
BRIEF SUMMARY

Folate and Vitamin E Deficiency Impair Cognitive Performance in Mice Subjected To Oxidative Stress

Differential Impact on Normal Mice and Mice Lacking Apolipoprotein E

**Shelia M. Mihalick, Daniela Ortiz, Ramya Kumar,
Eugene Rogers and Thomas B. Shea***

*Center for Cellular Neurobiology and Neurodegeneration Research Department
of Biological Sciences University of Massachusetts, Lowell, MA 01854*

Received June 13, 2003; Accepted July 7, 2003

Abstract

One factor contributing to the age-related decline in cognitive performance is increased oxidative stress, that can arise from environmental, nutritional, and/or genetic compromise. Folate deficiency has been linked to several age-related neurodegenerative conditions, including Alzheimer's disease (AD), at least in part by increasing oxidative stress. Folate deficiency also potentiates the impact of other known risk factors for AD. A decrease in function of apolipoprotein E (ApoE), is associated with increased oxidative stress and is a risk factor for AD. We tested the combined impact of dietary deficiencies in folate and vitamin E, coupled with exposure to high dietary iron as a pro-oxidant, on cognitive performance in normal and ApoE^{-/-} mice by monitoring the percent alternation in passive Y and T maze tests. Both normal and ApoE^{-/-} mice displayed some cognitive impairment when deprived of folate and vitamin E and exposed to iron, but ApoE^{-/-} mice were more severely affected. These findings highlight the potential combined impact of dietary deficiencies and genetic predisposition to neurodegeneration. They further leave open the possibility that one or more risk factors may remain latent, and neurodegeneration may ensue only following augmentation by one or more additional traumatic events or conditions.

Key Words: Apolipoprotein E; cognition; folate; iron; neurodegeneration; oxidative stress; vitamin E.

*Author to whom all correspondence and reprint requests should be addressed. E-mail: Thomas_Shea@uml.edu

Introduction

A factor contributing to the age-related decline in cognitive performance is increased oxidative stress, that can arise from environmental, nutritional and/or genetic compromise (Berr, 2002; Butterfield et al., 2002; Floyd and Hensley, 2002; Perry et al., 2002a, b). A growing body of evidence demonstrates that, in addition to causing developmental disorders of the nervous system, folate deficiency contributes to many age-related neurological and psychological disorders including dementia, impaired cognition, depression, psychosis, Alzheimer's disease (AD) and Parkinson's disease (Mizrahi et al., 2003; Mattson and Shea, 2002; Shea and Rogers, 2002a; Shea et al., 2002a). These deleterious effects arise at least in part by the increase in oxidative stress that accompanies folate deficiency. Folate deficiency increases neuronal oxidative stress by increasing levels of the neurotoxin homocysteine—levels that are related to the progression and severity of AD (Postiglione et al., 2001), decreasing endogenous antioxidants, and by inducing DNA damage and depleting energy reserves (Kruman et al., 2001; Ho et al., 2002, 2003). Folate deprivation also potentiates the deleterious impact of certain other risk factors for AD, including amyloid β , glutamate, and metal neurotoxicity (Ho et al., 2001, 2002, 2003; Kruman et al., 2000, 2002; White et al., 2001),

One genetic risk factor for sporadic and familial AD is the presence of the epsilon 4 (E4) allele of apolipoprotein E (ApoE; Growdon, 2001; Rebeck et al., 2002). Oxidative damage in the brain is elevated in AD patients, and the extent of this damage correlates with the presence of the E4 allele (Ramassamy et al., 1999). Transgenic mice lacking ApoE (ApoE^{-/-} mice) exhibit increased oxidative stress, and therefore represent a useful model for the impact of oxidative stress on neurodegeneration (e.g., Huang et al., 2000; Ramassamy et al., 1999, 2002; Shea and Rogers, 2002b; Shea et al., 2002b,c; Veinsbergs et al., 2000). We have demonstrated that folate deprivation potentiates the deleterious impact of ApoE deficiency. ApoE^{-/-} mice, but not normal mice, displayed oxidative damage to brain tissue within 1 mo of folate deprivation. Vitamin E deprivation alone did not induce detectable oxidative damage in this short-term trial; however, simultaneous deprivation of vitamin E potentiated the effects of folate deprivation (Shea and Rogers, 2002b;

