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Brain tocopherols related to Alzheimer's disease neuropathology in humans

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Abstract: Randomized trials of α-tocopherol supplements on cognitive decline are negative, whereas studies of dietary tocopherols have shown benefit. We investigated these inconsistencies by analyzing the relations of α- and γ-tocopherol brain concentrations to Alzheimer's disease (AD) neuropathology among 115 deceased participants of the prospective Rush Memory and Aging Project. Associations of amyloid load and neurofibrillary tangle severity with brain tocopherol concentrations were examined in separate adjusted linear regression models. γ-Tocopherol concentrations were associated with lower amyloid load (β = –2.10, P = .002) and lower neurofibrillary tangle severity (β = –1.16, P = .02). Concentrations of α-tocopherol were not associated with AD neuropathology, except as modified by γ-tocopherol: high α-tocopherol was associated with higher amyloid load when γ-tocopherol levels were low and with lower amyloid levels when γ-tocopherol levels were high (P for interaction = 0.03). Brain concentrations of γ- and α-tocopherols may be associated with AD neuropathology in interrelated, complex ways. Randomized trials should consider the contribution of γ-tocopherol.

Keywords: Nutritional; Alzheimer's disease; Cohort studies; Vitamin E; Tocopherols; Amyloid beta; Neurofiibrillary tangles

1. Introduction

There is an expansive literature on animal studies that demonstrate the importance of α-tocopherol to the healthy functioning of the brain, including protection against lipid peroxidation [1,2], neuron loss [3,4], β-amyloid deposition [2,5], DNA damage [6–9], mitochondrial dysfunction [10,11], and decline in memory and learning [1,12]. This literature is supported by prospective epidemiologic studies linking dietary intakes of vitamin E or combined tocopherols to slower rate of age-related cognitive decline [13–15] and lower risk of Alzheimer's disease (AD) [15–19]. It is actually γ-tocopherol that constitutes much of the vitamin E content in the U.S. food supply [20]. The vast majority of studies do not show cognitive benefit from supplements of α-tocopherol [21–27]. Recent completion of several randomized, controlled trials [22,23,28], all with negative results for a beneficial effect of high-dose α-tocopherol on cognitive decline, cast doubt in the scientific community on the potential beneficial effects of vitamin E. However, there are several aspects regarding the design of these randomized trials that may have resulted in the negative findings [29]. Among these are the trials' focus on high-dose α-tocopherol for treatment therapy as opposed to other tocopherol forms, and the lack of concern for baseline vitamin E status of the trial participants. At least one

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trial demonstrated a beneficial effect of α-tocopherol supplementation in the trial participants who had low dietary intakes (<6.1 mg/day) [28].

In this investigation we examined the relations of the predominant tocopherols, α and γ, to measures of AD neuropathology in a community study of residents who were initially free of AD at enrollment. Our findings help to shed light on the role of different levels and forms of tocopherols in human brain and in occurrence of AD.

2. Methods

2.1. Study sample

The Rush Memory and Aging Project (MAP) is an ongoing clinical–neuropathologic cohort study of persons living in Chicago continuous-care retirement communities and subsidized housing that began in 1997 [30]. MAP volunteers are dementia-free at enrollment and all agree to annual clinical neurologic evaluations and to brain autopsy upon death. Clinical diagnosis of AD is made by an expert neurologist blinded to neuropathologic findings and in accordance with accepted criteria [31] after review of annual clinical evaluations and neuropsychological performance testing, as previously described [30]. The average time interval between the final clinical evaluation and death was 10 months for the participants observed herein.

The ongoing tocopherol component to the MAP study began in 2004 and includes assessment of dietary and brain tissue levels of tocopherols. Of the 1555 individuals included in the MAP, 1360 were alive at initiation of the study and still actively participating (80 were deceased and 115 withdrew) and thus eligible to be part of the tocopherol investigation. Of these, 1089 (80%) agreed to participate. Since 2004, 278 of those enrolled in the tocopherol study have died, 232 autopsies have been performed (83%), and 115 have been analyzed for both brain tocopherols and neuropathology. Here we report on the first consecutive brain cases analyzed as part of the ongoing study. The study was approved by the institutional review board of Rush University Medical Center.

2.2. Brain neuropathology analyses

Brain autopsies were performed using standard procedures, as previously described [30]. Slabs from one cerebral hemisphere were placed in a freezer at –80°C. The brain tissue was used for tocopherol analysis. Slabs from the contralateral hemisphere were fixed in 4% paraformaldehyde and stored in 20% glycerol and 2% dimethysulfoxide (DSMO). For immunohistochemistry we used paraffin-embedded 20-micron sections from 2 or more blocks of the following regions of the brain: hippocampus CA1/subiculum; entorhinal cortex (Brodmann area or BA28); dorsolateral prefrontal; superior frontal (BA6/8); anterior cingulate (BA24); inferior temporal (BA20); angular/supramarginal (BA39/40); and calcarine (BA17) cortices. Aggregated amyloid-β was identified using 10D5 (dilution 1:300; courtesy of Elan Pharmaceuticals, South San Francisco, CA). Immunohistochemistry was performed on an automated immunostainer (Leica Microsystems, Inc., Bannockborn, IL).

To measure amyloid load, an investigator outlined the region of interest using a stereology system (MicroBrightfield, Inc., Colchester, VT) and an Olympus BX-51 microscope with a motorized stage. We used Stereo Investigator (version 9.0) software to systematically sample and then capture images. Area analysis was performed using Image J (version 1.42 g; http://rsbweb.nih.gov/ij/). A composite measure of the percent area occupied by amyloid-β was computed by averaging the values obtained by systematic computerized sampling of amyloid load in 8 cortical regions.

Neurofibrillary tangles (NFTs) were identified using Bielschowsky silver-stained 6-micron sections from hippocampal, entorhinal, midfrontal, midtemporal, and inferior parietal cortices. We computed Braak NFT stage using recommended criteria [32] that incorporates severity and regional progression of NFTs into one score on a scale of 0 (no NFT in any region) to 6 (frequent tangles across multiple regions).

All pathologic data collection was performed blinded to the clinical and tocopherol data. Fig. 1 shows examples of cases with low and high amyloid load in the neocortex and low and high Braak scores in the hippocampus.

2.3. Brain tocopherol analyses

Frozen brain tissue was thawed and analyzed for tocopherol concentrations using high-performance liquid chromatography coupled with electrochemical detection, according to previously described methods [33,34]. Tocopherol levels were measured in 2 cortical regions (inferior temporal and midfrontal) affected by AD and in 2 subcortical regions, the posterior putamen and ventromedial caudate, which are involved in motor function and cognitive behavior, respectively [35]. For analyses, tocopherol concentrations are expressed as picomoles (pmol) per milligram protein. Extraction losses were corrected for recovery of the internal standard, δ-tocopherol. We eliminated from the analyses 2 cases with extreme values (α-tocopherol >10,000 pmol/mg protein, γ-tocopherol >900 pmol/mg protein).

2.4. Vitamin E supplement use

Self-reported vitamin E supplement use (α-tocopherol) and dose from multivitamins and individual vitamin E supplements were obtained from a modified version of the Harvard Food Frequency Questionnaire, which has been validated in a sample of older Chicago residents [36]. Data on α-tocopherol intake levels from supplements were available for these analyses but not information on tocopherol intakes from food sources or