Ischemic Brain Injury in Hyperhomocysteinemia

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Cite this chapter as: Lehotsky J, Kovalska M, Baranovicova E, Hnilicova P, Kalenska D, Kaplan P. Ischemic Brain Injury in Hyperhomocysteinemia. In: Pluta R, editor. Cerebral Ischemia. Brisbane (AU): Exon Publications; 2021. Online first Sep 10.
Doi: https://doi.org/10.36255/exonpublications.cerebralischemia.2021.
hyperhomocysteinemia

Abstract: Homocysteine is an intermediate product of methionine metabolism. Hyperhomocysteinemia can be caused by high intake of methionine, deficiency of vitamin B₁₂, folate, or both. Hyperhomocysteinemia causes cardio- and cerebrovascular diseases, including ischemic stroke. Hyperhomocysteinemia-induced oxidative stress, inflammation, and endoplasmic reticulum stress play an important role in the pathogenesis of several neurodegenerative diseases. Pyramidal neurons of the hippocampus are sensitive to prolonged levels of homocysteine due to the absence of metabolization by transsulfuration as well as by folate- or
B$_{12}$-dependent remethylation. This chapter highlights the role of hyperhomocysteinemia in neurodegenerative changes following cerebral ischemia. An overview of how hyperhomocysteinemia by itself, or in combination with ischemia-reperfusion injury, exacerbates neurodegeneration is presented. The role of hyperhomocysteinemia in amyloid deposition and hyperphosphorylation of tau protein in the brain, along with plasma metabolic alterations in cerebral ischemia-reperfusion injury is reviewed. Prevention of hyperhomocysteinemia may have therapeutic implications in cerebral ischemic stroke and deserves investigation.

**Keywords:** cerebral ischemia-reperfusion injury; homocysteine; ischemic brain injury; methionine; hyperhomocysteinemia

**INTRODUCTION**

Many experimental and clinical studies show that co-morbid disorders are risk factors for developing vascular pathologies, such as stroke, in humans (1, 2). Mild hyperhomocysteinemia (hHcy) may increase the risk of stroke, probably due to the pleiotropic biochemical properties of homocysteine (Hcy) (2, 3). Hcy, a critical component of the one-carbon methionine (Met) metabolism, has been proposed to be an etiological agent of cerebrovascular disorders, such as ischemic stroke (2–4). Its toxicity is the result of auto-oxidation and free radical generation (3, 5). Intermediates of Hcy metabolism include Hcy-thiolactone and homocysteic acid. Increased lipoprotein and protein oxidation are directly involved in neuronal degeneration (6, 7). Posttranslational modifications of proteins, homocysteinylaton, and thiolation result in the impairment of functional proteins and enzyme inactivation (6). Overstimulation of the NMDA (N-Methyl-D-aspartic acid or N-Methyl-D-aspartate) and metabotropic receptors (mGluR), and the reduction of glutamate uptake in the cortex (8) and hippocampus (9), induce impairment of neuronal functions and the damage of glial cells (5, 10).

Cerebral ischemia induces neural damage through the depletion of cellular energy, release of excitatory amino acids, induction of mitochondrial dysfunction, and excessive generation of reactive oxygen (ROS) and nitrogen (RNS) species (7, 11). Preconditioning is one of the recognized neuroprotective strategies in which a period of sublethal insult (ischemia-ischemic preconditioning [IPC]) induces robust protection (tolerance) against subsequent injurious/lethal ischemic events (7, 11). The clinical relevance of hHcy in the development of human stroke and the toxicity of hHcy in the brain has been reported (11–13).