Shea et al., 2002b,c). Because long-term vitamin E deprivation has been shown to impair learning in ApoE^{-/-} mice (Veinsbergs et al., 2000), and folate deprivation has been shown to impair multiple aspects of learning and memory in experimental animals (Crowe and Ross, 1997; Lalonde et al., 1993) and humans (Hassing et al., 1999), we examined whether or not the increased oxidative damage in ApoE^{-/-} mouse central nervous system following folate and vitamin E deprivation was reflected by impaired cognitive performance.

Materials and Methods

ApoE^{-/-} mice (obtained from Jackson Laboratories, Bar Harbor, ME, strain number 2052 on a C57Bl6 background) and normal mice of the same genetic background were maintained on a basal, folate, and vitamin E-free chow and drinking water *ad libitum* for 1 mo ("AIN-76"; Purina/Mother Hubbard, Inc.; Shea and Rogers, 2002b; Shea et al., 2002b). For some groups, this basal diet was supplemented with folic acid (2mg/kg total diet wet weight), vitamin E (as α -tocopherol; 1g/kg total diet wet weight), and iron (50g/500g total diet); as a prooxidant (Shea and Rogers, 2002b; Shea et al., 2002b,c). Supplementation with folic acid and vitamin E without iron was defined as the complete diet; supplementation with iron without folic acid or vitamin E was defined as the deficient diet. Cages (3–4 mice each) were provided with an excess of chow and water each day that was weighed prior to dispensing and the remainder weighed the following day, allowing for calculation of the average amount consumed. After 1 mo on these diets, mice were subjected to standard Y maze and T maze tests (Corcoran et al., 2002; King et al., 1999 and refs. therein). For the Y maze, the pattern of exploration of the Y maze was recorded over 5 min intervals for each mouse. Under normal conditions, mice will preferentially explore the three arms of the maze in succession, for example, a mouse leaving the left arm and exploring the top arm should next enter the right arm rather than first returning to the left arm. The frequency that mice visited each of the three arms during any three-arm visitation sequence vs the total visitations defines the % alternation. We also examined cognitive deficiencies using the "T maze" reward-based system. Mice were placed at the bottom of a T-shaped maze, with one arm of the

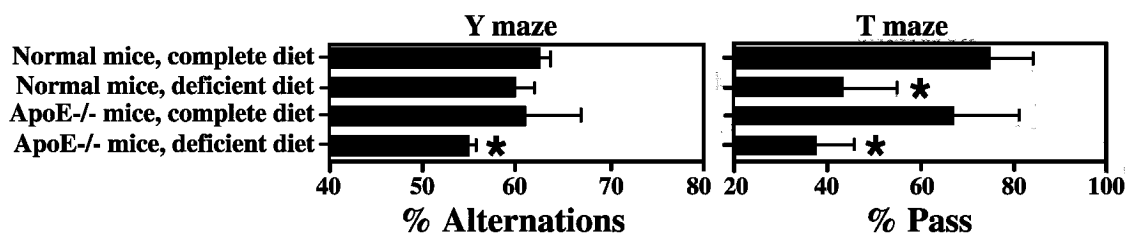


Fig. 1. Deprivation of folate and vitamin E differentially impairs cognitive performance of normal and ApoE^{-/-} mice subjected to oxidative challenge. Normal and ApoE^{-/-} mice were maintained on the indicated diets, then subjected to the Y and T maze tests ($n = 6-12$ mice, 3 independent experiments for the Y maze and 2 for the T maze). For the Y maze, the % alternations were determined for each group as described in Materials and Methods. For the T maze, each mouse was tested three times and the percentage of trials in which the mouse exhibited alternation (defined as passing) was determined as described in the Materials and Methods section. Note that both normal and ApoE^{-/-} mice demonstrate impaired performance on the deficient diet in the T maze ($p \leq 0.005$ vs normal mice receiving a complete diet; ANOVA), while only ApoE^{-/-} mice demonstrate impaired performance on the deficient diet in the Y maze ($p \leq 0.005$ vs normal mice receiving a complete diet; ANOVA).

