Elevation in S-Adenosylhomocysteine and DNA Hypomethylation: Potential Epigenetic Mechanism for Homocysteine-Related Pathology

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ABSTRACT Chronic nutritional deficiencies in folate, choline, methionine, vitamin B-6 and/or vitamin B-12 can perturb the complex regulatory network that maintains normal one-carbon metabolism and homocysteine homeostasis. Genetic polymorphisms in these pathways can act synergistically with nutritional deficiencies to accelerate metabolic pathology associated with atherothrombotic disease, birth defects and dementia. A major unanswered question is whether homocysteine is causally involved in disease pathogenesis or whether homocysteinemia is simply a passive and indirect indicator of a more complex mechanism. S-Adenosylmethionine and S-adenosylhomocysteine (SAH), as the substrate and product of methyltransferase reactions, are important metabolic indicators of cellular methylation status. Chronic elevation in homocysteine levels results in parallel increases in intracellular SAH and potent product inhibition of DNA methyltransferases. SAH-mediated DNA hypomethylation and associated alterations in gene expression and chromatin structure may provide new hypothesis for pathogenesis of diseases related to homocysteinemia. J. Nutr. 132: 2361S–2366S, 2002.

KEY WORDS: • homocysteine • S-adenosylhomocysteine • S-adenosylmethionine • DNA methylation

Regulatory determinants of homocysteine metabolism

The sole intracellular source of homocysteine (Hcy)3 is the hydrolysis of S-adenosylhomocysteine (SAH) by the enzyme SAH hydrolase (SAHH; EC 3.3.1.1) (1,2). Of the four enzymes capable of metabolizing Hcy, only the SAHH reaction is readily reversible (Fig. 1). Methionine synthase [MS(5-methyltetrahydrofolate-homocysteine S-methyltransferase); EC 2.1.1.13] and betaine-homocysteine methyltransferase (BHMT; EC 2.1.1.5) both remethylate Hcy to methionine, and both are unidirectional. Similarly, the permanent removal of Hcy from the methionine cycle by cystathionine β-synthase (CBS; EC 4.2.1.22) is a one-way reaction. Although the equilibrium dynamics of the SAHH reaction strongly favor SAH synthesis over Hcy synthesis, the efficient metabolic removal of Hcy and adenosine by the multiple pathways indicated in Figure 1 allows sustained Hcy synthesis to predominate (3). Remethylation of Hcy to methionine (the methionine cycle) predominates over the catabolic degradation of Hcy (transsulfuration) because of the order of magnitude difference in Km between MS and CBS (1). Genetic or nutritional perturbations that hinder efficient product removal of Hcy or adenosine will induce reversal of the SAHH reaction, leading to an intracellular accumulation of SAH (4). Chronic deficiencies in the nutrients folate, vitamin B-12, vitamin B-6, methionine or choline can independently and interactively disrupt normal metabolic flow and increase Hcy. Excess free intracellular Hcy is thought to readily cross the cell membrane into the plasma, although the precise mechanism is not known.

Genetic polymorphisms in genes coding for enzymes involved in these pathways interact with nutritional deficiencies to magnify imbalances in one-carbon metabolism that may promote several chronic disease states in humans (5,6). Gene-nutrient interactions that elevate Hcy levels have been associated with increased risk of cardiovascular disease (7), colon cancers (8), birth defects (9,10), recurrent early pregnancy loss (11), central nervous system (CNS) demyelination (12) and neuropsychiatric disease (13,14). Most recently, an increment of 5 μM plasma Hcy was associated with a 49% increase in
transfer is not known (19,20). SAH levels re
SAHH or, at high levels, there may be limited export into the
toxically accumulate of free SAH. It can
methylation ef
folding pattern at the catalytic domain is almost identical to
structure of the SAHH protein revealed that the polypeptide
reactions may therefore depend on close proximity between
in its high-affinity binding to the catalytic region of most
S-adenosylmethionine (SAM)-dependent methyltransferases, enabling it to act as a potent product inhibitor (17). For this reason, continual hydrolysis of SAH to Hcy and adenosine is essential to maintain normal methylation of DNA, RNA, protein, phospholipid, histones and neurotransmitters as well as a multitude of small molecules essential for normal cell function and viability. The efficiency of methyltransferase reactions may therefore depend on close proximity between methyltransferases and SAHH. A recent report of the crystal structure of the SAHH protein revealed that the polypeptide folding pattern at the catalytic domain is almost identical to that of bacterial methyltransferases, suggesting that close proximity would allow efficient exchange of SAH between the catalytic pockets of the two enzymes and thereby increase methylation efficiency (18).

