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Homocysteine and neuropsychiatric disorders

Homocisteína e transtornos psiquiátricos

Perminder Sachdev

School of Psychiatry, University of New South Wales, and the Neuropsychiatric Institute, Prince of Wales Hospital. Sydney, Australia

Abstract The author presents an overview of the current literature on homocysteine as a risk factor for neuropsychiatric disorders. The databases MEDLINE, Current Contents and EMBASE were searched (between 1966 and 2002) for English language publications with the key words 'Homocysteine' and 'Stroke'; 'Alzheimer Disease'; 'Cognitive Impairment'; 'Epilepsy'; 'Depression'; or 'Parkinson's disease'. Individual articles were hand searched for relevant cross-references. It is biologically plausible that high homocysteine levels may cause brain injury and neuropsychiatric disorders. Homocysteine is proatherogenic and prothrombotic, thereby increasing the risk of cerebrovascular disease, and may have a direct neurotoxic effect. Evidence for homocysteine as a risk factor for cerebral microvascular disease is conflicting but warrants further study. Cross-sectional and some longitudinal studies support increased prevalence of stroke and vascular dementia in hyperhomocysteinemic individuals. The evidence of increased neurodegeneration is accumulating. The relationship with depression is still tentative, as it is with epilepsy. Currently, treatment studies are necessary to place the evidence on a stronger footing, and maybe high-risk patients should be screened for hyperhomocysteinemia and this should be treated with folic acid. More research evidence is necessary before population screening can be recommended.

Keywords Homocysteine. Alzheimer's disease. Depression. Vascular dementia. Stroke. Alcoholism. Parkinson's disease. Folate. Vitamin B12. Vitamin B6.

Resumo O autor apresenta uma visão geral da literatura atual sobre homocisteína como um fator de risco para os transtornos neuropsiquiátricos. Foram pesquisados os bancos de dados MEDLINE, Current Contents e EMBASE (entre 1966 e 2002) para publicações em língua inglesa utilizando as palavras-chave "Homocisteína" e "AVC"; "Doença de Alzheimer"; "Déficit Cognitivo", "Epilepsia", "Depressão" ou "Doença de Parkinson". Artigos individuais foram pesquisados para referências cruzadas relevantes. É biologicamente plausível que altos níveis de homocisteína possam causar lesão cerebral e transtornos neuropsiquiátricos. A homocisteína é pró-aterogênica e pró-trombótica. Dessa forma, aumenta o risco de acidente vascular cerebral, podendo ter um efeito neurotóxico direto. Evidências de que a homocisteína seja um fator de risco para doença microvascular cerebral são conflitantes, mas justificam maiores estudos. Estudos transversais e alguns longitudinais suportam a crescente prevalência de acidente vascular cerebral e demência vascular em indivíduos com hiper-homocisteínemia. As evidências de crescente neurodegeneração estão se acumulando. A relação com a depressão ainda é experimental, da mesma forma como com a epilepsia. Atualmente, estudos sobre tratamentos são necessários para colocar as evidências sobre bases mais sólidas. Os pacientes de alto risco também devem ser pesquisados para hiper-homocisteínemia, cujo tratamento deve ser feito com ácido fólico. Mais evidências são necessárias antes que pesquisas populacionais possam ser recomendadas.

Descritores Homocisteína. Doença de Alzheimer. Depressão. Demência vascular. Acidente vascular cerebral. Alcoolismo. Doença de Parkinson. Folato. Vitamina B12. Vitamina B6.

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Introduction

The neuropsychiatric literature has recently seen a spate of papers on the role of homocysteine (Hcy), a sulphur-containing amino acid that is not a dietary constituent and does not form proteins.[1] Hcy is derived exclusively from the demethylation of methionine, an amino acid plentiful in both plant and animal proteins and the main source of protein sulphur atoms. The demethylation process is an important metabolic pathway resulting in vital methylation reactions in the body. The resulting Hcy is either remethylated to methionine, a process that utilises folate and vitamin B12 as cofactors, or catabolized by transsulfuration into cystathionine if excess Hcy is present, using vitamin B6 as a cofactor.[2] Three main enzymes are involved in the metabolism of Hcy: methionine synthase (MS) and 5-methyltetrahydrofolate reductase (MHTFR) in the remethylation, and cystathionine β-synthase (CBS) in transsulfuration. These enzymes, along with the coenzymes, maintain the intracellular concentration of Hcy within a narrow range, even though the plasma levels vary considerably.