Met is an essential amino acid present in food (14, 15). Intake of diet rich in Met, or the dysregulation of Met metabolism in the “Met-Hcy” cycle, can lead to the elevation of Hcy in plasma. S-adenosyl homocysteine (SAH), an intermediate of metabolic conversion of Hcy, regulates methylation signalling, and causes hypomethylation of DNA and proteins (16). Cellular clearance of Hcy is essential for genetic protection. Subcutaneous administration of Hcy in rats lead to mild hHcy, causes disintegration of neuronal tissue in the cortex and hippocampus, and triggers epigenetic changes via impairment in histone acetylation, likely by hHcy-initiated DNA hypomethylation (17–20). Met-rich diet induces pathological changes in the CA1 hippocampal area of rats.
Epigenetic mechanisms, such as DNA methylation, RNA editing, noncoding RNAs (ncRNAs), and microRNAs (miRNAs) are involved in the pathogenesis of ischemic stroke (13). Hcy is toxic to neuronal and endothelial cells (3, 4). A link between hHcy and vascular diseases, cardiovascular symptoms, and neurological disorders such as cerebral atrophy and seizures has been recognized (21–23). Metabolic conversion of Hcy requires the presence of dietary vitamin B12 (cobalamin) and folic acid for methyl group transfer and re-methylation of N-5-methyltetrahydrofolate-Hcy methyltransferase activity. Transsulfuration reaction of Hcy depends on the presence of vitamin B6. The reaction is absent in the brain, and thus, the remethylation pathway depends on exogenous folate and cobalamin. This fact has clinical relevance due to the lower intake of vitamins in older age, decreased absorption by the gastrointestinal mucosa, or low stores of vitamin B12 in the brain. The neurotoxicity of Hcy affects neuronal survival, the ability of neurons to transmit signals, and alters neural networks and circuitry (24, 25).

Hcy can be transported through the blood-brain barrier via a specific saturable transporter, and accumulate in the brain (4). It induces dysfunction of endothelial, astrocytic, and neuronal cells in brain (3, 24). In the hippocampus, hHcy induces lipoperoxidation, apoptosis, and neuronal degeneration (3, 7, 11, 26–28). In the cortex, secretary pathway calcium-ATPase 2 and Mn²⁺ superoxide dismutase activity are reduced (26) and Golgi stress is increased because of redox dysbalance (3, 29, 30). hHcy reduces mitochondrial respiration, increases the electron transport chain complex II, and inhibits complex IV activity (31). It also induces a decline in electron transport chain activities of the heart through the expression of proteins responsible for cellular stress response and redox balance (32).

Met plays a critical role in cell physiology (33). Its plasma level is the result of Met metabolism, daily intake, and protein degradation (34). An excess of Met could be detrimental and might increase the risk of developing several diseases, including toxicity to central nervous system, DNA and dendritic spine density damage, and synaptic remodeling (33, 35). Faulty Met metabolism results in the accumulation of its metabolites in plasma, mostly Hcy, as part of the Met-Hcy cycle. If high dietary intake of Met exceeds the transsulfuration capacity, the Hcy blood concentration increases (15, 33). Data suggest that hHcy caused by Met diet leads to neuroinflammation, microhemorrhages, apoptosis, and synaptic remodeling (33, 35, 36). Furthermore, modifications in the “one-carbon metabolism” may exacerbate the toxic potential of Hcy and its metabolites, and affect the “methylation index” with an impact on gene regulation (16, 33, 35). In our studies, a high-Met diet induced neuropathological changes in the CA1 hippocampal area and impaired spatial and learning memory acquisition—likely due to Met-induced changes in “methylation index” of hippocampus and participation in the Met-Hcy cycle (37). 1H NMR spectroscopy with 7T MR scanner showed alterations in metabolic profile, increased hippocampal volume, and modifications in the number and morphology of astrocytes and neurons of CA1 hippocampus (20, 37). Subcutaneous injection of Hcy in rats led to neurodegeneration, altered morphology of the hippocampus, entorhinal, parietal and motor cortex, and accumulation of amyloid plaques and hyperphosphorylated tau protein (18, 38, 39). Met diet also increases H₂S production.
and inflammatory factors, and decreases mitochondrial function (40). High-Met diet-induced mild hHcy (20, 33, 41–43) can cause vascular cognitive impairment, neuroinflammation, and tau protein phosphorylation (44, 45). These can in turn affect astrocytes, microglia, and neurons (17, 18, 20, 42, 46). Gestational, neonatal, or adult hMet in rats and mice increases autophagosomes, apoptosis, and caspase activity (35, 47).

NMR metabolomic analysis of plasma showed alteration in energy metabolism in rats treated with Met diet (37). Decreased utilization of glucose is balanced with increased utilization of triacylglycerols to coordinate cellular function and facilitate neuronal survival (48–50) and compensate abnormalities in behavioral tests (33, 48). Because memory impairment is frequently the earliest symptom of dementia (51, 52), we hypothesize that Met induces neuropathological changes in the hippocampus which leads to memory impairment at the very early stages of Met/Hcy neurotoxicity. The clinical relevance is that a diet, which is high in Met and low in B vitamins, is a risk factor for the development of human neurodegenerative disorders.

