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Short Communication

Folate, vitamin E, and acetyl-L-carnitine provide synergistic protection against oxidative stress resulting from exposure of human neuroblastoma cells to amyloid-beta

Sirikarnt Dhitavat[a,b], Daniela Ortiz[a], Eugene Rogers[a,b,c], Ezequiel Rivera[d], Thomas B. Shea[a,b,d,*]

[a]Center for Cellular Neurobiology and Neurodegeneration Research, University of Massachusetts-Lowell, Lowell, MA 01854, USA
[b]Department of Biochemistry, University of Massachusetts-Lowell, Lowell, MA 01854, USA
[c]Department of Health and Clinical Sciences, University of Massachusetts-Lowell, Lowell, MA 01854, USA
[d]Department of Biological Sciences, University of Massachusetts-Lowell, Lowell, MA 01854, USA

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Abstract

Oxidative stress is an early and pivotal factor in Alzheimer's disease (AD). The neurotoxic peptide amyloid-beta (Abeta) contributes to oxidative damage in AD by inducing lipid peroxidation, which in turn generates additional downstream cytosolic free radicals and reactive oxygen species (ROS), leading to mitochondrial and cytoskeletal compromise, depletion of ATP, and ultimate apoptosis. Timely application of antioxidants can prevent all downstream consequences of Abeta exposure in culture, but in situ efficacy is limited, due in part to prior damage as well as difficulty in delivery. Herein, we demonstrate that administration of a combination of vitamin E (which prevents de novo membrane oxidative damage), folate (which maintains levels of the endogenous antioxidant glutathione), and acetyl-L-carnitine (which prevents Abeta-induced mitochondrial damage and ATP depletion) provides superior protection to that derived from each agent alone. These findings support a combinatorial approach in Alzheimer's therapy.

Theme: Disorders of the nervous system
Topic: Degenerative disease: Alzheimer's—miscellaneous

Keywords: Abeta; Oxidative damage; Folate; Vitamin E; Acetyl-L-carnitine; Alzheimer's disease

Oxidative stress is a pivotal factor in Alzheimer's disease (AD), and is evident prior to cytopathological hallmarks of the disorder [17,30]. The neurotoxic peptide amyloid-beta (Abeta) that accumulates in AD [12] contributes to oxidative damage by inducing lipid peroxidation [3,13], which in turn generates additional downstream cytosolic free radicals and reactive oxygen species (ROS; [26]). Endogenous antioxidant systems normally provide sufficient neuroprotection, but may fail to compensate for the increased generation of reactive oxygen species (ROS) that accompanies neurodegeneration [24]. One limiting factor in dietary consumption of antioxidants may be the difficulty in attaining appropriate concentrations within brain, although recent studies suggest that systemic antioxidant levels are helpful [23]. The antioxidant vitamin E provides some, but not dramatic, neuroprotection in AD [1,8,27,29]; the efficacy of vitamin E is likely to be limited by its lipophilic nature, which retards its ability to quench cytosolic ROS, including those generated from antecedent membrane oxidative damage [26]. In this regard, vitamin E prevents Abeta-induced ROS in cultured SH-SY-5Y human neuroblastoma cells only if present prior to, or applied simultaneously with,

* Corresponding author. Department of Biological Sciences, University of Massachusetts-Lowell, Lowell, MA 01854, USA.
E-mail address: thomas_shea@uml.edu (T.B. Shea).

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Abeta treatment, while antioxidants capable of entering the cytosolic compartment of these cells are protective even if applied some time after Abeta exposure [4,7].

Oxidative damage induced by Abeta can be exacerbated by other AD risk factors such as folate deficiency contributes to AD [18,19]. The neurotoxic amino acid homocysteine (HC), which increases during folate deprivation, induces ROS and potentiates Abeta neurotoxicity [9,10,14,15,28]. Combined exposure to Abeta and HC generated synergistic levels of ROS even at Abeta and HC concentrations that were benign individually [9]. Folate deficiency is also accompanied by a decrease in the endogenous cytosolic antioxidant glutathione (GSH), which may contribute to its potentiation of Abeta toxicity [11], since folate deprivation would compromise the neuron's capacity to buffer the consequences of oxidative stress.

Abeta neurotoxicity also leads to ATP depletion and mitochondrial compromise [16,22]. Supplementation with acetyl-L-carnitine (ALCAR) can prevent both of these deleterious effects [5] and has demonstrated some efficacy against AD in clinical trials [2,20,21].

Herein, we examined the impact of combined treatment with folate, vitamin E, and ALCAR on Abeta-induced ROS in culture.

