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Hyperhomocysteinemia and Neurologic Disorders: a Review

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Homocysteine (Hcy) is a sulfur-containing amino acid that is generated during methionine metabolism. It has a physiologic role in DNA metabolism via methylation, a process governed by the presentation of folate, and vitamins B6 and B12. Physiologic Hcy levels are determined primarily by dietary intake and vitamin status. Elevated plasma levels of Hcy (eHcy) can be caused by deficiency of either vitamin B12 or folate, or a combination thereof. Certain genetic factors also cause eHcy, such as C667T substitution of the gene encoding methylenetetrahydrofolate reductase. eHcy has been observed in several medical conditions, such as cardiovascular disorders, atherosclerosis, myocardial infarction, stroke, minimal cognitive impairment, dementia, Parkinson's disease, multiple sclerosis, epilepsy, and eclampsia. There is evidence from laboratory and clinical studies that Hcy, and especially eHcy, exerts direct toxic effects on both the vascular and nervous systems. This article provides a review of the current literature on the possible roles of eHcy relevant to various neurologic disorders. **J Clin Neurol 2014;10(4):281-288**

Key Words hyperhomocysteinemia, neurologic disorders, pregnancy.

Introduction

Homocysteine (Hcy) is a sulfur-containing amino acid that is generated from an essential amino acid: methionine. Hcy concentrations are maintained dynamically by either a transsulfuration or remethylation pathway. The enzyme methionine synthase, with vitamin B12 (hereafter referred to as B12) as a cofactor, remethylates Hcy back to methionine; folate is also required for this reaction. Hcy levels can also be affected by the activities of the enzymes methylenetetrahyrdofolate reductase (MTHFR) and cystathione beta-synthase (CBS). CBS controls the transsulfuration of Hcy to cystathione, and is dependent on vitamin B6 (hereafter referred to simply as B6) as a cofactor.[1,2] The metabolic regulation of Hcy is based on the distribution of available Hcy between remethylation and transsulfuration to cystathionine. Hcy is a precursor of S-adenosylmethionine (AdoMet) and a metabolite of S-adenosylhomocysteine (AdoHcy). The ratio of AdoMet to AdoHcy is defined as the methylation potential (MP).[3] The two pathways (i.e., remethylation and transsulfuration) are coordinated by AdoHcy, which acts as an allosteric inhibitor of the MTHFR reaction and as an activator of CBS.[4]

The physiologic levels of Hcy in healthy populations are determined primarily by the dietary intakes of methionine,[5] folate,[5] and B12.[6] It is thought that lifestyle conditions such as excessive coffee or alcohol consumption, cigarette smoking, and physical inactivity may play a role in modulating the plasma level of Hcy,[7] although the evidence remains controversial.[8] Diets abundant in vegetables, fruit, and bread can result in reduction in the plasma level of Hcy.[9] Elevated levels of Hcy (eHcy, or hyperhomocysteinemia) occur with aging and decreased renal function.[8-11]

There is strong evidence from laboratory and clinical studies that eHcy is an independent risk factor for cardiovascular disease, although some recent studies appear to refute this claim.[12] The findings of these laboratory studies and clinical observations suggest that eHcy exerts toxic effects on endothelial cells, the vascular wall structure, and the blood coagulation system.[4] The actions of eHcy on vascular endothelial cells lead to the proliferation of smooth-muscle cells, promote the oxidation of low-density lipoprotein, and increase colla-