**HYPERHOMOCYSTEINEMIA AS A DETRIMENTAL FACTOR IN CEREBRAL ISCHEMIA-REPERFUSION INJURY**

Apart from being neurotoxic by itself, hHcy exerts synergistic detrimental toxicity in cerebral ischemic experimental models of human stroke (Figure 1) (53). Ischemia-reperfusion injury (IRI) induces degeneration of hippocampal neurons (17, 54). The synergistic effect of both stressors, hHcy and IRI, leads to the aggravation of neuronal and glial morphological changes in the hippocampus and the cortex (17, 42, 54). Astrocytes, as dynamic cells, affect intercellular communication with surrounding synapses (10, 55). The synergistic effect of ischemia and hHcy modifies the expression of the mitogen-activated kinase (17, 54, 56) and enhances the severity of tissue injury (12, 57). hHcy aggravates cortical cell injury after ischemia via autophagy, blood-brain barrier disruption, and homocysteinyl-ation of cytochrome c, which in turn induces autophagy (58). Hcy reduces the number of reparatory endothelial cells in stroke patients (59), silences coagulation genes by hypermethylation (60), activates dysregulation of the ubiquitin system (61), and suppresses NO synthesis to impair circulation (62). Hypermethioninemia (hMet) is linked to memory deficits and morphological changes in the hippocampus (63). Chronic hMet and its sulfoxide product, induces oxidative stress (64) and contributes to brain pathology (65).

Combination of hHcy and IRI aggravates the neurodegenerative processes and might eventually lead to the development of Alzheimer’s disease-like neuropathology (18, 38). In rats that were fed a high-Met diet, IRI further increased Hcy levels, aggravated the degeneration of the hippocampal neurons, decreased grey matter volume, and altered the metabolic ratio (42, 47). Furthermore, many studies have shown the deleterious role of hHcy on cognition and cerebral microbleeds (45, 66–70).

Astrocytes activation can precede neuronal loss and aggravate ischemia-induced brain injury (71). In animals fed Met diet and subjected to IRI, short, thicker, and branched processes of astrocytes were found (20, 72).
Ischemic Brain Injury in Hyperhomocysteinemia

Cognitive decline ↑
βA and pTau
Metabolomic changes ↑
Microglia activation ↑
Cognitive decline ↑
Astrocyte changes ↑
TUNEL + ↑
Fluro Jade-C + ↑
Metabolomic changes ↑
βA and pTau
BBB permeability ↑
Homocysteinylation of mitochondrial proteins
Histone acetylation ↑
SPCA pump ↓
Mn-SOD ↑ Catalase ↓
Lipoperoxidation ↑ Protein oxidation ↑
Homocysteinylation of mitochondrial proteins
Hcy + IRI

Figure 1. **Mechanisms leading to the neurotoxicity in hHcy + IRI conditions.** hHcy+IRI-mediated neurotoxicity is the result of a plethora of dysregulated pathways including redox dysbalance, lipoperoxidation, protein oxidation, secretory pathway Ca^{2+}/Mn^{2+}-ATPase, and p38 MAPK. Together, they cause neurodegeneration through a variety of mechanisms including the induction of apoptosis, disruption of the blood brain barrier, β amyloid and Tau accumulation, and alterations in metabolome. (↑), increased number of cells and/or activity; (↓), decreased number of cells and/or activity. βA, beta amyloid; BBB, blood-brain barrier; pTau, phosphorylated tau protein (3, 5, 7, 10, 15, 17–20, 26, 28, 37–43, 46, 53, 54, 58, 62).
Other studies demonstrate neuroinflammatory and neurodegenerative changes in various neuronal cell types (42, 73, 74). A time-dependent decrease of the tNAA/tCr ratio (N-acetylaspartate/total creatine [marker of metabolic alterations]) was also observed following IRI (37, 42, 70, 75). Choline, considered a marker of membrane integrity (76), i.e., phospholipids synthesis and degradation, suggests the process of hippocampal re/ de-myelination. An increased ratio of mlIns/tCr (myo-Inositol [mlIns]), total creatine [tCreatine]), suggests changes in the number and morphology of hippocampal astrocytes and the process of de-myelination (75, 77). Collectively, the results of metabolic analysis indicate that IRI with Met diet initiates progressive metabolic disturbances with the dysregulation of the myelinated tract in the hippocampus (42). While hHcy alone leads to an increase in the hippocampal volume (20, 42), in conjunction with IRI, it further increases cerebral edema on day 3, followed by a decrease on day 7—probably a sign of neurodegeneration (69, 78, 79). Sustained edema results from the blood-brain barrier disruption caused by hHcy, or its metabolites, or the excitotoxic effect of Hcy (47, 62). Most of the blood-brain barrier damage usually occurs before 48 h post-stroke, as shown in the embolic ischemic model in rats (69, 78). Studies also suggest that mild hHcy impairs cardiac contractility, alters metabolism, and causes cardiac muscle remodeling and dysfunction (32, 80), and abnormally activated MMP-9 (81). Collectively, a mild hHcy in combination with IRI generates a toxic environment with detrimental impact on neuronal tissue, volume disturbances, attenuated neurites, and activation of astrocytes in hippocampus (42).

