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S-ADENOSYL METHIONINE: A CONNECTION BETWEEN NUTRITIONAL AND GENETIC RISK FACTORS FOR NEURODEGENERATION IN ALZHEIMER'S DISEASE

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Abstract: Clinical manifestation of Alzheimer's disease may depend upon interaction among its risk factors. Apolipoprotein E-deficient mice undergo oxidative damage and cognitive impairment when deprived of folate. We demonstrate herein that these mice were depleted in the methyl donor S-adenosyl methionine (SAM), which inhibited glutathione S-transferase, since this enzyme requires methylation of oxidative species prior to glutathione-dependent reduction. Dietary supplementation with SAM alleviated neuropathology. Since SAM deficiency promotes presenilin-1 overexpression, which increases gamma-secretase expression and Abeta generation, these findings directly link nutritional deficiency and genetic risk factors, and support supplementation with SAM for Alzheimer's therapy.

Key words: S-adenosyl methionine, folate, oxidative damage, glutathione, methylation, apoliprotein E, Alzheimer's disease.

Introduction

Alzheimer's disease (AD) has an apparent multifactoral etiology that encompasses nutritional, genetic, and environmental risk factors, none of which is sufficient to account for all cases of AD (1). The convergence of one or more risk factors may therefore represent a key determinant leading to clinical manifestation of AD. One possibility is that genetic predispositions may remain latent pending age-related decline in nutrition and/or homeostasis (2).

A gradual hypomethylation of DNA accompanies aging (3). This is due at least in part to a deficiency of S-adenosyl methionine (SAM), the major methyl donor, in normal aging and in AD (4). A recent study indicates that insufficient levels of SAM may lead to overexpression of presenilin-1 (PS1), a gene linked to AD (1). SAM mediates numerous transmethylation reactions, including methylation of proteins, phospholipids, neurotransmitters and nucleic acids. In addition, S-adenosyl homocysteine (SAH), the downstream metabolic product resulting from SAM-mediated transmethylation reactions, is present at relatively elevated levels in AD brains (5). SAH is a competitive inhibitor of methyl transferases that utilize SAM and therefore further inhibits the already-diminished levels of methylation in AD brain (5). In addition, the activity of the enzyme responsible for SAM generation (methionine-S-adenosyltransferase), is decreased in neurodegeneration (6).

One common age-related compromise in nutrition that adversely impacts SAM levels is folate deficiency, which contributes to many neurological and psychological disorders including dementia, impaired cognition, depression, psychosis, AD and Parkinson's disease (2). Folate- and B12-dependent reactions convert homocysteine to methionine, which in turn is converted to SAM. Folate deficiency therefore not only increases levels of the neurotoxin homocysteine, which increases oxidative stress and related to the severity and progression of AD, but also reduces levels of SAM (2).

Deficiencies in apolipoprotein E (ApoE) function increase oxidative stress and are associated with AD (7,8). The deleterious effects of folate deprivation are potentiated by deficiency in apolipoprotein E. Deficiencies in apolipoprotein E (ApoE) function increase oxidative stress and are associated with AD in a gene-dose manner (7,9-11). Transgenic mice homozygously-lacking apolipoprotein E (ApoE-/-; 12-14) undergo markedly more severe oxidative damage and cognitive impairment when maintained on a folate-deficient diet than do normal mice (15-17). These mice upregulate transcription and activity of glutathione synthase (GS) and display increased glutathione (GSH) in brain tissue in an apparently unsuccessful attempt to compensate for the increased oxidative damage resulting from deficiency in folate and ApoE (18). Damage to brain tissue despite upregulation in GSH production and levels prompted the consideration that ApoE-/- mice maintained on the deficient diet may be impaired in usage of GSH. We therefore examined herein the activity of the GSH-dependent enzymes glutathione peroxidase (GPX), glutathione reductase (GR) and glutathione S-transferase (GST) (19) in brain tissue of mice maintained under these conditions. Previous studies have demonstrated that SAM decreased oxidatie damage and stimulated the GSH system (20-23). Moreover, prior studies have indicated that certain potential GST substrates such as arsenic must undergo one or more SAM-dependent methylations (24-30) that are stimulated by GSH (31). We therefore considered that SAM may be an essential cofactor for more GST-GSH-dependent reductions than has been previously appreciated. Since GST requires prior methylation of its