Three mechanisms exist in mammalian cells to avert potentially toxic intracellular accumulation of free SAH. It can be bound to available intracellular proteins or hydrolyzed by SAHH or, at high levels, there may be limited export into the plasma, although the exact mechanism for transmembrane transfer is not known (19,20). SAH levels reflect the cumulative balance among the activities of the multiple methyltransferases, the rates of synthesis and hydrolysis by SAHH and the efficiency of Hcy and adenosine product removal. It is important to note that tissues lacking CBS and a catabolic outlet for elevated Hcy would be expected to be more sensitive to SAHH reversal and intracellular accumulation of SAH. Only liver, pancreas, kidney and intestine have been reported to express the complete transsulfuration pathway. Therefore, the majority of cells lack CBS expression and may be particularly vulnerable to SAH-mediated methyltransferase inhibition, depending on the tissue-specific $K_c$. In an excellent review of SAM-dependent methyltransferases, Clarke and Banfield (21) report that the $K_c$ for SAH is less than the $K_m$ for SAM for many of the methyltransferases.

Figure 1 emphasizes an unusual metabolic branch point created by SAHH in which the product reactions are bidirectional. Thus, elevation of either Hcy or adenosine or both will promote intracellular SAH accumulation (20). Increased levels of SAH have been reported to upregulate CBS and downregulate methylentetrahydrofolate reductase (MTHFR; EC 1.5.1.20), MS and BHMT (3). In CBS-expressing cells, these regulatory functions would act in concert to reduce methio-
nine remethylation and expedite Hcy removal in an attempt to normalize one-carbon flow. A fascinating adaptive response to SAH toxicity in SAHH-inhibited neuroblastoma cells is an upregulation of methionine adenosyltransferase II (MATII; EC 2.5.1.6) mRNA and activity (22,23). The resulting in-
crease in SAM synthesis effectively counterbalances the in-
crease in SAH and permits cell survival. Follow-up studies are required to determine whether this response is unique or whether upregulation of MATII could represent a new regu-
ulatory function for SAH. Altogether, these results support a bioregulatory role for SAH in maintaining normal one-carbon metabolism as first proposed by Cantoni (24).

HUMAN STUDIES

Elevated plasma Hcy, SAH accumulation and hypomethylation

Using a sensitive new high pressure liquid chromatography method with electrochemical detection, a recent study provided evidence that moderate elevations in plasma total Hcy (tHcy) were highly correlated with parallel elevations in plasma SAH but not SAM (Fig. 2A) (4). Furthermore, increased plasma and lymphocyte SAH levels were associated with increased DNA hypomethylation (Fig. 2B). These results suggest the interesting possibility that elevated plasma tHcy may be an indirect indicator of elevated intracellular SAH and compromised cellular methylation capacity. In addition to DNA (cytosine-5) methyltransferase (EC.2.1.1.137) (25–27), SAH has been reported to act as a potent product inhibitor of catechol O-methyltransferase (EC.2.1.1.6) (28), phosphati-
dylethanolamine N-methyltransferase (EC.2.1.1.17) (29), his-
tone-lysine N-methyltransferase (EC.2.1.1.43) (25), tRNA and mRNA methyltransferases (30,31), acetylseryotonin O-
methyltransferase (EC.2.1.1.4) (32) and histamine N-methyl-
transferase (EC.2.1.1.8) (33). The functional consequences of reduced methylation capacity are significant and include CNS demyelination (34,35), reduced neurotransmitter syn-
thesis (28,32), decreased chenotaxis and macrophage phago-
cytosis (36), altered membrane phospholipid composition (37,38) and membrane fluidity (34,39), gene expression (40–
42) and cell differentiation (43,44). The $K_c$ for SAH varies with different cellular methyltransferases (21) and also varies according to tissue priorities and subcellular methyltransferase distribution (45); thus, sensitivity to hypomethylation is likely to be tissue specific.

Vascular endothelial cells, lacking both CBS and BHMT, are limited in their capacity to metabolize Hcy to either remethylation via MS or SAHH reversal (46). Because the transulfuration pathway provides an important route to remove excess Hcy, cells lacking CBS would be expected to be more prone to SAH accumulation. Supporting an increased
sensitivity to SAH, Wang et al. (47) found that physiologic levels of Hcy, but not cysteine, decreased carboxyl methylation of p21ras in cultured human vascular endothelial cells, consistent with SAH-mediated inhibition of the protein methyltransferase. Supporting a role for SAH in cardiovascular disease risk, Wagner et al. (48) presented convincing evidence that an increase in plasma SAH is a much more sensitive indicator of cardiovascular disease than an increase in plasma tHcy. In hyperhomocysteinemic patients with occlusive vascular disease, Loehrer et al. (49) found a reduced ratio of SAM:SAH in both plasma and erythrocytes that was due to elevated SAH levels. In kidney failure patients with hyperhomocysteinemia, Perna and colleagues (50,51) observed a four- to eightfold increase in intracellular SAH, minimal change in SAM and a decrease in both DNA methylation and protein methylation. Finally, Gonzales et al. (52) recently reported that colorectal polyp biopsies had significantly lower folate levels, twofold higher SAH levels and global DNA hypomethylation relative to control biopsies from the same patient. Thus, data from several recent studies strongly suggest that hyperhomocysteinemia is accompanied by significant increases in plasma and intracellular SAH that can negatively affect cellular methylation potential in humans.