SH-SY-5Y human neuroblastoma cells were cultured in DMEM containing 10% fetal calf serum and were differentiated for 7 days with 10 μM retinoic acid [7]. Treatment with retinoic acid for this time stops cell division and induces differentiation as judged by the elaboration of neurites of length ≥ 2 respective somal diameters in 90.9 ± 3.4% of cells (mean ± standard deviation of the mean, $n \geq 35$ cells from 5 different experiments for a total $n = 238$ cells; [7]). Cultures were approximately 70% confluent at the end of the differentiation period. Medium was then replaced with medium containing or lacking 4 mg/l folate and 0.15 mg/l vitamin E (as alpha-tocopherol; [6,7,25]), ALCAR (50 μM; [5]), and/or amyloid-beta 25–35 (Abeta; 20 μM; [7]) and cultures were incubated for an additional 2 h; retinoic acid and serum were maintained during this 2-h incubation. The dosages of folate, vitamin E, and ALCAR utilized were derived from those dosages demonstrated efficacious in prior studies of each individual agent [4,5,11]. Since the concentration of folate in serum is approximately 10 μg/l; folate-free medium supplemented with 10% serum therefore contains approximately 1 μg/l folate; this level is vastly reduced compared to that contained in folate-supplemented medium, and the presence or absence of serum was likely to have minimal, if any, impact upon cells in folate-free medium [11]. Reference to such conditions as "folate-deprived" or "receiving medium lacking folate" is intended to reflect this major difference in folate concentration rather than necessarily referring to a complete absence of folate. Moreover, cells are likely to retain some intracellular folate that may provide for certain metabolic processes during the relatively short incubation period (2 h) utilized in most of these experiments.

For monitoring of intracellular peroxide concentrations as an index of ROS, cultures received 10 μl/ml DFCD (2′7′-dichlorofluorescein diacetate; Kodak) for 20 min. Cultures were then visualized under fluorescein UV optics [7]. Images were captured using a Dage CCL-72 camera operated by NIH Image via a Scion LG-3 frame grabber and stored as PICT files on a PowerPC Macintosh. Identical illumination and capture settings were used for all images. Multiple [1,3,8,23,24,26] randomly selected fields visualized at 20×, typically containing 25–50 cells, were captured from duplicate or triplicate cultures. Relative

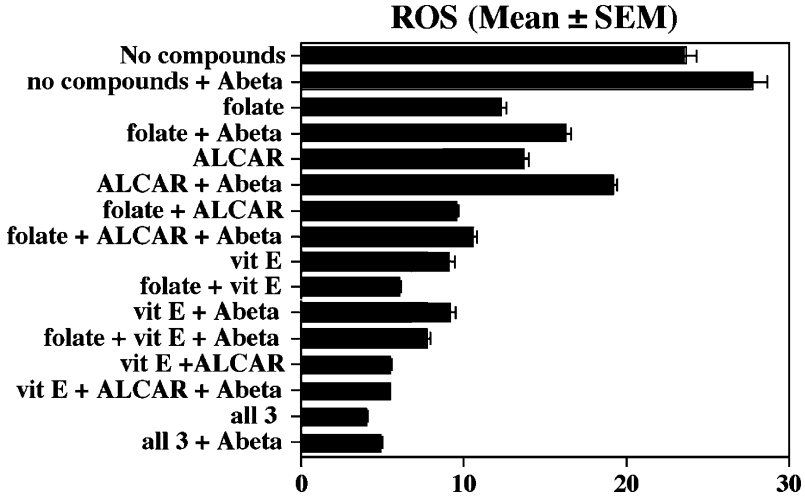

Fig. 1. Combined treatment with folate, vitamin E, and ALCAR provides superior neuroprotection against Abeta-induced ROS. Differentiated SH-SY-5Y human neuroblastoma cells were treated for 2 h with or without Abeta, folate, vitamin E, and/or ALCAR then processed for ROS via DCFD as described in the text. Values are presented in arbitrary densitometric units. Folate, vitamin E, and ALCAR each statistically ($P < 0.05$) reduced the extent of ROS otherwise induced by Abeta, with statistically ($P < 0.05$) increased protection in the order folate < ALCAR < folate < vit E for the concentrations tested. Combinations of two of these agents provided statistically ($P < 0.05$) further reduction than either agent alone, with increased protection in the order [folate + ALCAR] < [folate + vit E] < [vit E + ALCAR]. Optimal protection was provided by simultaneous treatment with all agents, which statistically ($P < 0.05$) reduced ROS below all other treatments both in the absence and presence of Abeta.