The plasma Hcy is about 70% bound to albumin and exists in many forms: reduced Hcy, oxidised Hcy, and mixed disulphides comprising Hcy and other thiols. Most laboratories measure the total pool of Hcy (tHcy) in the plasma, usually from a fasting sample.[3] A challenge test may be performed by measuring tHcy 2 to 8 hours after an oral methionine load (100 mg methionine per Kg body weight). The normal plasma range for tHcy is accepted as between 5 and 15 μmol/L.[4] Hyperhomocysteinemia is common, and elevated Hcy levels may be moderate (15-30 μmol/L), intermediate (31-100 μmol/L) or severe (>100 μmol/L).[4] The causes of elevated Hcy levels are multiple and may be genetic or acquired. They are listed in Table 1.

The most common genetic abnormality of homocysteine metabolism is a substitution at nucleotide 677 (C677T) in the gene encoding for the enzyme MTHFR, rendering it about 50% less active.[5] In population studies in Western countries, 9-17% of the population was homozygous for this mutant enzyme and 30-41% was heterozygous.[5-8] Homozygous deficiency of CBS is rare and causes homocystinuria, but its heterozygous deficiency, leading to moderate elevation of Hcy, is present in 1 in 300 individuals.[6] Acquired causes of hyperhomocysteinemia include deficiencies of the cofactors (vitamins B12, B6 and folate), increasing age, diseases such as renal failure and hypothyroidism, medications that interfere with the metabolism of vitamin B12, B6 or folate, and lifestyle factors such as cigarette smoking, alcoholism, diet and physical inactivity.

Homocysteine and neuropsychiatric disorders

Epidemiological evidence has gradually accumulated to implicate Hcy in the pathophysiology of many neuropsychiatric disorders.[9] Hcy is recognised to be pro-atherogenic and prothrombotic. The association of Hcy with atherosclerosis was first demonstrated by McCully[10] in an infant with homocystinuria, and later demonstrated for coronary artery disease (CAD) by Wilcken and Wilcken.[11] Hcy is recognised as an independent risk factor for atherosclerosis of coronary, cerebral and peripheral arteries.[12] High levels of Hcy cause endothelial cell injury by impairing endothelial-dependent vasodilatation[13] and endogenous tissue-type plasminogen activator activity, and decreasing endothelial DNA synthesis.[14] High Hcy levels also lead to lower intracellular levels of adenosine, which has a cardio- and vaso-protective effect.[15] Hcy causes the release of many inflammatory mediators that play an active role in atherosclerosis, such as TNFα and receptor for advanced glycation end-products (RAGE) and its signal-transducing ligand (EN-RAGE).[16] Thrombosis is increased by effects on platelet aggregation – increased synthesis of thromboxan A_2 and decreased synthesis of prostacyclin – and the clotting cascade – activation of factors V, X, and XII and inhibition of natural anticoagulants.[1] Hcy promotes smooth muscle growth[14] and the binding of lipoprotein(a) to fibrin.[17]

Hcy may also have direct neurotoxic effects through the induction of apoptosis[18] and NMDA mediated excitotoxicity,[19] and its metabolite homocysteic acid is also excitotoxic. Exposure of rat hippocampal neurones to Hcy has been shown to lead to activation of poly-ADP-ribose polymerise (PARP) and NAD depletion, which precede mitochondrial dysfunction, oxidative stress, caspase activation and neuronal apoptosis.[18] A limitation of all this evidence is that most studies were in animals with very high levels of Hcy, and the findings may not be generalisable to humans with moderate elevations of Hcy.

Table 1 - Causes of hyperhomocysteinemia.

Genetic causes	
	MTHFR deficiency (homozygous thermolabile C677T mutation) (10%)
	MTHFR defect (homozygous thermostable mutation) (rare)
	CBS deficiency (heterozygotes) (0.5-1.5%)
	CBS defect (homocystinuria – homozygotes) (rare)
	Functional methionine synthase deficiency (rare)
Nutritional causes	
	Folic acid deficiency
	Vitamin B12 deficiency
	Vitamin B6 deficiency
	Excessive methionine-rich animal protein
Systemic disorders	
	Renal disease
	Cancer
	Hypothyroidism
	Psoriasis
	Diabetes mellitus
	Acute phase of stroke or myocardial infarction
Physiological factors	
	Increased age
	Male sex
	Menopause
	Race
Drugs	
	Anticonvulsants (phenytoin, carbamazepine)
	Oral contraceptives
	Methotrexate
	Nitrous oxide
	Trimethoprim
	Sulfasalazine
	Lipid lowering drugs
Lifestyle factors	
	Tobacco smoking
	Alcohol abuse
	Physical inactivity
	Diet