gen synthesis and procoagulant activity, with all of these actions accounting for the development of atherosclerosis.[13] Polymorphism of the C677T genotype of the gene encoding MTHFR (*MTHFR*) generally plays a minor role in determining Hcy levels in healthy individuals,[14,15] although a meta-analysis found that it may cause mild eHcy without increasing the risk of cardiovascular disorders.[16] Furthermore, another meta-analysis found that the homogenous *MTHFR* 677TT genotype is associated with an increased risk of coronary artery disease.[17,18] Chronic eHcy has been shown to affect MP and DNA methylation, while *in vitro* acute treatment of lymphocytes from healthy male volunteers with high concentrations of Hcy (i.e., >20 μmol/L for 8 h) failed to cause any change in MP or DNA hypomethylation, indicating that eHcy-induced toxicity is most likely the result of a chronic, rather than acute, process.[17] Supplementation with folate reduces Hcy levels, but a consensus has yet to be reached as to whether it reduces vascular risk.[1-5,19] Recent clinical studies have shown that supplementation with folate alone does not reduce the risks of coronary artery disease and stroke.[18,20] Although eHcy is observed in many pathophysiologic conditions, there is a dearth of knowledge regarding the role of Hcy (and eHcy) in neurologic disorders. This article provides a review of the current literature on eHcy in various neurologic conditions, including stroke, minimal cognitive impairment, dementia, Parkinson's disease (PD), multiple sclerosis (MS), epilepsy, and pregnancy.

eHcy and stroke

Stroke is the second leading cause of death worldwide and the leading cause of adult disability in many countries. A stroke can be either an ischemic or hemorrhagic event that disturbs the blood flow to part of the brain, such as via the occlusion or rupture of a blood vessel. Prospective and retrospective clinical studies have shown that eHcy is a preclinical marker of stroke, and may be the cause of stroke-related thrombophilia.[21] A review of the clinical data on the relationship between eHcy and thrombosis revealed a positive association, with odds ratios (ORs) ranging from 2 to 13 in eight out of ten studies.[22] Facilitated formation of 8-iso-prostaglandin F (2alpha), a measurable marker of lipid peroxidation[23] (representing the peroxidation of platelet-derived arachidonic acid), was observed in eHcy patients with homozygous CBS deficiency, suggesting that enhanced lipid peroxidation from platelet activation is involved in eHcy-related ischemic stroke in CBS-deficient subjects. Interestingly, the frequency of craniocervical arterial dissection has been found to be elevated in stroke patients with even mildly increased Hcy.[23,24] This finding was supported by a prospective case-control study showing that Hcy levels were significantly higher in patients with spontaneous cervical artery dissection than in normal subjects, while no significant difference was found between stroke patients with spontaneous cervical artery dissection and those with atherothrombosis without dissection. Ocular involvement in eHcy has been reported in case reports of recurrent nonarteritic anterior ischemic optic neuropathy[24] and CBS deficiency causing retinal embolism due to dissection of the cervical carotid artery.[25]

The magnitude of the plasma Hcy elevation was observed to be associated with a graded increase in the pulsatility index (which is a measure of the vascular resistance distal to an examined artery) in all intracranial arteries in patients with ischemic stroke where there was no combined internal carotid arterial steno-occlusion (ICS).[25] The level of Hcy was significantly higher in ischemic stroke patients with ICS than in those without ICS. An Hcy of greater than 14.0 μmol/L is significantly associated with the progression of aortic arch atheroma, which is an independent risk factor for recurrent vascular events in transient ischemic attack and stroke patients.[26] These findings suggest that eHcy serves as a mediator of aortic plaque progression.

Since eHcy can be generated by a vitamin deficiency (folate, B12, or B6), vitamin supplementation is the option of choice for treating the condition, with the expectation of reducing the risk of associated morbidities, such as stroke. A consistent finding from clinical data is that folate supplementation is the most effective agent for lowering mild-to-moderate eHcy, with maximal benefit occurring in individuals with a higher pretreatment Hcy or lower pretreatment folate levels. B12 supplementation confers a minor additional benefit, whereas B6 supplementation has not been demonstrated to confer any further benefit. Daily supplemental multivitamins with folate at 400−1,000 μg, B12 at 400−600 μg, and B6 at 2−10 mg have been suggested as primary prophylaxis for individuals with known cerebrovascular disease and eHcy.[26,27] However, reports of convincing therapeutic effects of this regimen for stroke prevention have been ambiguous or lacking.

eHcy and mild cognitive impairment

Mild cognitive impairment (MCI) falls between the cognitive impairments encountered with normal aging and early dementia. Individuals experiencing MCI are still able to perform day-to-day activities in the setting of evident decline in memory or other cognitive faculties. MCI is divided into two subtypes (amnestic and nonamnestic), but neither type meets the diagnostic criteria for dementia. Rates of conversion from MCI to dementia are greater than from normal cognition, thus implying that MCI is a prodrome to dementia.

