Hyperhomocysteinemia, Oxidative Stress, and Cerebral Vascular Dysfunction

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An elevated circulating concentration of the sulfur-containing amino acid homocysteine, hyperhomocysteinemia, produces complex changes within the blood vessel wall. In the peripheral circulation, these changes include oxidative stress, proinflammatory effects such as expression of tumor necrosis factor-α and inducible nitric oxide (NO) synthase (iNOS), and endothelial dysfunction.1-6 Hyperhomocysteinemia-induced oxidative stress may occur as a result of decreased expression and/or activity of key antioxidant enzymes as well as increased enzymatic generation of superoxide anion (the precursor for multiple reactive oxygen and reactive nitrogen species).2,4

Do hyperhomocysteinemia-induced changes occur in the cerebral circulation and why are they potentially important? Hyperhomocysteinemia is a an emerging risk factor for carotid artery disease (atherosclerosis) and stroke and is associated with Alzheimer’s disease and vascular dementia.7–11 It was not until relatively recently, however, that experimental studies began to define the impact of hyperhomocysteinemia on cerebral vascular biology and the molecular mechanisms that account for these changes. This work has been facilitated by the development of mouse models with genetic alterations in different components of the homocysteine metabolic pathway (see below).

Early work in the cerebral microcirculation observed that acute local administration of a very high concentration of homocysteine (1 mmol/L) in the presence of exogenous Cu2+ produced superoxide-mediated reductions in resting cerebral blood flow (CBF) as well as attenuation of endothelium-dependent and NO-mediated responses.12 Our group was among the first to show that mild chronic hyperhomocysteinemia produces endothelial dysfunction in the carotid artery.1,13 Moderate hyperhomocysteinemia, induced by acute methionine loading, produces impaired autoregulatory responses in older humans.14 When considering the consequences of impairment of normal endothelial function, it is important to recall that genetic analyses have demonstrated cosegregation of endothelial dysfunction with a stroke-prone phenotype.15 and endothelial dysfunction is emerging as an independent predictor of clinical events including stroke.16

Levels of circulating homocysteine are determined by multiple mechanisms including genetic and dietary factors. Several genetically altered mice deficient in key enzymes within the homocysteine metabolic pathway have now been developed.17 These include mice deficient in expression of cystathionine β-synthase (CBS), methylenetetrahydrofolate reductase (MTHFR), and methione synthase (MS).17 All these genetic alterations result in hyperhomocysteinemia, the magnitude of which is dependent on the content of methionine, folate, and choline in the diet. The development of these animals, along with combined dietary interventions, now allows mechanistic studies of effects of mild to moderate hyperhomocysteinemia on the vasculature. We have used these novel genetic models in studies of the cerebral circulation, and several major concepts have emerged from this effort. First, we obtained evidence for endothelial dysfunction in cerebral arterioles in CBS-, MTHFR-, and MS-deficient mice18,19 (and unpublished observations) (Figure). In these studies, endothelial dysfunction correlated with levels of plasma total homocysteine, regardless of the combination of genetic and dietary factors used to manipulate homocysteine levels. This finding suggests that homocysteine per se, or a closely related metabolite such as S-adenosylhomocysteine, may be the mediator that produces cerebral vascular dysfunction. Second, the level of hyperhomocysteinemia needed to produce endothelial dysfunction in cerebral microvessels appears to be lower than that needed to produce similar dysfunction in aorta.6,18,19 The mechanism(s) responsible for apparent increased sensitivity of cerebral arterioles to hyperhomocysteinemia is unknown. Some clinical observations support the concept that hyperhomocysteinemia produces inflammation and endothelial dysfunction in cerebral microvessels in humans.20 Third, studies in CBS-deficient mice revealed that very modest hyperhomocysteinemia produces hypertrophy and altered mechanics in the cerebral microcirculation (Figure).21 Increases in cross-sectional area of the vessel wall (hypertrophy) may have functional consequences because hypertrophy of vascular muscle can impair maximal vasodilator capacity. These results compliment findings obtained in a rat model of hyperhomocysteinemia in which morphological evidence for cerebral endothelial and mitochondrial injury was obtained.22

What mechanisms are responsible for producing hyperhomocysteinemia-induced vascular changes? Work in aorta and other peripheral blood vessels suggests that these mechanisms are complex and probably multifactorial.2 Many studies in experimental animals and humans in extracranial blood vessels support the concept that oxidative and/or
Peroxynitrite is known to activate poly (ADP-ribose) polymerase (PARP), which is known to activate PARP, at least in neurons, suggesting that PARP may potentially be involved in cerebral vascular dysfunction during hyperhomocysteinemia. Peroxynitrite can also produce vascular dysfunction and oxidative stress by nitration of tyrosine residues in select proteins including the mitochondrial isoform of superoxide dismutase (Mn-SOD), and by possibly promoting the uncoupling of NO synthases, a circumstance in which the normal flow of electrons within NO synthase is diverted so that the enzyme produces superoxide rather than NO.

Hyperhomocysteinemia may produce vascular dysfunction and promote oxidative stress by increasing levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthases (Figure). Our group and others have shown that ADMA levels are increased in hyperhomocysteinemia, possibly due to hyperhomocysteinemia-induced inhibition of dimethylarginine dimethylaminohydrolases (DDAH), the enzymes that normally degrade ADMA. We have shown that ADMA inhibits basal and stimulated endothelium-dependent relaxation in cerebral blood vessels. ADMA may inhibit production of NO by endothelial NOS (eNOS) and also may promote eNOS uncoupling.

DNA methylation is a critical component of epigenetic regulation of gene expression, particularly during development, but may also affect expression of genes during disease states such as atherosclerosis. DNA hypomethylation is induced by increases in homocysteine and S-adenosylhomocysteine, an intermediate in homocysteine metabolism and inhibitor of methylation. Thus, global or selective DNA methylation may contribute to alterations in gene expression and vascular changes during hyperhomocysteinemia (Figure).

In summary, hyperhomocysteinemia produces changes instructure and function of cerebral blood vessels. Oxidative stress appears to play a major role in mediating these changes. The role of specific reactive oxygen and/or reactive nitrogen species as well as the importance of mechanisms such as inflammation and increased levels of ADMA have not been defined. A major question for future studies will be to define what mechanisms account for apparent increased sensitivity of cerebral microvessels, as opposed to extracranial blood vessels, to hyperhomocysteinemia. The recent development of genetically altered mouse models that allow the study of chronic effects of a range of homocysteine levels should facilitate such efforts.

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References


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