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Antioxidants for Alzheimer Disease

A Randomized Clinical Trial With Cerebrospinal Fluid Biomarker Measures

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Objective: To evaluate whether antioxidant supplements presumed to target specific cellular compartments affected cerebrospinal fluid (CSF) biomarkers.

Design: Double-blind, placebo-controlled clinical trial.

Setting: Academic medical centers.

Participants: Subjects with mild to moderate Alzheimer disease.

Intervention: Random assignment to treatment for 16 weeks with 800 IU/d of vitamin E (α -tocopherol) plus 500 mg/d of vitamin C plus 900 mg/d of α -lipoic acid (E/C/ALA); 400 mg of coenzyme Q 3 times/d; or placebo.

Main Outcome Measures: Changes from baseline to 16 weeks in CSF biomarkers related to Alzheimer disease and oxidative stress, cognition (Mini-Mental State Examination), and function (Alzheimer's Disease Cooperative Study Activities of Daily Living Scale).

Results: Seventy-eight subjects were randomized; 66 provided serial CSF specimens adequate for biochemical

analyses. Study drugs were well tolerated, but accelerated decline in Mini-Mental State Examination scores occurred in the E/C/ALA group, a potential safety concern. Changes in CSF A β 42, tau, and P-tau₁₈₁ levels did not differ between the 3 groups. Cerebrospinal fluid F2-isoprostane levels, an oxidative stress biomarker, decreased on average by 19% from baseline to week 16 in the E/C/ALA group but were unchanged in the other groups.

Conclusions: Antioxidants did not influence CSF biomarkers related to amyloid or tau pathology. Lowering of CSF F2-isoprostane levels in the E/C/ALA group suggests reduction of oxidative stress in the brain. However, this treatment raised the caution of faster cognitive decline, which would need careful assessment if longer-term clinical trials are conducted.

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OXIDATIVE DAMAGE IS ASSOCIATED with aging and is widespread in the brain in Alzheimer disease (AD).¹ Free radical species mediate damage to proteins, lipids, mitochondria, and DNA and may activate the cell cycle; overwhelm endogenous antioxidant defenses in the brain; and contribute to neuronal damage.^{1,2} Observational studies suggest that an antioxidant-rich diet may reduce the risk of AD.³ However, prevention trials of combinations of antioxidant dietary supplements in elderly subjects did not influence age-associated cognitive decline.^{4,5} Antioxidant randomized clinical trials in AD have had mixed results. High doses of vitamin E (α -tocopherol) in moderate to severe AD delayed clinical milestones without benefiting cognition.⁶ High-dose α -tocopherol did not

influence progression in amnesic mild cognitive impairment.⁷

We hypothesized that if antioxidant treatment affects key pathogenic mechanisms in patients with AD, this would be reflected by cerebrospinal fluid (CSF) biomarker changes. Aided by an expert panel of advisors, we reviewed antioxidant studies for evidence of benefit in transgenic mouse and other models relevant to AD, determined dose ranges likely to be safe and potentially efficacious in humans, and identified combinations that could provide synergism⁸ (eg, vitamin C helps to quench reactive oxygen intermediates when combined with vitamin E).⁹ We decided to study a combination of vitamin E, vitamin C, and α -lipoic acid (ALA); coenzyme Q (CoQ) alone (to our knowledge, this study would provide the first safety data in AD), and placebo.

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Group Information: The Alzheimer's Disease Cooperative Study (ADCS) investigators and sites are listed at the end of this article.

In transgenic mouse models of amyloid deposition, effects of antioxidant supplements depend on time and dose. In an amyloid protein precursor transgenic mouse, early treatment with vitamin E (α -tocopherol) attenuated plaque formation but was not effective if started after amyloid deposits were present.¹⁰ Treatment with vitamin C plus a medium dose of vitamin E decreased oxidative damage and improved performance on tests of spatial memory; these benefits were not seen with vitamin C plus a high dose of vitamin E.¹¹ Treatment with vitamin E improved the morphology of neurites surrounding plaque cores.¹²

α -Lipoic acid is a mitochondrial coenzyme with antioxidant actions; it also induces the transcription factor Nrf2, which induces many antioxidant enzymes.⁹ Aged dogs given a diet rich in antioxidant supplements, including ALA, showed better cognitive performance, decreased amyloid burden, and reduced oxidative damage.¹³ Studies of long-term dietary supplementation with ALA in amyloid protein precursor transgenic mice have reported amelioration of deficits on memory testing¹⁴ and decreased oxidative markers without changes in amyloid pathology.¹⁵ A cohort of patients with mild AD who received 600 mg/d of ALA, compared with patients who did not receive ALA, showed cognitive stabilization over 48 months.¹⁶