Clinical studies have shown that eHcy is associated with the transition from being cognitively healthy to developing dementia,[27-29] and that eHcy is an independent risk factor for the decline of cognitive performance in normal elderly sub-

jects and patients with Alzheimer's disease (AD). Furthermore, eHcy was found in both healthy elderly with and without MCI.[30] A correlation between hippocampal function, generalization performance, and eHcy was demonstrated, demonstrating a role of eHcy in declining cognitive function in both healthy controls and patients with MCI.[28] A double-blind, randomized controlled study revealed a positive correlation between the presence of eHcy and the rate of brain atrophy in elderly with MCI.[29] Administration of folate exerts demonstrable protective effects, preventing or delaying the brain atrophy process associated with eHcy in MCI with respect to conversion to dementia.[31] On the other hand, abnormal levels of plasma folate, B12, and Hcy are associated with declining cognitive function in cognitively impaired elderly and AD patients.[32]

However, there exists controversy regarding the evidence for associations between MCI and eHcy. McMahon and colleagues performed a 2-year, double-blind, placebo-controlled, randomized clinical trial involving 276 healthy participants aged at least 65 years who had plasma Hcy concentrations of at least 13 μmol/L. The Hcy-lowering treatment comprised a daily supplement of folate (1,000 μg), B12 (500 μg), and B6 (10 mg). Cognition was tested at baseline and after 1 and 2 years of treatment, with treatment effects adjusted for baseline values, sex, and education. Although a significant reduction of Hcy was observed in the group treated with Hcy-lowering agents, the trial failed to show differences in cognition between the non-demented participants with and without treatment.[29,31,32] Reitz et al.[33] performed a longitudinal prospective observational cohort study on early intervention and the relationship between the transition from normal cognition to MCI to prevent subsequent decline into AD. They found no correlation between elevated baseline Hcy levels and conversion to all-cause MCI among either the amnestic or non-amnestic type, and concluded that plasma Hcy levels measured at baseline were not related to MCI or its subtypes in an elderly multiethnic cohort.[34] The conflicting findings regarding the association between eHcy and MCI warrant further investigations.

Hcy and dementia

Dementia is an acquired, generalized, and usually progressive impairment of cognitive function that affects the awareness of surroundings. It is a medical condition caused by structural and/or irreversible functional disturbances of the cerebral cortex, its subcortical connections, or both. Dementia has been estimated to affect 5−20% of individuals older than 65 years,[34,35] and its incidence increases with age.

Clinical observations have disclosed that eHcy is frequently associated with dementia. eHcy is a predictive factor for AD, which is the most common cause of dementia in the elderly.[36] The findings of several prospective clinical studies indicate that eHcy is an independent risk factor not only for promoting dementia,[36,37] but also for facilitating medial temporal lobe atrophy and the evolution from MCI into AD. Interestingly, eHcy potentially impairs cognitive function in AD patients in the setting of elevated plasma amyloid beta 42 levels.[27,30] Notably, eHcy is correlated with the duration rather than the onset of AD.[35]