maze blocked. 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 similar to the alternation described above for the Y maze; given a choice, mice under normal conditions will demonstrate a greater tendency to explore a novel area rather than re-explore previously visited territory. Mice were tested three 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. Notably, the same mice were subjected to both maze trials on successive days; mice were subjected to the Y maze prior to the T maze to avoid any influence of reward expectations on the behavior in the Y maze.

Results and Discussion

Normal mice demonstrated identical performance in the Y maze on either diet, while the deficient diet impaired the performance of normal mice in the T maze (Fig. 1). The differential performance of normal mice in these two tests highlights that

these tests are not identical and that they assay distinct (although overlapping) aspects of cognitive performance. In contrast to normal mice, ApoE^{-/-} mice displayed reduced performance in both tests when maintained on the deficient diet (Fig. 1-/-). No significant difference in consumption of food and water was observed among groups receiving different diets (Table 1).

Our prior analyses have demonstrated that following folate and vitamin E deprivation, and inclusion of excess dietary iron (as a pro-oxidant), ApoE^{-/-} mice, but not normal mice, exhibit oxidative damage to brain tissue (Shea and Rogers, 2002b). Both normal and ApoE^{-/-} mice exhibit increased glutathione levels when maintained under this diet in an apparent attempt to compensate for increased oxidative stress. Increased thiobarbituric acid-reactive substance (TBARs) in brain tissue of ApoE^{-/-} mice indicates that this increase in glutathione is insufficient to compensate for the combined impact of ApoE, folate, and vitamin E deficiency, yet is able to compensate for individual dietary or genetic deficiencies. The present findings in the Y and T-mazes demonstrate that the oxidative damage resulting from the combined impact of folate and vitamin E deficiencies along with dietary iron is reflected in impaired cognitive performance, and that deficiency in ApoE function imparts a further deleterious impact on cognitive performance. It remains unclear whether the increased risk of AD associated with the E4 is

Table 1
Normal and ApoE^{-/-} Mice Consume Identical Amounts of Food and Water
When Maintained on the Complete or Deficient Diet

	Chow(g)/day	Water (mL)/day
Normal, complete	10.7 ± 1.9	9.7 ± 2.3
Normal, deficient	12 ± 1.0	11 ± 0.7
ApoE ^{-/-} , complete	10.0 ± 2.0	10.8 ± 2.7
ApoE ^{-/-} , deficient	10.8 ± 1.1	10.2 ± 0.8

Note: Groups of mice maintained under complete and deficient diets were provided with a known amount of fresh chow and water each day in excess of their average consumption. The remainder of each was quantified the next day. Values consumed per mouse (presented as mean ± standard deviation of the mean) were calculated by dividing the number of mice per cage and were averaged over the month-long feeding regimen. Note identical consumption of food and water for both mouse strains under all diets.

derived from the diminished function(s) of ApoE4, or from the absence of protective effects provided by ApoE3 and/or ApoE2, or derives from the actual presence of ApoE4 (Rebecki et al., 2002). Because we utilized ApoE^{-/-} mice, our studies address only the lack of ApoE function. Additional studies should aim to carry out similar studies with transgenic mice expressing human E4 vs E3 and/or E2.