Stroke

A meta-analysis of 27 case control and cross-sectional studies by Boushey and colleagues[12] demonstrated that Hcy was an independent risk factor for cerebrovascular disease, with a summary odds ratio of 1.9 per 5 μmol/L increment in plasma homocysteine. A more recent meta-analysis reported an odds ratio for cerebrovascular disease with a tHcy above the 95th percentile of 3.97.[20] The results of prospective studies have been mixed. Of the 3 negative studies, two had surprisingly low serum total tHcy levels[21,22] but one had average levels.[23] Of the prospective studies reporting a positive association, Perry and colleagues[24] found a significant trend of increasing risk stroke with increasing tHcy, while the Women's Health Study[25] found an increased risk for myocardial infarction and stroke only in patients whose homocysteine levels were at least 13.26 μmol/L. In the Framingham[26] and Rotterdam[27] studies, the risk of all strokes was significantly greater for the highest compared to the lowest quartile of Hcy. The Rotterdam study found a positive correlation between increasing tHcy and risk of stroke, particularly lacunar strokes, with a 7% increase in risk for all strokes per 1 μmol/L rise in tHcy.

The differences in the above findings may be due to the following: i) The proportion of subjects with elevated levels of Hcy (e.g. 25% in the Framingham study, but only 10% in the Finnish study).[21] ii) Age of the sample, although the evidence for this is mixed.[21-23] iii) Other risk factors for cerebrovascular disease, such as hypertension, may play a role. iv) Genetic factors may be important. However, individuals with the C677T MTHFR mutation do not appear to have an increased risk of ischaemic events,[28-30] probably because elevated homocysteine levels arise only in the presence of environmental factors, such as folate deficiency.

In conclusion, there is good epidemiological evidence which suggests that elevated homocysteine is a significant risk factor for stoke. Conclusive proof will come from intervention trials aimed at examining the effects of reduced levels of Hcy, such as the VITATOPS and the VISP trials.

Vascular dementia (VaD)

The association of Hcy with stroke would make it a risk factor for VaD as well, and this has been examined empirically in at least 7 studies,[31-37] although in none of these was the diagnosis confirmed by neuropathological examination. One study examined a clinically homogeneous group of elderly patients with subcortical vascular encephalopathy, and found tHcy to be a stronger risk factor than age, hypertension, diabetes or smoking, with an odds ratio of 5.7 (95% CI 2.5-12.9).[32] The relationship of Hcy to increased small vessel disease in this study was not supported by our own work.[37] However, in a younger (60-64 years) community sample, we found Hcy to be a significant determinant of deep but not periventricular hyperintensities.[38] A study in Alzheimer's disease patients reported an OR for leukoaraiosis of 1.40 for every 5 μmol/L rise on hcy levels.[39] The role of Hcy in cerebral microvascular disease is therefore uncertain and needs further study. Elevated homocysteine levels were reported in patients with NINDS-AIREN diagnosed VaD compared with controls.[34] Another study also found significantly higher homocysteine levels in memory clinic patients with vascular dementia, Alzheimer's dementia and dysmentia compared to those with subjective memory impairment only, with the highest values seen in the group with vascular dementia.[33]

Alzheimer's dementia (AD)

That Hcy may be related to neurodegenerative disease is an intriguing finding that has been supported by many lines of evidence. Of ten cross sectional studies that examined the association between AD and Hcy levels,[33,34,40-47] eight reported higher Hcy levels in AD patients compared to controls. A number of authors have demonstrated a correlation between homocysteine levels and severity of cognitive impairment[33,43,45] except in the very very old.[48] The report by Clarke and colleagues[42] is worthy of special comment. These authors examined 164 patients with AD, with histological confirmation in 76, and found that those with baseline tHcy in the top two tertiles had significantly more temporal lobe atrophy after 3 years than those in the lowest tertile, suggesting that elevated tHcy levels may determine rate of progression of the disease. A recent cross-sectional MRI study described a similar correlation between high homocysteine levels and cerebral atrophy in healthy elderly individuals, suggesting

Table 2 - Pathogenetic mechanisms for the effect of high homocysteine on the brain.