Coenzyme Q is a naturally occurring antioxidant that helps to protect mitochondria from reactive oxidative species generated during oxidative metabolism. It is currently under investigation in amyotrophic lateral sclerosis and Parkinson disease.¹⁷ Administration of CoQ to aged amyloid protein precursor/presenilin 1 mice led to a decrease in cortical levels of A β 42 and reduction of markers of oxidative stress.¹⁸ Aged mice treated with CoQ showed decreased levels of brain carbonyls and other oxidative stress markers.¹⁹

METHODS

PARTICIPANTS

The Alzheimer's Disease Cooperative Study (ADCS) Antioxidant Biomarker study was a double-blind, randomized multicenter clinical trial, conducted between 2006 and 2008 at 12 centers across the United States, funded by the National Institute on Aging. Subjects diagnosed with probable AD²⁰ with Mini-Mental State Examination (MMSE)²¹ scores of 16 or more of 30 were enrolled. Further inclusion criteria were age between 50 and 85 years, presence of a caregiver to supervise study medications, and neuroimaging (computed tomography or magnetic resonance imaging) results within the past 24 months consistent with AD but lacking evidence of significant vascular disease (infarction that could cause dementia) or other intracranial disease processes. Stable anti-AD treatment with a cholinesterase inhibitor, memantine, or both for at least 3 months was required. Medications for behavioral symptoms were allowed only if doses were stable for at least 4 weeks before screening.

Subjects were excluded if they had a diagnosis of dementia other than AD (eg, vascular dementia), a neurological disorder or major psychiatric disorder, or drug or alcohol abuse or dependence. Subjects with contraindications to lumbar puncture were excluded, eg, treatment with oral anticoagulants, prior surgery or deformity of the lumbosacral spine, or a platelet count

less than $100 \times 10^3/\mu\text{L}$. Medical factors or conditions that could increase systemic oxidative stress were also exclusions: diabetes mellitus; obesity (body mass index >30 [calculated as weight in kilograms divided by height in meters squared]); cardiac, renal, or pulmonary failure; smoking; inflammatory diseases; and regular use of nonsteroidal anti-inflammatory drugs, immunosuppressive drugs, or drugs for Parkinson disease. Because supplements and vitamins are widely used in elderly individuals, subjects were allowed to continue taking their current antioxidant supplements provided that daily doses were less than the following thresholds: 100 IU/d or less for α -tocopherol, 200 mg/d or less for vitamin C, 60 mg/d or less for CoQ, and 100 IU/d or less for ALA.

STUDY DESIGN

Subjects who met study criteria and provided informed consent were assigned to a 16-week double-blind period of treatment in 1 of 3 arms: 800 IU/d of vitamin E (α -tocopherol) plus 500 mg/d of vitamin C plus 900 mg/d of α -lipoic acid (E/C/ALA); 400 mg of CoQ 3 times/d; or placebo. To maintain blinding, all subjects received 3-times/d dosing, including placebos matching CoQ and the E/C/ALA combination as needed. Study drugs were dispensed in coded containers.

Study visits occurred at screening, baseline (within 4 weeks of screening), 8 weeks, and 16 weeks. Lumbar punctures were performed at the baseline and 16-week visits. Investigators received training regarding research lumbar punctures, including the use of atraumatic small-gauge needles. Vitamine Inc. provided CoQ and a matching placebo, in the form of chewable wafers, taken at mealtime. They also supplied encapsulated α -tocopherol, vitamin C, and racemic ALA and matching placebo capsules. Subjects, caregivers, and investigators were blind to treatment allocation.

The study was conducted in accordance with the principles of the Declaration of Helsinki and with the laws and regulations of the locality in which the research was conducted. The study protocol was approved by the institutional review boards of the participating centers. The study was conducted under an Investigational New Drug Application from the Food and Drug Administration. A data safety monitoring board reviewed the study data every 3 months.

OUTCOME MEASURES

Safety was assessed by interviewing subjects and informants about adverse events, measuring vital signs, brief physical examination, and a panel of blood tests at baseline, week 8, and week 16. Electrocardiograms were obtained at baseline and 16 weeks. Adverse experiences were recorded at each study visit and defined according to Medical Dictionary for Regulatory Activities terminology.