Table 1

Dietary supplementation with SAM alleviates oxidative damage and restores GSH homeostasis following folate and vitamin E deficiency Normal and ApoE -/- mice were maintained on complete or deficient diets, or deficient diets supplemented with SAM for 1 month. Total brain tissue was harvested and analyzed as described in the text. Note that supplementation with SAM prevented the increase in TBARs and GSH, restored performance in the T maze, resorted normal activity of GPX, GR and GST, and restored normal levels of GS transcription. SAM did not reduce GS activity. Values represent the mean (± SEM) increase versus normal mice on the complete diet compiled from 2-4 independent experiments, n = 2-3 mice/diet/experiment (total n=8-12. Values with an asterisk differed significantly ($p<0.05$; Student's t test) from those of normal mice maintained on the complete diet

Mouse strain	Diet	TBARs	T Maze % pass	GSH	GS trans.	GS activity	GPX activity	GR activity	GST activity
Normal	Complete	15.2 ± 0.6	67 ± 16	55 ± 6.7	77.2 ± 3.3	0.99 ± 0.1	0.32 ± 0.02	0.38 ± 0.01	0.22 ± .01
ApoE-/-	Deficient	20.6 ± 0.2*	42 ± 18	93 ± 18*	109.2 ± 5.7	1.2 ± 0.2*	0.51 ± 0.06*	0.66 ± 0.03*	0.07 ± 0.1*
ApoE-/-	Deficient + SAM	16.0 ± 0.2	67 ± 15	52 ± 3.1	67.5 ± 7.8	1.2 ± 0.1*	0.38 ± 0.03	0.42 ± 0.06	0.31 ± 0.0

substrates, we also examined whether or not a critical decrease in SAM accompanied folate deprivation, and whether any such decrease could be compensated for by dietary supplementation with SAM.

Materiels and Methods

Normal and ApoE-/- ("knockout") mice (9-12 months of age; 2-4 independent experiments, n = 2-3 mice/diet/experiment; total n=8-12) received basal, vitamin-free diets ("AIN-76"; Purina/Mother Hubbard, Inc.) either supplemented with folic acid (40 mg/kg), and vitamin E (1g/kg total diet wet weight) (defined as the "complete diet"), or lacking folate and vitamin E and containing iron (50g/500g total diet;) as a pro-oxidant (defined as the "deficient diet") with and without SAM (80mg/kg diet) for 1 month commencing at approx. 9 months of age (15,16). Overall oxidative damage was monitored in total brain homogenates by quantifying thiobarbituric acid-reactive substances (TBARs; 15).

Cognitive impairment was monitored using a standard reward-based T maze test (17). Briefly, mice were placed at the bottom of a T-shaped maze, with one arm of the maze blocked, and therefore could explore only one arm. Each arm of the maze contained a depression containing a small amount of sweetened milk. Mice were allowed to locate and consume the milk in the available arm, then were returned to the bottom of the maze and the block was removed from the other arm. If the mouse entered the opposite (newly-unblocked arm), it was scored as passing; if it instead re-explored the previously-visited arm, it was scored as failing. The rationale for these criteria are that mice under normal conditions will demonstrate a greater tendency to explore a novel area rather than re-explore previously-visited territory. Mice were tested 3 times, and the percentage of passing trials calculated for each mouse. Mazes were cleaned and dried between tests to avoid influence of the prior mouse on subsequent exploration (17, and refs. therein).

GSH levels, GS activity and GS transcription were assayed as described (18). GST GPX and GR activity was monitored by commercial activity kits (Cayman, Ann Arbor, MI) using 1-chloro-2,4-dinitrodenzene as a substrate. SAM and SAH were assayed by HPLC (4); purified SAM and SAH (ICN, Costa Mesa, CA) were utilized as standards.

Results

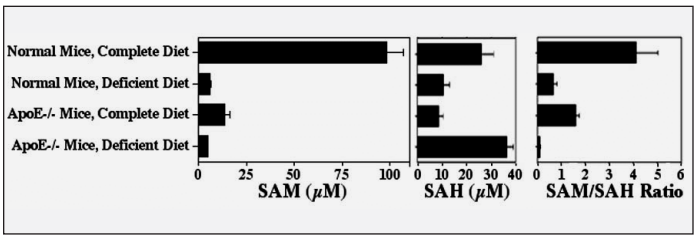
ApoE-/- mice maintained on the deficient diet demonstrated a significant ($p<0.05$) increase in activity of GPX and GR (approx 60% and 70% higher than that of normal mice on the complete diet). yet unexpectedly display a significant decrease (67%; $p<0.05$) in GST activity despite the presence of increased oxidative damage and increased GSH (Table 1). This situation is similar to that observed in AD, in which GPX and GR display increased activity yet GST undergoes a paradoxical decrease in activity despite increased oxidative damage (19-33).