The mechanism underlying the effect of eHcy on dementia is receiving intense interest. Hcy may act like an excitatory neurotransmitter by competing with inhibitory neurotransmitters such as gamma-aminobutyric acid (GABA).[36] In addition, eHcy induces microvascular permeability by attenuating the GABA-A/B receptors and increasing redox stress, in turn activating a disintegrin and metalloproteinase, which suppresses tissue inhibitors of metalloproteinase. This process causes disruption of the matrix in the blood-brain barrier[37] and contributes to vascular dementia. Since eHcy is usually associated with low vitamin status, it is unclear whether eHcy or vitamin deficiency, either alone or in combination, is responsible for cognitive decline. Low folate levels are a known risk factor for cognitive decline in high-functioning older adults with concomitant eHcy.[37] The risk of developing cognitive decline may be modified by the dietary folate intake. Indeed, administration of folate with vitamin-B-complex exerts a protective effect on the brain atrophy associated with eHcy in MCI with respect to conversion to dementia[31,38] and delays cognitive deterioration in cognitively impaired elderly and AD patients.[32] However, in a separate clinical trial, daily supplementation of folate at 2 mg, B6 at 25 mg, and B12 at 400 μg over 2 years failed to exert a therapeutic effect in the cognitive subscale of the Alzheimer's Disease Assessment Scale compared with the placebo-treated group.[38] Similar observations were made by other investigators.[39] Ironically, it has been reported that supplementation with high doses of folate may worsen long-term episodic memory, total episodic memory, and global cognition, although these findings require validation.[40] In addition, there was no significant decrease in the risk of cognitive impairment [OR, 0.72; 95% confidence interval (CI), 0.25−2.09] and dementia (hazard ratio, 0.72; 95% CI, 0.29−1.78) over 8 years of follow-up.[40,41]

Hcy and PD

Parkinson's disease is a progressive neurodegenerative disorder that is characterized by akinesia/bradykinesia, tremors, postural instability, and rigidity due to the loss of dopaminergic neurons in the substantia nigra in the midbrain. The etiology of PD remains unclear, but innate and environmental factors including aging, genetics,[40] toxins such as pesticides, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP),[41] metals and

other toxicants, traumatic brain injury, and deficiency of trophic factors are thought to play a part. Lifestyle factors including cigarette smoking[42] and coffee consumption,[43] with a gender bias,[44] may also play a role in the development of PD.[45] Recent clinical studies have shown that eHcy is observed in PD patients and may be involved in the pathogenesis of the PD neurodegeneration.[46]

Cultured human dopaminergic neurons and animal models are useful tools for studying the pathogenesis of PD.[47] Mice on a folate-deficient diet exhibit eHcy and increased sensitivity to the neurotoxin MPTP, as well as PD-like pathology and motor dysfunction, suggesting that eHcy increases the susceptibility of dopaminergic neurons to damage and hastens the onset and progression of PD.[48] The adverse effect of eHcy on dopaminergic cells in these mouse models is ameliorated by administration of folate and antioxidants, such as uric acid, and an inhibitor of poly-adenosine diphosphate-ribose polymerase.[49,50] The increased susceptibility of dopaminergic neurons to oxidative stress in the presence of eHcy suggests a mechanism whereby dietary folate influences the risk of PD.[49]

Current treatments for PD exert complicated effects on plasma Hcy levels. Levodopa (L-DOPA), the most effective known treatment for PD, causes the formation of eHcy due to methylation of L-DOPA via catechol-O-methyltransferase (COMT).[50-52] It was demonstrated in a human study that eHcy is produced in L-DOPA-treated patients.[53] The occurrence of L-DOPA-induced eHcy can be prevented by giving patients tolcapone, which is a COMT enzyme inhibitor.[54,55] However, multiple human and clinical studies on the genetic and environmental factors associated with eHcy and its clinical implications in PD failed to provide evidence for eHcy as an independent risk factor for PD. The possibility that eHcy may contribute to the pathogenesis of PD remains to be confirmed.[56,57]

Hcy and MS

Multiple sclerosis is an autoimmune-mediated inflammatory demyelinating disease of the central nervous system. An interplay between genetic and environmental factors may trigger the inflammatory process. Interestingly, eHcy has been observed in patients with MS, but appears to be unrelated to immune activation, oxidative stress, or a deficiency in B6, B12, or folate, indicating a role of eHcy in the pathogenesis of MS.[58-61] Neurons may be particularly susceptible to eHcy-induced excitotoxicity[62,63] since eHcy compromises methionine availability, which in turn interferes with methyl group donor in many biochemical processes. Hypomethylation of myelin basic protein could result in less stable myelin structures that are amenable to degeneration.

