enhance seizure activity and neurodegeneration in pilocarpine-treated rats and it can be suggested that similar detrimental effects might occur in patients affected by temporal lobe epilepsy (73). Homocysteine-derived chemically reactive metabolites are suggested to play an important role in Hcy induced seizures.

Protection by selected compounds, the role of antioxidants

My studies proved that a number of substances can decrease the plasma level of the Hcy. For example, resveratrol was shown to inhibit Hcy-induced oxidative stress, apoptosis and cognitive impairment. Resveratrol is a polyphenol compound, which is an integral component of human diet naturally found in fruits, nuts, red wine and bark of different plants. Resveratrol strongly, but not completely, reduced platelet apoptosis induced by Hcy or Hcy-thiolactone (74). It has drawn attention because of its potential benefits against cancer, cardiovascular and neurological diseases. Resveratrol reduced cortical and hippocampal neuronal loss, improved motor performance and spatial memory (75), increased level of anti-oxidant enzymes SOD, CAT and peroxidase activities in rat brain (76) and in mouse brain (77). Hamlaoui *et al.* (78) evaluated the toxic effect of doxorubicin (Dox) and showed that Dox decreased plasma CAT and SOD activity but unexpectedly increased peroxidase activity. Co-treatment with resveratrol counteracted almost all Dox's effects, which confirmed real antioxidant properties of resveratrol (78). It has also been shown that the antiapoptotic effect of resveratrol was linked to its antioxidant actions (79) and that the neuroprotective effect of resveratrol is dependent on specific induction of heme oxygenase 1 enzyme in the brain (80, 81).

In humans, causality of the hHcy could be both, 1) genetic deficiencies in the enzymes (CBS and MTHFR) responsible for the remethylation or transsulfuration of Hcy, and 2) nutritional (B6, B12, choline and folate) deficiencies of vitamins serving as cofactors for the enzymes. These dietary nutrients are the best sources to influence the supply of methyl groups and regulate the biochemical pathways for methylation processes. Supplementation with natural folate-rich foods, folic acid and 5-MTHF reached a similar reduction in Hcy concentrations (82). The efficacy of 5-MTHF has been compared with that of folic acid in several studies with contrasting results: Fohr *et al.* (83) showed that in women folic acid was more effective than MTHF in lowering plasma total Hcy, while Venn *et al.* (84) reported that a low-dose of 5-MTHF was at least as effective as folic acid in reducing total Hcy concentrations in healthy subjects. In contrast, 5-MTHF was more effective than folic acid in increasing plasma folate levels in the study of Prinz-Langenohl (85) both in TT and CC subjects.

The human body evolved the ability to eliminate one of the metabolites of Hcy, Hcy-thiolactone. A high-density lipoprotein (HDL)-associated enzyme, Hcy-thiolactonase/paraoxonase 1 (PON1) is able to hydrolyze this toxic metabolite (Hcy-thiolactone) in human serum (86). More recently, Hcy-thiolactonase/bleomycin hydrolase (BLH) was found to hydrolyze Hcy-thiolactone intracellularly (87). Borowczyk *et al.* suggested that bleomycin hydrolase (Blmh), named for its ability to hydrolyze the anticancer drug bleomycin, protects against protein N-homocysteinylation by hydrolyzing Hcy-thiolactone *in vivo* (88). This indicates that at least in mice, PON1 protects mice against Hcy-thiolactone neurotoxicity by hydrolyzing it in the brain (89). hHcy contributes to progressive ageing, autophagy and ischemia/reperfusion injury (90, 91) and causes increased cerebrovascular permeability. Tetrahydrocurcumin (THC)-herbal antioxidant ameliorates homocysteinylation-mediated autophagy in hyperhomocysteinemic mice after cerebral ischemia (92). THC may be an effective prophylactic agent in the prevention of oxidative stress by Hcy.

CONCLUSION

Elevated level of the Hcy is now the recognized risk factor in the development of various diseases, including neuronal ones.

Plasma hyperhomocysteinemia leads to an increase in cerebrovascular permeability and causes thiolation and homocysteinylation to proteins and enzymes in the brain. As a consequence, these post-translational modifications affect the function and activity of different oxidant and anti-oxidant enzymes like SOD, CAT or GPx. This process leads to the redox imbalance and to an increased oxidative stress and formation of reactive oxygen and nitrogen species, followed by the lipoperoxidation, protein oxidation, all of the factors included in the brain damage. Accumulation of these toxic free radicals plays an essential role in blood brain barrier pathology. hHcy also causes the endothelial dysfunction and increases the risk of atherothrombosis and the other diseases, such as diabetes mellitus, renal diseases, Alzheimer's disease and dementia. As the elevated levels of Hcy can result from the deficiency of one or more enzyme's vitamin cofactor involved in its metabolism, it would be very important to find strategies to decrease Hcy in blood thus preventing further damage of the CNS structures.

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