Supplementation of the deficient diet with SAM restored GST activity to that of normal mice maintained on the normal diet. SAM also prevented oxidative damage and cognitive impairment, reduced GS transcription, and reduced GSH levels to those of mice maintained on the complete diet, but did not reduce GS activity (Table 1). We therefore considered that ApoE-/- mice maintained on the deficient diet may have a critical deficiency in SAM. HPLC analyses of brain homogenates demonstrated that ApoE-/- mice exhibited significantly reduced SAM on either diet, and that the deficient diet depleted SAM in normal mice, and also increased SAH in ApoE-/- mice (Fig. 1).

Finally, we added 3mM SAM to the homogenate derived from ApoE-/- mice maintained on the deficient diet, which also restored GST activity to that of control mice receiving the complete diet (0.20 ± 0.08 versus 0.22 ± 0.1). This latter finding demonstrates that GST within these mice remained capable of activity during the 1-month dietary deficiency, and that the GSH present within brain tissue of these mice was sufficient to mediate reduction of oxidative species.

Figure 1

SAM levels in brain tissue are subject to dietary and genetic influences. Homogenates from normal and ApoE-/- mice maintained on the indicated diets were subjected to HPLC according to Morrison et al. (1996). Values represent the mean area (± standard error of the mean) of SAM and SAH peaks, identified by purified SAM and SAH chromatographed under identical conditions, from 4 brains from 2 independent experiments.



Discussion

Decreased GSH represents an early event in neurodegeneration (19). The findings of the present study demonstrate that perturbation in the ability to utilize GSH can also lead to neurodegeneration, and that this can derive from critical alteration in relative levels of SAM and SAH. The reduced level of SAM resulting from the deficient diet was apparently not problematic in and of itself, since a similar decrease in SAM was observed for normal and ApoE-/- mice, yet normal mice did not exhibit neuropathology (15-18). However, unlike normal mice, ApoE-/- mice maintained on the deficient diet exhibited a significant increase in SAH. Since SAH is a competitive inhibitor of methyltransferases, a critical reduction in the ratio of SAM/SAH ratio is apparently what inhibits transmethylation reactions including those in AD (5); perturbation of this ratio is apparently what inhibited GST activity in ApoE-/- mice on the deficient diet, leading to increased oxidative damage despite the presence of excess GSH. This latter conclusion is supported by the beneficial effects of dietary supplementation with SAM and following its addition to the GST assay itself.

Our findings underscore how nutritional deficiency in folate may initiate a cascade of alterations in genetic expression that may promote the development of AD. Since SAM deficiency, perhaps coupled with SAH accumulation, increases PS-1 expression via hypomethylation of the PS-1 promoter (34 folate deficiency, by decreasing SAM (2) can lead to increased PS-1 expression. Increased PS-1 expression promotes increased expression of gamma-secretase, which, by increasing cleavage of the amyloid precursor protein, generates increased Abeta (1). Additional studies provide a potential link between ApoE and PS-1 in AD; the occurrence of some (35) kindreds of PS-linked familial AD is enhanced by the presence of the E4 allele of APoE in a gene-dosage manner (i.e., individuals homozygous for E4 being more susceptible than those heterozygous for E4). The findings of the present study, which demonstrate a significant decline in SAM levels in ApoE-/- brain tissue following nutritional compromise, provide one mechanism by which deficiencies in ApoE activity could foster increased expression of PS-1. Since critical gene expression can be repressed by remethylation (36), our findings suggest that dietary supplementation with SAM, shown to be effective against depression (37) also holds promise as a therapeutic approach to prevent or delay AD. A preliminary study (38) indicates that SAM can provide some cognitive improvement in AD patients. Notably, polymorphisms in the enzyme that utilizes folate to generate methionine, methylene tetrahydrofolate reductase, which diminish folate usage even in the presence of otherwise adequate dietary folate, also result in diminished SAM levels (39). Such polymorphisms are present in as high as 20% of some populations (40) and are and represent synergistic risk factors for AD along with the presence of ApoE4 (8); SAM deficiency may be one critical aspect of this synergistic risk.

In summary, these findings have (1) demonstrated a novel mechanism by which SAM can provide neuroprotection, (2) directly connected nutritional deficiency and the known genetic predispositions for AD, and (3) demonstrated that an individual may generate sufficient antioxidants to prevent neurodegeneration even under adverse conditions, yet not be able to use them due to insufficient methylation.

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