Several clinical studies have found Hcy levels to be significantly higher in MS patients than in controls without B12 and/or folate deficiency, in the absence of *MTHFR* mutation;[62-65] however, one study involving a population of Greek MS patients found no eHcy.[64] In MS, eHcy is correlated with clinical progression[65] and worsening cognitive function in nonverbal reasoning, visual attention, visual-spatial memory, and visual spatial ability, compared to MS patients with normal Hcy levels. Variables associated with MCI or moderate cognitive impairment reportedly include chronic progressive MS, longer duration of disease, moderate or severe disability, higher plasma Hcy levels, and chronicity of eHcy.[66] Notably, administration of folate does not appear to reduce intracellular Hcy, but may adversely affect intracellular one-carbon metabolism.[67]

eHcy in pregnant women and neural-tube defects in their newborn

In physiologic conditions, Hcy concentrations fall during normal pregnancy.[68,69] However, eHcy is associated with common pregnancy complications such as preeclampsia, and adverse pregnancy outcomes such as newborns with neural-tube defects (NTDs).[70,71] NTDs occur in the first month of pregnancy, with a reported incidence in the United States of 1 in 1,000−1,500 live births.[8,72,73] The two most common NTDs are spina bifida, which occurs when the fetal spinal column does not close completely, and anencephaly, in which the majority of the brain and skull do not develop; newborns with anencephaly are either stillborn or die shortly after birth. In addition, adverse fetal outcomes during pregnancy may be related to placental pathology. For example, prothrombotic states such as thrombophilia, antiphospholipid antibody syndrome, and eHcy can cause placental vascular insufficiency. The causes of NTDs in pregnant women may include obesity, poorly controlled diabetes, and adverse effects of medications such as the antiepileptic drug valproic acid, which may all be linked to eHcy. Environmental and lifestyle factors such as dietary and lifestyle preferences, excessive coffee or alcohol consumption, cigarette smoking, and absence of exercise may play a role in generating eHcy.[7,73] Smoking during pregnancy has been demonstrated to cause eHcy in newborns,[74] and deficiency of folate and B12 metabolism may cause eHcy with an NTD. Supplementation of folate before pregnancy can reduce the risk of having a baby with an NTD.[75,76] Pregnant mothers with an *MTHFR* 677 C→T polymorphism are likely to have significantly elevated Hcy and an increased likelihood of spina bifida in their offspring (OR, 1.7; 95% CI, 1.1−2.6).[77]

Clinical studies have shown that eHcy is associated with an increased risk of preeclampsia and pregnancy loss, which may be caused by abnormalities of the placental vasculature due to placental vascular endothelial dysfunction.[78,79] Signifi-

cant eHcy was observed in preeclamptic women versus controls (Hcy: 16.39 μmol/L vs 9.45 μmol/L; $p \leq 0.001$).[80] It is estimated that an increase in plasma Hcy of 1 μmol/L corresponds to a 151-g reduction in birth weight in the third trimester for Japanese women with a purely dietary intake of folate.[81] However, no association between eHcy and gestational hypertension has been found.[81,82]

Since eHcy is a recognized risk factor for cardiovascular diseases in adults,[77] the effects of eHcy on the occurrence of congenital heart defects (CHDs) in newborns have been investigated. eHcy was evident and CHDs were more likely to occur in the offspring of women who smoked prior to conception, and especially in those with the *MTHFR* 677C-T genotype (OR, 11.8; 95% CI, 2.59−53.3),[82] although the *MTHFR* polymorphism alone does not appear to represent an individual risk factor for CHD.[80-82] Copper/zinc-superoxide dismutase activity and MT-1 (metallothionein) mRNA expression, which are two biomarkers of oxidative stress, are abnormal decreased and increased in eHcy fetuses with CHD, respectively. Supplementation with zinc and folate normalizes these markers in rat fetuses with eHcy.[83]

The precise mechanism by which eHcy provokes preeclampsia in pregnant mothers and produces NTDs or CHDs in their newborns remains unclear. Clinical observations suggest that the adverse effects of eHcy play an important role in the pathogenesis of these conditions, but the underlying mechanisms remain to be elucidated.