Increased risk of cerebrovascular disease and injury	
	Impairs endothelial-dependent vasodilatation
	Impairs endogenous tissue-type plasminogen activator activity
	Decreases endothelial DNA synthesis
	Lowers intracellular levels of adenosine
	Promotes inflammatory mediations such as TNFa, RAGE and EN-RAGE
	Alters expression of genes regulating cell growth and differentiation
	Inhibits nitric oxide synthase
	Enhances lipid peroxidation
	Impairs cellular antioxidative potential
	Increased synthesis of thromboxan A_2 and decreased synthesis of prostacyclin, thereby increasing platelet aggregation
	Activation of factors V, X, and XII and inhibition of natural anticoagulants
	Enhances binding of lipoprotein(a) to fibrin
Direct neurotoxicity	
	Increased oxidative stress, and Impaired cellular antioxidative potential
	Activation of poly-ADP-ribose polymerise (PARP) and NAD depletion
	Caspase activation and neuronal apoptosis
	NMDA mediated excitotoxicity

the possibility that hyperhomocysteinemia is neurotoxic.[49] Another report from the Framingham study was that the risk of developing dementia (RR 1.3 [95%CI, 1.1-1.6]) and specifically AD (RR 1.9; [95%CI, 1.2-3.0]) during a follow up period averaging 8 years was almost doubled in those whose homocysteine levels were above 14 μmol/ L.[50] In this study, the risk of AD increased by 40% for every 5 μmol/L increase in tHcy, after adjustment for confounding variables. These findings are intriguing enough to recommend more prospective studies, and perhaps placebo-controlled intervention studies to definitely establish the relationship between Hcy and AD.

Cognitive impairment

The evidence that elevated homocysteine is a risk factor for cognitive impairment in non-demented subjects remains controversial and limited by the small number of studies. All the cross-sectional studies[33,37,51-54] reported an inverse association between elevated levels of homocysteine and measures of cognitive impairment, but the deficits differed in each study. The study by Duthie et al[54] found that Hcy accounted for approximately 7-8% of the variance in cognitive performance in elderly subjects. In the Sydney Stroke Study, we found that Hcy levels were associated with cognitive impairment, after controlling for age and folate levels, over and above what could be accounted for by MRI-visible cerebrovascular disease, and this was strongest in the top quartile of Hcy levels.[37] Of the prospective studies, one failed to find any association between homocysteine and concurrent cognitive impairment or decline, as assessed by Mini-Mental State Examination (MMSE), after follow-up of almost three years.[55] Another, using a battery of cognitive tests, found an association between tHcy and baseline cognitive impairment, but none with cognitive decline over 3 and 7 years.[56] A small (n=32) study concluded that high baseline tHcy scores predicted decline in MMSE scores after 5 years.[43] Unfortunately the median decline in MMSE was one point, and the sensitivity of a small change in MMSE is poor. In summary, cross-sectional studies suggest a relationship between homocysteine and cognitive impairment, but the evidence from longitudinal studies is not consistent.

Depression

The relationship between depression and B vitamins, in particular folic acid and B12, has been of interest for much longer than the focus on Hcy. Studies that have examined the association between homocysteine and B group vitamin levels and depression have reported no consistent finding. In two large correlational studies, the association of depression was found with folic acid[57] and B12[58] rather than with Hcy levels. The study by Bottiglieri et al[59] is interesting in that of 46 inpatients for depression, about one-half had elevated homocysteine levels compared with controls, with only a few subjects with low red cell folate, and subjects with high homocysteine levels had more severe Hamilton Depression Rating Scale (HDRS) scores. A study of late-onset depression[60] found higher rates of C677T MHTFR mutation in this group compared to early-onset depression, but tHcy levels were not reported.

The relatively high numbers of depressed patients with subclinical B group vitamin deficiencies in these studies suggests they may result from depression-associated anorexia rather than be involved in the pathogenesis of depression. It is noteworthy that folate deficiency has been associated with poor response to antidepressant medication, suggesting that augmentation with folate may improve resolution of depression. One study has examined the effect of folate as an adjunct to antidepressant medication by supplementing fluoxetine with 0.5 mg of folic acid in a randomised placebo controlled trial.[61] Of interest, only women demonstrated significant improvement on this combination. The decrease in plasma Hcy of the women taking folate and fluoxetine correlated with improvement, while vitamin B12 levels were not associated with clinical response. The authors speculated that the differential response of women to folate supplementation might be because males require more folate supplementation to significantly alter serum folate and Hcy levels. In summary, although no prospective studies have been performed, there is some evidence that folate and Hcy status predicts depression severity and response to antidepressant treatment and that folate supplementation effectively improves response to fluoxetine. The suggestion has also been made that Hcy may play a role in the elevated rates of cardiovascular mortality in depression.[62]

Alcoholism

Hyperhomocysteinemia has been reported in chronic alcoholism.[63] A recent study[64] suggested that the brain atrophy seen in patients with alcoholism may be related to high Hcy levels. This cross-sectional study warrants confirmation, preferably in a longitudinal design.