ANIMAL STUDIES

Tissue-specific sensitivity to SAH accumulation and hypomethylation

Because SAM and SAH are the substrate and product of essential methyltransferase reactions, the ratio of SAM:SAH is frequently used as an indicator of cellular methylation potential. The ratio alone, however, does not indicate whether substrate insufficiency, product inhibition or both are required to negatively affect cellular methylation capacity. To examine this question, CBS heterozygous and wild-type mice were fed a control or methyl-deficient diet for 24 wk (53). The independent and combined effect of genotype and diet on SAM, SAH and the SAM:SAH ratio were assessed in liver, kidney, brain and testes and correlated with relative changes in tissue-specific global DNA methylation. In Figure 3, the correlation between SAH and DNA hypomethylation in liver, kidney, brain and testes is presented. Overall, the results in different tissues indicated that a decrease in SAM alone was not sufficient to affect DNA methylation in this model, whereas an

![FIGURE 2](https://academic.oup.com/jn/article-abstract/132/8/2361S/4687579)

**FIGURE 2** (A) The correlation between plasma Hcy and plasma SAH in a cohort of healthy young women (4). (B) The correlation between plasma SAH and DNA lymphocyte hypomethylation in the same cohort of healthy young women. DPM, Disintegrations per minute; r, correlation coefficient; p, P-value; SAH, S-adenosylhomocysteine.

![FIGURE 3](https://academic.oup.com/jn/article-abstract/132/8/2361S/4687579)

**FIGURE 3** The correlation between DNA hypomethylation as a function of intracellular SAH concentration in liver, kidney, brain and testes from mice heterozygous for CBS inactivation (53). DPM, Disintegrations per minute; r, correlation coefficient; p, P-value; SAH, S-adenosylhomocysteine.
increase in SAH, either alone or associated with a decrease in SAM, was most consistently associated with DNA hypomethylation. A decrease in SAM:SAH ratio was predictive of reduced methylation capacity only when associated with an increase in SAH; a decrease in the SAM:SAH ratio due to SAM depletion alone was not sufficient to affect DNA methylation in this model. Plasma Hcy levels were positively correlated with intracellular SAH levels in all tissues except kidney. These results add further support to the possibility that plasma SAH concentrations may provide a sensitive biomarker for cellular methylation status.

Another study using the heterozygous CBS-deficient mouse model examined endothelial cell dysfunction in folate-replete hyperhomocysteinemic mice (54). Plasma tHcy correlated positively with SAH in the liver and brain. Endothelium-dependent relaxation and thrombomodulin anticoagulant activity were significantly impaired in aortas from the homocysteinemic mice with elevated SAH, suggesting that altered methylation potential may contribute to endothelial dysfunction associated with hyperhomocysteinemia. In a study of MTHFR knockout mice, both heterozygous and homozygous mice exhibited increased plasma tHcy, decreased SAM and increased SAH levels that were associated with global hypomethylation in liver, brain and ovaries (55). In a study of SAHH inhibition in isolated rat acinar cells, the absolute value of SAH was found to be more predictive of reduced methylation-dependent excocrine secretion than was the SAM:SAH ratio (56). Similarly, Weir et al. (57) found that the reduced SAM:SAH ratio in the brain and spinal fluid of nitrous oxide-exposed pigs was driven by the increase in SAH rather than a decrease in SAM. These in vivo and in vitro animal studies add further support to the pivotal importance of intracellular SAH as a mediator of methylation inhibition and cellular dysfunction.

