Homocysteine Gets to the Brain

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Interest in the possible vascular effects of hyperhomocysteinemia is reflected in the growing flux of published studies in the last decade focusing on this sulfur-amino acid. Elevated homocysteine levels were first implicated in the pathology of atherosclerosis in the pioneering manuscript of McCully over 30 years ago [1]. However, only recently accumulated epidemiologic data from at least 14 prospective cohort or nested case-control surveys and from about 70 cross-sectional and retrospective studies suggest that the risk of atherothrombotic events of the heart, brain and limbs is increased due to high blood HCys.

In the current issue of *IMAJ* two manuscripts address the possible role of elevated HCys in two supposedly unrelated brain pathologies: stroke in celiac disease and cognitive decay in Alzheimer's disease. Gefel and colleagues [2] report a case of a young man hospitalized with right hemiparesis of sudden onset, which was preceded by an earlier stroke of the left hemisphere. Electrocardiography was suggestive of an old myocardial infarction, while echocardiography revealed a thrombus of the left ventricle. Angiography demonstrated diffuse arteriosclerosis of the left anterior descending coronary artery and a total occlusion of the right carotid artery. A high titer of anti-endomysial and gliadin antibodies and villous atrophy were compatible with the diagnosis of celiac disease. Several potential risk factors for such severe vascular lesions were excluded in this patient. Fibrinogen, activated protein C, antithrombin III, anticardiolipin, C-reactive protein, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol, and triglycerides were all normal. An evaluation of a possible deficient vitamin status due to malabsorption revealed an exceedingly low level of folic acid causing secondary hyperhomocysteinemia. Indeed, folic acid supplement in combination with anticoagulant treatment and gluten-free diet brought about a 4 year remission with normal vascular and neurologic states. The issue of hyperhomocysteinemia in celiac disease was addressed only recently by Grigg [3] in a patient with a presenting feature of deep venous thrombosis, yet the present report by Gefel et al. underscores the multiple vascular lesions culminating in two stroke events that could be influenced by elevated HCys. Thromboembolic complications, though not frequent, have been described in various enteropathies mainly of the inflammatory type [4], which may be related to low folate and the resulting high HCys levels. Similar inverse correlation of these two analytes was reported in Crohn's disease [5], where folic acid deficiency contributed more significantly to the high HCys levels than did that of B12, as was evident in the presently reported celiac case.

Stroke is the second most common cause of mortality in the world and a major factor in long-term disability [6]. Meta-analyses strongly suggest that even mild to moderate elevation of HCys confers an independent risk for arteriosclerotic outcomes, including stroke [7]. Interestingly, there is a strong, graded association between increased plasma HCys and ischemic stroke due to

HCys = homocysteine

atherosclerosis of large arteries, and to a much lesser extent due to that of small arteries. Since cardioembolic or other etiologic subtypes of ischemic stroke were not associated with high HCys in first-ever ischemic stroke cases, it is suggested that the deleterious effect of elevated HCys is mediated primarily via a pro-atherogenic rather than prothrombotic mechanism [6]. The unique effect of HCys in the mechanism of ischemic stroke is further supported by two recent articles published in *Stroke*. Multivariate analysis of patients with significant carotid stenosis leading to cerebrovascular events revealed that elevated HCys was associated with an odds ratio of 4.07. Other thrombophilic factors, i.e., C677T mutation in MTHFR, G20210A mutation of factor II, factor V Leiden and antiphospholipid antibodies, did not differ significantly between stroke patients and asymptomatic controls [8]. A very similar conclusion emerges from a survey of young adults (<51 years of age) with a history of ischemic stroke, showing that moderate hyperhomocysteinemia is the only variable correlated with that cerebrovascular event, while other inherited prothrombotic parameters did not [9]. The elevation in HCys levels is also significantly associated with silent brain infarction, usually identified with brain lacunar infarction [10]. Magnetic resonance imaging reveals that lesions of silent brain infarction are common in the elderly, and it is presently assumed that such "subclinical" infarction increases the risk of progression to clinically apparent stroke and cognitive decline.

Several experimental systems, some of which examined homocysteine influence *in vitro*, yielded numerous possible mechanisms to account for its vascular effects. HCys has mitogenic activity in vascular smooth muscle cells which could cause arterial wall thickening. HCys can also induce intracellular release of calcium in vascular smooth muscle cells, hence increasing their proliferation and the mass of extracellular matrix [11]. Other possible adverse mechanisms of hyperhomocysteinemia relate to its conferring oxidative injury to endothelial cells and enhancing the peroxidation of LDL, thus promoting the atheromatous process. Increased HCys could also augment thrombotic events as it inhibits the expression of thrombomodulin secreted by the endothelial cells to prevent the activation of protein C. In addition, HCys enhances the activity of factors V and VII and the adhesion of platelets to the endothelium. A tempting hypothesis proposes that elevated HCys reduces the availability of nitric oxide, the recognized endothelium relaxing factor [12]. Interestingly, *in vitro* studies have indicated that HCys induces the release of NO from endothelial cells. Indeed, HCys can react with NO to produce S-nitrosohomocysteine, which promotes the anti-platelet and vasodilatory effects of NO. However, when NO is exposed to high concentrations of HCys over time, it is depleted gradually and its vaso-relaxation effect is diminished. Another interesting recent study in elderly patients with stroke found a concomitant increase in plasma levels of HCys and asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase [13]. Multiple logistic regression analysis revealed that hyperhomocysteinemia was a significant predictor of elevated asymmetric

dimethylarginine, which increases the risk for ischemic stroke in the elderly.