Epilepsy

Patients with homocystinuria, with high serum levels of Hcy (50-200 μmol/L), have seizures in 20% cases,[65] but this relationship with lower Hcy levels has not been established. The administration of high doses of Hcy to animals results in convulsive seizure.[66] Furthermore, many anticonvulsants reduce folic acid levels, thereby raising Hcy levels and increasing the risk of vascular disease and cognitive impairment. High Hcy levels in epileptics have also been implicated in the teratogenic effects of anticonvulsants, leading to the recommendation by the American Academy of Neurology[67] that all women of childbearing potential who are taking anticonvulsants consume at least 0.4 mg/day of folic acid.

Parkinson's disease

Hyperhomocysteinemia has been described in patients with Parkinson's disease (PD), and is likely to result from the formation of S-adenylhomocysteine during the metabolism of levodopa, i.e. it is secondary to treatment with levodopa. Hyperhomocysteinemia is more severe in PD patients homozygous for the C667T MTHFR mutation,[68,69] but even heterozygotes have than those without the mutation.[69] Levodopa treated patients may therefore be at a higher risk for cognitive impairment and atherosclerotic disease, especially those with the T/T

genotype. In addition, if homocysteine is neurotoxic, levodopa treatment could worsen nigrostriatal degeneration.

Conclusions

It is obvious from the above review that Hcy is an intriguing amino acid that will continue to excite neuropsychiatric research. Its role in vascular disease is well-supported, but there are still some questions about its direct neurotoxic effects, requiring further studies. The best evidence will come from studies that aim to examine the effect of Hcy-lowering treatments. Since the relationship of Hcy levels with vascular pathology and cognitive impairment is linear, the 'normal' range of Hcy levels must be closely examined, as lower levels may be advantageous. The analogy here is with blood pressure and cholesterol.

Fortunately, Hcy levels can be easily reduced by supplementation with folic acid, and to a lesser extent B12 and B6. Several studies have demonstrated that folate supplementation is the most effective means of intervention. The Homocysteine Lowering Trialists' Collaboration[70] published a meta-analysis of 12 studies involving 1114 subjects, and concluded that folic acid at doses between 0.5 and 5 mg folate per day lowered homocysteine levels by 25%, (95%CI: 23-28%). A study comparing doses of 0.2, 0.4, 0.6, 0.8 and 1.0 mg of folic acid per day demonstrated maximum Hcy reduction with the 0.8 mg dose.[71] Both studies reported a more profound effect in those with higher pre-treatment homocysteine concentrations, and doses of at least 0.5 mg/day of folic acid were necessary, with betaine being less effective.[72] The long-term efficacy of folic acid in reducing Hcy levels is not fully known.

The benefits of vitamins B12 and B6 are more modest. Meta-analysis of the effect of vitamin B12 suggested that a dose of 0.02 to 1 mg/day produced an additional reduction of Hcy by about 7% (95% CI: 3-10%), but vitamin B6 had no additional effect.[70] In an open study,[73] the effects of folate and cobalamin supplementation in 33 people with mild to moderate dementia were studied. After two months almost all of the patients with elevated tHcy pre-treatment were cognitively and behaviourally improved, while the majority of patients with normal pre-treatment tHcy did not improve. Treatment with folic acid carries the risk of precipitating subacute combined degeneration of the spinal cord in individuals with subclinical vitamin B12 deficiency. This can be avoided by either excluding vitamin B12 deficiency prior to the initiation of folate therapy, or supplementing folic acid with vitamin B12 at a dose of 400 µg/day or greater. Chronic treatment with vitamin B6 at doses greater than 400 mg/day can cause sensory peripheral neuropathy. In the United States of America there is a mandatory requirement that flour and cereals be fortified with140 µg of folic acid per 100 g of flour, and it is estimated that this is associated with a 3% reduction in the risk of homocysteine associated coronary artery stenosis.[74]

How should this information influence current clinical practice? We recommend that patients with stroke, symptoms of cerebrovascular disease, AD or mild cognitive impairment should be screened for hyperhomocysteinemia, and high Hcy treated with folic acid. Conditions known to be associated with high Hcy levels (e.g. renal failure, hypothyroidism, alcoholism etc.) should be similarly treated. Population screening for hyperhomocysteinemia cannot at this stage be justified although in the Framingham study, 29% of the elderly cohort had levels above 14 µmol/L.[75] Further research is necessary before this can be recommended. While the focus of folic acid fortification of cereals has so far been neural tube defects, the potential of reducing Hcy levels in the population[76] should also be a factor in the determination of governmental policy.

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Correspondence:
Perminder Sachdev
NPI, Prince of Wales Hospital, Barker Street
RANDWICK NSW 2031, Austrália
E-mail: p.sachdev@unsw.edu.au