eHcy and epilepsy

Epilepsy is characterized by recurrent, unprovoked seizures due to excessively abnormal discharge of the cerebral cortical neurons. It may manifest as a convulsion, hypertonic movements or stereotyped movement, disturbances in sensation, alteration of perception, or loss of consciousness. eHcy has been observed in patients with epilepsy, but this may also result from the adverse effects of chronic use of antiepileptic drugs such as valproic acid, topiramate, and oxcarbazepine,[84] especially in those with *MTHFR* 677C-T or 1298A-C polymorphisms.[85] As mentioned above, the decreased *MTHFR* enzymatic activity in these patients[86] causes eHcy. Gorgone et al.[87] found that the rate of brain atrophy was higher in epilepsy patients with eHcy in an MRI study of 58 patients with epilepsy who were taking antiepileptic drugs and, for comparison, 60 age- and sex-matched controls. They concluded that both Hcy and polypharmacy contribute to brain atrophy in epileptic patients.[87]

It has been shown that elevated levels of homocysteic acid and Hcy sulfinic acid in pediatric epilepsy with homocysteinuria are excitotoxic via N-methyl-D-aspartate (NMDA) and non-NMDA receptors. Moreover, eHcy inhibits glutamate decarboxylase activity and disturbs glutamate-glutamine metabolism.[88]

eHcy and peripheral neuropathy

Peripheral neuropathy (PN) is a common neurologic disorder that is more common among the elderly. Depending upon the type of nerve fibers involved, PN can cause symptoms of sensory, motor, and autonomic dysfunction. It has many possible etiologies: metabolic, infectious, inflammatory, toxic (including adverse effects of certain drugs and radiation), malnutritional, inherited, or autoimmune-mediated. Recent clinical studies have revealed that eHcy exaggerates the prevalence of PN in diabetics and exacerbates any preexisting diabetic neuropathy.[89-91] In a community-based study of 483 adults, Bruce and Young[89] observed a high prevalence of PN among those with undiagnosed diabetes. After multivariate logistic regression analysis and controls for age, female sex, low education, glycated hemoglobin, and smoking, it was found that eHcy was independently associated with PN.[89] The notion of eHcy as an independent variable associated with increased prevalence of diabetic PN has been supported by subsequent clinical studies.[90-93]

Elevated levels of Hcy may be a risk factor for diabetic autonomic neuropathy. It was estimated that for each 1-mmol/L increase in Hcy, there is a 7.1% increased risk in diabetic autonomic neuropathy ($p<0.05$), while a 5 μmol/L increase in Hcy levels would increase the OR to 2.6 for PN (95% CI, 1.07−6.33) in diabetic patients.[94] Our group recently reported that isolated eHcy without any concurrently identifiable risk factors for PN is an independent risk factor for the development of PN.[94] Previous animal studies have shown a marked elevation of AdoHcy in the neural tissues in a methylation-deficiency pig model, which suggested that eHcy is able to cause PN through a raised AdoHcy level in those tissues.[95] Therefore, eHcy-induced PN may be a potentially treatable entity.[96] Interestingly, anti-PD medication causes eHcy, and an increased frequency of PN was evident in PD patients with eHcy who had been treated with L-DOPA,[97] suggesting a link between eHcy and the development of PN.

Summary

Elevated level of Hcy has been observed in many medical conditions including various neurologic disorders. The pathogenesis of eHcy is currently attracting considerable research interest, simply because early intervention to normalize the Hcy level may be beneficial to these patients and prevent them from suffering eHcy-induced additional cell damage and dysfunction. However, current clinical observations have produced inconsistent conclusions. Although controversy ex-

ists, this review on the relationship between eHcy (namely hyperhomocysteinemia) and neurologic disorders may help toward a better understanding of the current knowledge and rationales for further clinical and basic research. A simple blood test that can easily detect eHcy may be useful during the initial workup of common neurologic disorders. The strategy of attempting to normalize eHcy using a simple and relatively harmless multivitamin regimen can be tried in addition to the usual standard of care for that particular disorder. The usefulness of this potential strategy should be addressed in future studies.

Conflicts of Interest

The authors have no financial conflicts of interest.

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