The abundance of theories attempting to explain the vascular activity of elevated HCys emphasizes the lack of a consensus and the complexity of the issue. An alternative view of homocysteine was hypothesized by Dudman [14], who suggested that the overwhelming epidemiologic support for hyperhomocysteinemia and vascular events is not due to a direct atherogenic or thrombogenic effect of this molecule. Rather, increased HCys is none other than a marker for tissue damage and repair in retrospect, which takes place during myocardial infarction or stroke and the recovery from such traumatic events. Tissue damage accelerates mainly reactions of nucleic acid methylation, thus generating S-adenosylhomocysteine and releasing HCys. In addition, enhanced methylation activity would require an increased supply of folic acid, and would reduce the availability of this nutrient in MTHFR-dependent remethylation of HCys to methionine, causing an elevation in plasma HCys. This crucial mechanistic dilemma of whether HCys is a cause of vascular damage, or merely its outcome, is even more intensely debated by some who believe that elevated HCys merely mirrors a nutritional deficient status of folic acid and vitamins B12 and B6, all of which are required for the conversion of HCys to other metabolic intermediates. This issue may be resolved by the results of ongoing prospective nutritional and clinical trials worldwide. A 5 year program of food fortification with folic acid, which began in the U.S. in January 1998, aimed at increasing the daily intake of this nutrient by approximately 130 µg will be later analyzed for its possible effect on the statistics of vascular events in the ensuing years.

Peterson and Spence [15] reported that supplementing middle-aged individuals with a combination of folic acid, pyridoxine and cobalamin halted progression of the carotid atherosclerotic plaque. This report stimulated the design of a multi-center study in the U.S. and Canada, designated as VISP (**V**itamin **I**ntervention for **S**troke **P**revention), in which patients with non-disabling ischemic stroke will be supplemented with either high or low doses of the B vitamin cocktail to determine the possible reduction of recurrent stroke events and parallel lowering of HCys levels [16].

Another paper in this issue of *IMAJ*, by Mizrahi and co-workers [17], reviews the possibility of HCys being a risk factor for Alzheimer's disease. Dementia is a common and devastating problem affecting up to 10% of individuals over the age of 65. Classically, vascular dementia, referred to also as multi-infarct dementia, due to brain ischemia resulting from cerebrovascular disease is distinguished from AD with its characteristic amyloid plaques and neurofibrillary tangles. Recent studies suggest, however, that cardiovascular disease, atherosclerosis and abnormalities in the cerebral microvasculature contribute to the cause of AD [18]. Furthermore, the ε4 allele of apolipoprotein E is a risk factor not only for Alzheimer but also for cardiovascular disease, and the presence of apoE4 in combination with atherosclerosis increases synergistically the risk of cognitive decline [19]. Evidence

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LDL = low density lipoprotein
NO = nitric oxide

AD = Alzheimer's disease

in support of a relationship between hyperhomocysteinemia and Alzheimer disease accumulated during the 1990s. Based on the Cambridge Cognitive Examination Score [20–22], increased HCys correlates inversely with cognitive performance, and appears to be an independent predictor of decline in parameters such as word recall, orientation and constructional praxis. A case-control study by Clarke et al. [23] in AD patients and age-matched controls strongly supports the notion that hyperhomocysteinemia may contribute to cognitive impairment and AD. An intriguing finding is that patients with high HCys at the first visit showed a more rapid atrophy of the medial temporal lobe during a 3 year follow-up compared to those with lower HCys levels. The significance of such an observation may be evaluated in prospective surveys. A crucial question is whether increased HCys is a cause or consequence of Alzheimer's disease. One argument, for instance, is that dementia leads to a reduced dietary intake of vitamin B12 and folate, thus elevating HCys levels. Large-scale trials in high risk populations will indicate whether lowering blood homocysteine will indeed reduce the risk of AD. Data from a case-control study by Postiglione and colleagues [24] suggest that rather than a risk factor for AD, hyperhomocysteinemia is related to its progression and increasing severity.

Several plausible mechanisms were proposed to explain how HCys could adversely affect cognition. This amino acid or its excitotoxic derivatives could activate N-methyl-D-aspartate receptors, resulting in neuronal death [25]. Another report [26] demonstrates even more directly that HCys induces apoptosis in hippocampal neurons, and that it can break DNA strands and lead to mitochondrial dysfunction by caspase activation. The increased vulnerability of hippocampal neurons to oxidative injury induced by HCys *in vivo* could be relevant to the mechanism by which HCys contributes to neurodegenerative disorders. Even the well-documented neurotoxicity of β-amyloid, the hallmark of Alzheimer disease, is potentiated by HCys, via a mechanism of increasing intracellular calcium influx and oxidative stress, thus enhancing neuronal excitotoxicity [27]. The real significance of these neurodegenerative mechanisms of HCys in Alzeimer dementia will eventually be clarified only following prospective, large-scale folate and vitamin B treatment to lower HCys in the population.

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ApoE = apolipoprotein E