Hypoxic or ischemic preconditioning is a widely recognized strategy that eventually leads to ischemic tolerance (82, 83). As discussed above, ischemiareperfusion injury leads to neurodegeneration of hippocampal neurons in the CA1 region (46). Preconditioning remarkably reduces neurodegeneration and confers neuroprotection (17, 28, 54). The combination of hHcy with ischemic injury further increases the extent of neurodegeneration, and preconditioning suppresses neuronal degeneration (3, 7, 17, 26, 28). Combination of both stressors (ischemia+hHcy) leads to the massive activation of phospho-p38 MAPK (17). A recent study (83), using NMR to assess metabolomic changes in rat plasma, showed disturbed glycolysis pathway, and increased ketone bodies along with increased utilization of triacylglycerols. A decreased level of glycolytic intermediates (lactate, pyruvate, acetate) with an increased glucose was found in ischemic and preconditioned animals (83). HHcy also induced alterations in intracellular signaling, epigenetic dysregulation in methylation or acetylation status, microRNAs (13, 84, 85), and one-carbon metabolism, as part of Hcy metabolism (3, 5, 13, 84). Remarkably, demethylation of SAM (S-adenosylmethionine) to SAH is the sole source of de novo methyl groups for the cell. Thus, in hHcy conditions, dysregulation of this step might have an implication for many cellular processes, including modulation of gene expression via epigenetic regulation (13, 84). Collectively, responses of neuronal cells to hHcy, IRI, and preischemic challenge in rats suggest a correlation of several etiological factors, such as antioxidant defense (3), alterations in the mechanisms of Ca2+ transport (26), DNA methylation, and chromatin remodeling. In summary, the combination of hHcy with ischemic injury increases the extent of neurodegeneration and preconditioning reduces neuronal degeneration (3, 7, 17, 26, 28, 83).
CONCLUSION

The prevalence of hHcy and its role in the pathogenesis of cerebrovascular disorders is still not fully explored. hHcy has a role in the etiology of neurological damages due to its toxic effect on neurons, glia and vascular endothelium. Furthermore, hHcy can cause hippocampal edema, metabolic depletion, and cognitive decline, which is subsequently exacerbated by ischemia-reperfusion insult. However, most of our current knowledge is largely based on experimental findings and strategies to decrease plasma Hcy level did not reach conclusive clinical outcomes (6, 66, 86). The efficacy of combined folic acid, B₆, and B₁₂-vitamin supplementation to reduce hHcy is clinically inconclusive. Prevention of hHcy has the potential to prevent human stroke and Alzheimer’s disease incidence, and deserves further investigation. Epigenetic DNA methylation as a consequence of hHcy by endogenous (polymorphism of Hcy and folate pathways genes) and/or exogenous factors (dietary Met intake or/and deficiency of folate and vitamins) might be involved in hHcy pathogenesis. Exploration of the methyl balance and understanding the pathophysiology of diseases from a “methylation point of view”, although challenging, is a worthwhile effort.

Acknowledgment: The authors acknowledge the financial support from grant VEGA, No : 1/0230/20 and APVV, grant No. 15/107.

Conflict of Interest: The authors declare no potential conflicts of interest concerning research, authorship, and/or publication of this chapter.

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