Oxidative stress, and deficiencies in folate, vitamin E, and ApoE function all represent risk factors for AD (Growdon, 2001; Mattson and Shea, 2002; Perry et al., 2002a,b). These findings stress that age-related cognitive impairment can arise from the combined impact of genetic predisposition, oxidative stress, and dietary deficiencies. These impairments can arise under conditions where no apparent trauma results from any one of the above risk factors in isolation. These findings leave open the possibility that one or more risk factors may remain latent and neurodegeneration may ensue only following augmentation by one or more additional traumatic events or conditions. In this regard, improved dietary supplementation with folate has fostered an increase among certain populations of individuals expressing polymorphisms of the folate-dependent enzyme 5',10'-methylene tetrahydrofolate reductase that exhibit decreased activity (Reyes-Engel et al., 2002). Although such individuals, who would otherwise have presented acute developmental disorders, appear normal, they may harbor an increased latent risk for neurodegeneration that may manifest only following age-related compromise in nutrition. Further studies are required to address the synergistic impact of known

and potential risk factors on age-related neurodegeneration and cognitive impairment.

Acknowledgments

This research was supported by the Alzheimer's Association and the Office of Collaborative Research of the University of Massachusetts Lowell Research Foundation.

References

- Berr, C. (2002) Oxidative stress and cognitive impairment in the elderly. *J. Nutr. Health Aging* **6**, 261–266.
- Butterfield, D. A. and Lauderback, C. M. (2002) Lipid peroxidation and protein oxidation in Alzheimer's disease: potential causes and consequences involving amyloid-beta peptide-associated free radical oxidative stress. *Free Rad. Biol. Med.* **32**, 1050–1060.
- Corcoran, K. A., Lu, Y., Turner, R.S., and Maren, S. (2002) Overexpression of hAPPswe impairs rewarded alternation and contextual fear conditioning in a transgenic mouse model of Alzheimer's disease. *Learn. Mem.* **9**, 243–252.
- Crowe, S. F. and Ross, C. K. (1997) Effect of folate deficiency and folate and B₁₂ excess on memory functioning in young chicks. *Pharmacol. Biochem. Behav.* **56**, 189–197.
- Floyd, R. A. and Hensley, K. (2002) Oxidative stress in brain aging: implications for therapeutics of neurodegenerative diseases. *Neurobiol. Aging* **23**, 795–807.
- Growdon, J. (2001) Incorporating biomarkers into clinical drug trials in Alzheimer's disease. *J. Alz. Dis.* **3**, 287–292.

