ELSEVIER

Neurobiology of Aging 28 (2007) 213–225

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Chronic dietary α-lipoic acid reduces deficits in hippocampal memory of aged Tg2576 mice

Joseph F. Quinn[a,b], Joseph R. Bussiere[c], Rebecca S. Hammond[c], Thomas J. Montine[d], Edward Henson[e], Richard E. Jones[a,b], Robert W. Stackman Jr.[c,*]

[a] *Portland Veterans Affairs Medical Center, P3 R&D Portland, OR, United States*
[b] *Department of Neurology, Oregon Health and Science University, Portland, OR, United States*
[c] *Department of Behavioral Neuroscience, Oregon Health and Science University, Portland, OR, United States*
[d] *Department of Pathology, University of Washington, Seattle, WA, United States*
[e] *Department of Pediatrics, Oregon Health and Science University, Portland, OR, United States*

Received 2 August 2005; received in revised form 19 November 2005; accepted 16 December 2005
Available online 31 January 2006

Abstract

Oxidative stress may play a key role in Alzheimer's disease (AD) neuropathology. Here, the effects of the antioxidant, α-lipoic acid (ALA) were tested on the Tg2576 mouse, a transgenic model of cerebral amyloidosis associated with AD. Ten-month old Tg2576 and wild type mice were fed an ALA-containing diet (0.1%) or control diet for 6 months and then assessed for the influence of diet on memory and neuropathology. ALA-treated Tg2576 mice exhibited significantly improved learning, and memory retention in the Morris water maze task compared to untreated Tg2576 mice. Twenty-four hours after contextual fear conditioning, untreated Tg2576 mice exhibited significantly impaired context-dependent freezing. ALA-treated Tg2576 mice exhibited significantly more context freezing than the untreated Tg2576 mice. Assessment of brain soluble and insoluble β-amyloid levels revealed no differences between ALA-treated and untreated Tg2576 mice. Brain levels of nitrotyrosine, a marker of nitrative stress, were elevated in Tg2576 mice, while F2 isoprostanes and neuroprostanes, oxidative stress markers, were not elevated in the Tg2576 mice relative to wild type. These data indicate that chronic dietary ALA can reduce hippocampal-dependent memory deficits of Tg2576 mice without affecting β-amyloid levels or plaque deposition.

Keywords: Spatial memory; Context memory; Hippocampus; Antioxidant; Alzheimer's disease; Amyloid; Nitrotyrosine

1. Introduction

Alzheimer's disease (AD) is characterized by impairments in memory, elevated levels of brain β-amyloid, and the formation of β-amyloid-containing plaques, and neurofibrillary tangles in forebrain regions such as the hippocampus, entorhinal cortex, and amygdala—regions important for memory. Considerable evidence indicates that oxidative stress may contribute to the neurodegenerative process in AD [49]. β-amyloid proteins stimulate the formation of free radicals in several in vitro model systems [7,26], and several antioxidants limit the neurotoxicity of β-amyloid [6,9,33,48,91]. Furthermore, post-mortem analyses of AD brain tissue reveal characteristic indices of oxidative stress including increased oxidation of proteins [3,75] and DNA [20], lipid peroxidation [54,63], and decreased polyunsaturated fatty acids [83]. Furthermore, levels of nitrotyrosine, a marker of increased protein oxidation or nitration, have been reported in cortical tissue [77] and cerebrospinal fluid of AD patients [82], indicating the potential involvement of reactive nitrogen species in the progression of the disease. Treatment of AD patients with the antioxidant, vitamin E has been shown to slow the progression of AD symptoms [73] as well as reduce the oxidative stress induced by β-amyloid plaque formation [89]. These findings lend support to the potential

* Corresponding author at: Department of Psychology, BS 101, Florida Atlantic University, 777 Glades Road, Boca Raton, FL 33431-0991, United States. Tel.: +1 561 297 2270; fax: +1 561 297 2160.
E-mail address: rstackma@fau.edu (R.W. Stackman Jr.).

0197-4580/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2005.12.014

beneficial effects of antioxidants as treatments to limit AD symptomology.

The discovery of genetic mutations and polymorphisms that are risk factors for familial AD or that may underlie AD has permitted the development of rational transgenic mouse models of AD [31]. One such model, the Tg2576 mouse, expresses a double mutation on the human amyloid precursor protein (APP_{695}), and exhibits a progressive impairment of spatial and contextual memory, elevation of brain β-amyloid levels, and deposition of β-amyloid plaques in cortex and hippocampus [28,40,88]. The hippocampal and cortical β-amyloid plaque deposits begin to appear in the Tg2576 mice at approximately 9–10 months of age [35]. Analyses of brain tissue from aged Tg2576 mice also reveal marked brain oxidative stress [65,67,76]. The AD-like abnormalities present in the Tg2576 mouse do not completely model the human condition; yet provide a useful model to examine interventions to correct the cerebral amyloidosis of AD.