**MECHANISTIC ASPECTS OF SAH-MEDIATED HYPMETHYLATION IN THE PATHOGENESIS OF CHRONIC DISEASE**

**Hcy, SAH-mediated methyltransferase inhibition and altered gene expression**

Supplemental Hcy and adenosine or the administration of chemical SAHH inhibitors to cells or animals has provided further insights into the interaction among SAH accumulation, hypomethylation and cellular dysfunction. Among the modifications associated with both increased intracellular SAH and DNA hypomethylation are alterations in the expression of specific genes, the induction of cellular differentiation, alterations in chromatin conformation and cell phenotypic changes (24,40,58). Although high levels of Hcy alone will induce similar changes in gene expression and mRNA levels, the reversibility of the SAHH reaction in intact cells makes it impossible to differentiate a direct effect of Hcy from an indirect effect of SAH accumulation. Increased intracellular SAH will upregulate CBS activity to provide some protection against the toxic accumulation of Hcy and SAH; however, only tissues such as liver, pancreas, kidney and brain that express CBS would be responsive to SAH-mediated regulation (3). In fact, the majority of mammalian cell types do not express CBS and therefore would be particularly sensitive to Hcy/SAH accumulation, DNA methyltransferase inhibition and associated alterations in gene expression. In these cells, extracellular export of Hcy may provide the only means to control toxic intracellular accumulation of SAH. Thus, it is likely that cells that do not express CBS provide the major source of plasma tHcy and SAH and would also be most sensitive to SAH toxicity. Alterations in DNA methylation and gene expression secondary to SAH accumulation should provide a fertile area for future research, especially as related to the pathogenesis of chronic diseases associated with hyperhomocysteinemia.

**Hcy-induced oxidative stress plus SAH-induced DNA hypomethylation: interaction may potentiate DNA damage associated with chronic disease**

Oxidative damage to cells has been associated with elevated plasma tHcy and is attributed to auto-oxidation and the production of reactive oxygen species (58,59). Hyperhomocysteinemia and oxidative damage to DNA have been implicated in the pathogenesis of several chronic diseases of aging, including occlusive cardiovascular disease, certain cancers and Alzheimer’s disease. An interesting and as yet unexplored possibility is that SAH-mediated DNA hypomethylation could increase vulnerability and sensitivity of the DNA to Hcy-induced free radicals. Because Hcy and SAH tend to increase in parallel, especially in cells lacking CBS, an interactive contribution to DNA damage is a plausible notion. It is well established that hypomethylated DNA is associated with hyperacetylated and decondensed chromatin due to decreased binding of methyl-sensitive proteins such as methyl-CpG-binding protein and histone deacetylase (60). For example, chromatin decondensation is induced by hypomethylating agents such as 5-azacytidine and SAH. The more open DNA conformation associated with hypomethylated chromatin is much more sensitive (vulnerable) to endonuclease cleavage (61) and oxidative stress (62). Interestingly, hyperhomocysteinemia and folate deficiency have been associated with several chronic diseases of aging, DNA hypomethylation and increased DNA strand breakage (62,63). Increased levels of DNA strand breaks and apoptosis are induced by both Hcy (64) and SAH (65) and are present in preneoplastic cells (62,66), in alcoholic liver disease (67), in atherosclerotic lesions (68) and in brain cells of Alzheimer’s patients (69). The possibility that SAH-mediated DNA hypomethylation, secondary to elevated intracellular Hcy, results in increased susceptibility to DNA damage and apoptosis from Hcy-induced free radicals is an unexplored area for future research.

**Plasma Hcy: exportable control of SAH-mediated hypomethylation?**

Recent evidence from both human studies and animal studies suggests that reversal of the SAHH hydrolase reaction readily occurs under conditions of elevated intracellular Hcy. Both Hcy and SAH are present in plasma; however, the concentration of Hcy exceeds that of SAH by almost 3 orders of magnitude. This difference most likely reflects the more facile transport of Hcy across the plasma membrane compared to SAH. Consistent with SAHH reversal, the recent study of Yi et al. (4) showed that plasma SAH increases in parallel with tHcy within normal physiologic ranges in healthy young women and was associated with lymphocyte DNA hypomethylation. In one additional study, healthy postmenopausal women on controlled folate depletion exhibited moderate elevation in tHcy and lymphocyte DNA hypomethylation (70,71). Moderate increases in plasma tHcy also occur with homozygosity for the MTHFR C677T polymorphism in ~10% of Caucasians. When folate status is low (5), this polymorphism has been shown to increase risk of several
Hcy-related diseases and interestingly was recently shown to be associated with global DNA hypomethylation (72).

The cellular toxicity of SAH as a result of the inhibition of SAM-dependent cellular methyltransferases is pleiotropic and could disrupt a) membrane fluidity and signal transduction (phosphatidylethanolamine methyltransferase); b) protein synthesis and repair (RNA methyltransferases and protein L-isospartate-methyltransferase); c) chromatin conformation and gene expression (DNA and histone methyltransferases); and d) neurotransmitter synthesis (catecholamine O-methyltransferase). Given the potential toxicity of intracellular SAH, it is plausible to speculate that the more facile transport of Hcy out of the cell provides an additional mechanism to prevent toxic effect of SAH. This would suggest that one function of Hcy may be to provide an exportable form of SAH.

LITERATURE CITED