- Huang, G. S., Yang, S. M., Hong, M. Y., Yang, P. C., and Liu, Y. C. (2000) Differential gene expression of livers from ApoE deficient mice. *Life Sci.* **68**, 19–28.
- Ho, P.I., Collins, S. C., Dhitavat, S, et al. (2001) Homocysteine potentiates beta-amyloid neurotoxicity: role of oxidative stress. *J. Neurochem.* **78**, 249–253.
- Ho, P.I., Ortiz, D., Rogers, E., and Shea, T. B. (2002) Multiple aspects of homocysteine neurotoxicity: glutamate excitotoxicity, kinase hyperactivation and DNA damage. *J. Neurosci. Res.* **70**, 694–702.
- Ho, P., Ashline, D., Dhitavat, S. et al. (2003) Folate deprivation induces neurodegeneration: roles of oxidative stress and increased homocysteine. *Neurobiol. Dis.* **1**, 32–42.
- King, D. L., Arendash, G. W., Crawford, F., Sterk, T., Menendez, J., Mullan, M. J. (1999) Progressive and gender-dependent cognitive impairment in the APP(SW) transgenic mouse model for Alzheimer's disease. *Behav. Brain Res.* **103**, 145–162
- Kruman, II, Culmsee, C., Chan, S. L., et al. (2000) Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. *J. Neurosci.* **20**, 6920–6926
- Kruman, II, Kumaravel, T. S., Lohani, A. et al. (2002) Folic acid deficiency and homocysteine impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of Alzheimer's disease. *J. Neurosci.* **22**, 1752–1762.
- Lalonde, R. (2002). The neurobiological basis of spontaneous alternation. *Neurosci. Biobehav. Rev.* **26**, 91–104.
- Hassing, L., Wahlin, A., Winblad, B., and Backman, L. (1999) Further evidence on the effects of vitamin B₁₂ and folate levels on episodic memory functioning: a population-based study of healthy very old adults. *Biol. Psychiatry* **45**, 1472–1480.
- Mattson, M. M. and Shea, T.B. (2002) Folate and homocysteine in neural plasticity and neurodegenerative disorders. *Trends Neurosci.* **26**, 137–146.
- Mizrahi, E. H., Jacobsen, D. W., Debanne, S. M., et al. (2003) Plasma total homocysteine levels, dietary vitamin B₆ and folate intake in AD and healthy aging. *J. Nutr. Health Aging* **3**, 160–165.
- Perry, G., Cash, A. D., Smith, M. A. (2002a) Alzheimer disease and oxidative stress. *J. Biomed. Biotechnol.* **2**, 120–123.
- Perry, G., Nunomura, A., Hirai, K., et al. (2002b) Is oxidative damage the fundamental pathogenic mechanism of Alzheimer's and other neurodegenerative diseases? *Free Radic. Biol. Med.* **33**, 1475–1479.
- Postiglione, A., Milan, G., Ruocco, A., et al. (2001) Plasma folate, vitamin B(12), and total homocysteine and homozygosity for the C677T mutation of the 5,10-methylene tetrahydrofolate reductase gene in patients with Alzheimer's dementia: a case-control study. *Gerontology* **6**, 324–329.
- Ramassamy, C., Averill, D., Beffert, L., et al. (1999) Oxidative damage and protection by antioxidants in the frontal cortex of Alzheimer's disease is related to the apolipoprotein E genotype. *Free Radic. Biol. Med.* **27**, 544–553.
- Ramassamy, C., Krzywkowski, P., Averill, D., et al. (2002) Impact of apolipoprotein E deficiency on oxidative insults and antioxidant levels in the brain. *Mol. Brain Res.* **86**, 76–83
- Rebeck, G. W., Kindy, M., and LaDu, M. J. (2002) Apolipoprotein E and Alzheimer's disease: the protective effects of ApoE2 and E3. *J. Alz. Dis.* **4**, 145–154.
- Shea, T. B. and Rogers, E. (2002a) Homocysteine as a risk factor for Alzheimer's disease. *New Eng. J. Med.* **346**, 2007.
- Shea, T. B. and Rogers, E. (2002b) Folate quenches oxidative damage in brains of apolipoprotein E-deficient mice: augmentation by vitamin E. *Mol. Brain Res.* **108**, 1–6.
- Shea, T. B., Lyons-Wieler, J., and Rogers, E. (2002a) Homocysteine, folate deprivation and Alzheimer's disease. *J. Alz. Dis.* **4**, 261–268
- Shea, T. B., Rogers, E., Ortiz, D., and Sheu, M. S. (2002b) Apolipoprotein E deficiency promotes increased oxidative stress and compensatory increases in antioxidants in brain tissue. *Free Rad. Biol. Med.* **33**, 1115–1120.
- Shea, T. B., Rogers, E., Ashline, D., et al. (2002c) Vitamin E deficiency does not induce compensatory antioxidant increases in central nervous system tissue of apolipoprotein E-deficient mice. *J. Alz. Dis.* **4**, 1–6.
- Reyes-Engel, A., Munoz, E., Gaitan, M. J., et al. (2002) Implications on human fertility of the 677C delta T and 1298A delta C polymorphisms of the MTHFR gene: consequences of a possible genetic selection. *Mol. Hum. Reprod.* **8**, 952–957.
- Veinbergs, I., Mallory, M., Sagara, Y., and Masliah, E. (2000) Vitamin E supplementation prevents spatial learning deficits and dendritic alterations in aged apolipoprotein E-deficient mice. *Eur. J. Neurosci.* **12**, 4541–4546
- White, A. R., Huang, X., Jobling, M. F., et al. (2001) Homocysteine potentiates copper- and amyloid beta peptide-mediated toxicity in primary cultures: possible risk factors in the Alzheimer's-type neurodegenerative pathways. *J. Neurochem.* **76**, 1509–1520.