We previously reported that chronic treatment with *Ginkgo biloba* extract (∼70 mg/kg/day, oral) prevented the spatial learning and memory deficits that are seen in aged Tg2576 mice, but had no effect on the deposition of hippocampal β-amyloid plaques [78]. There are several constituents present in *G. biloba* extract including those with antioxidant properties. Thus, it is difficult to ascribe the beneficial effects of *G. biloba* extract to a single mechanism. The present study focused on the effect of a well-characterized natural antioxidant, α-lipoic acid (ALA), upon learning and memory deficits of aged Tg2576 mice. Both ALA and its active metabolite dihydrolipoic acid potently interrupt deleterious cellular oxidative processes [64]. In vitro studies relevant to AD mechanisms have revealed that ALA protects cultured hippocampal neurons from β-amyloid-induced neurotoxicity [46]. Chronic dietary ALA significantly improves metabolic activity and cognitive function and reduces brain oxidative stress in aged mice, rats, and dogs [16,18,22,44,79]. For the present study, 10-month old female Tg2576 and wild type mice were fed an ALA-containing diet (0.1%) or control diet for 6 months and then assessed for the influence of diet on age-related learning and memory deficits. Histological assessments of β-amyloid plaques and brain levels of soluble and insoluble β-amyloid, and markers of oxidative and nitrosative damage were determined at the completion of behavioral testing.

2. Methods

2.1. Animals and diet

Transgenic mice were bred from a breeding pair of Tg2576 mice generously provided by Dr. Karen Hsiao-Ashe (Mayo Clinic, MN). The transgene is carried on a mixed genetic background of C57BL/6J × SJL. Litters were genotyped within 2 weeks of birth, weaned, and group-housed (4–6/cage) until commencement of experiments. All mice were maintained in a central vivarium on a 12 h light/dark cycle and allowed ad libitum access to rodent chow and tap water. All of the mice in this study were female since male Tg2576 mice are prone to marked aggression towards cage mates. Forty-one female mice were initially used in this study. Nine mice were excluded from behavioral testing due to blindness or observed motor difficulties (see below). Within the wild type group ($n = 17$), 9 were treated with chronic dietary ALA and 8 were given a control diet. Within the Tg2576 group ($n = 15$), 9 mice were treated with chronic dietary ALA, while 6 were given a control diet. ALA-supplemented mouse chow was prepared by Dyets Inc. (Bethlehem, PA) using DL-ALA from Sigma. An ALA concentration of 0.1% was selected based on published experience with aging rats [23,44]. Control diet mice were fed a chow that was identical except for ALA. Chow was weighed and replaced once a week to determine amounts consumed.

2.2. Morris water maze

Mice were trained in a standard Morris water maze task [56] using the same apparatus and protocol as in our previous study [78]. Briefly, a cylindrical tank (109 cm diameter) constructed of seamless white high-density polyethylene was positioned in a testing room surrounded by various distinct extra maze cues (posters, curtain panels, chair, desk, computer screen, door, etc.). The tank was filled to a depth of 33 cm with water rendered opaque by the addition of white non-toxic Tempra paint. A 13 cm diameter escape platform was constructed of clear Plexiglas and submerged 1 cm below the surface of the water.

2.2.1. Non-spatial pre-training

Each mouse received two habituation trials (1/day) in the Morris water maze prior to the commencement of spatial training. Each habituation trial comprised placing the mouse on the submerged platform in the center of the pool for 60 s. Floor-to-ceiling curtains were drawn around the maze to restrict the animals' view of extra-maze cues. After 60 s, the mouse was introduced into the pool in close proximity (within 2–3 cm) to the platform and allowed to climb onto the platform from four release points in the water.

2.2.2. Spatial training

Training on the hidden platform water maze task began 24 h after the last habituation trial. Floor-to-ceiling curtains were opened to permit the animals' view of extra-maze cues. Mice were trained for 36 trials (4/day) to learn the location of a submerged escape platform in the center of the SE quadrant of the pool. The platform position remained fixed throughout training. During a given trial, the mouse was introduced into the pool, at one of four pseudorandomly chosen start points (N, S, W, E), and allowed 60 s to find the platform. If the mouse did not find the platform after 60 s, it was placed on