Cognition was assessed with the MMSE at baseline and 16 weeks; scores range from 0 to 30. Functional abilities were rated by informant interview using the ADCS Activities of Daily Living Scale (ADCS-ADL); scores range from 0 to 78.²²

The primary outcome measures were levels of 4 biochemical markers measured in CSF.²³ A β 42 is a 42-amino acid peptide that aggregates and is deposited in the brain to form plaques in AD. Cerebrospinal fluid levels of A β 42 are decreased in AD, presumably because binding of this peptide to aggregated amyloid in the brain results in less being cleared into the CSF.²⁴ The microtubule-associated protein tau is an integral component of neurofibrillary tangles and neuritic dystrophy in AD. Cerebrospinal fluid levels of tau and tau phosphorylated at specific sites (P-tau) are increased in AD, related to damage to neurons and their axonal processes.²⁴ To evaluate oxidative dam-

age to the central nervous system, F2-isoprostane levels, which are stably oxidized forms of arachidonic acid, were measured.²⁵ Brain levels of F2-isoprostanes increase in patients with AD and in transgenic mouse models.²⁶ Cerebrospinal fluid levels of F2-isoprostanes are increased in AD^{25,26} and in cognitively intact subjects who carry mutations in the amyloid protein precursor or presenilin 1 genes associated with familial AD.²⁷

Cerebrospinal fluid cell counts and total protein and glucose levels were measured by local laboratories. Only samples with a red blood cell count less than 500/mL were used for biomarker measurements. Within 30 minutes of the lumbar puncture, CSF samples were aliquoted into polypropylene cryotubes, frozen, shipped to a central laboratory, then sent to the University of California, San Diego, and stored at -80°C until biomarkers were measured. Levels of A β ₄₂, total tau, and P-tau₁₈₁ were measured using multiplex sandwich assays (INNO-BIA AlzBio3; Innogenetics), following established methods.²⁸ F2-isoprostane levels were measured in the laboratory of one of us (T.J.M., University of Washington, Seattle) by stable isotope dilution assay using gas chromatography–mass spectrometry with selective ion monitoring.²⁶ Assays were measured by technicians who were blind to subjects and treatment groups.

DATA ANALYSIS

Statistical analyses were conducted by the ADCS Data Core. The primary efficacy analysis was a between-group comparison of the changes from baseline in CSF levels of A β ₄₂, total tau, P-tau₁₈₁, and F2-isoprostanes. Secondary analyses compared safety data and changes in MMSE and ADCS-ADL scores among the 3 groups. The primary analyses were performed in subjects who completed a baseline and follow-up lumbar puncture that yielded analyzable CSF. For clinical and safety outcomes, modified intention-to-treat analyses were performed, including subjects who received at least 1 dose of double-blind medication, had a baseline evaluation, and had at least 1 evaluation after the start of treatment.

Baseline data were compared between groups, using the Kruskal-Wallis test for continuous variables and the Fisher exact test for categorical variables. For analysis of change in CSF biomarkers, analysis of covariance was used. Age, sex, and baseline MMSE score did not differ across groups, and models with and without these as covariates were analyzed. Models were fitted comparing each treatment group with placebo, and the *P* values were adjusted using the Hochberg method for multiple comparison.²⁹ The adjusted *P* values were compared with $\alpha = .05$.

Power calculations for CSF biomarkers were based on published data on serial CSF levels of tau.³⁰ With 25 subjects in each treatment arm, there would be 80% power to detect a 40% or more change in CSF tau level, with $\alpha = .05$ and σ^2 (variance) = 382.

RESULTS

Eighty-seven subjects were screened at 12 sites, and 78 were randomized to E/C/ALA, CoQ, or placebo, using permuted block randomization (blocks of 3 subjects at each site), generated by the Coordinating Center. The flow of subjects is shown in the **Figure**, and demographic and clinical data, in **Table 1**. The groups were well matched at baseline.

The study drugs were well tolerated. There were no significant changes in vital signs or body mass index from baseline to the end of the study in any treatment arm and no group differences in the frequencies of reported adverse events. There were no significant changes in results of a panel of hematological and biochemical blood tests in any

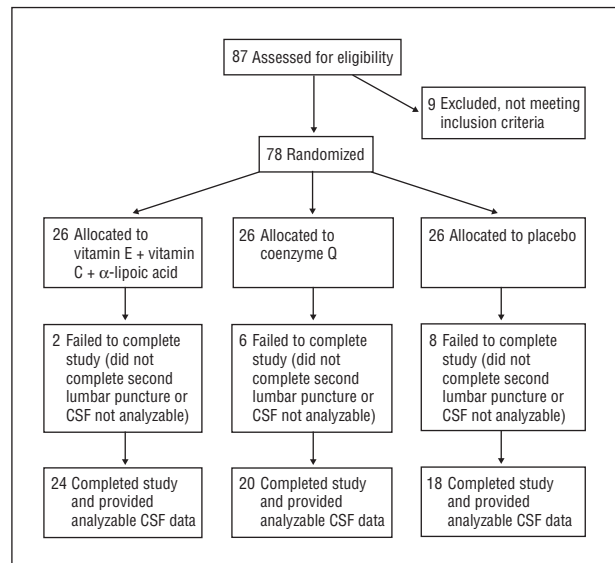


Figure. Flowchart of subject participation in the study. CSF indicates cerebrospinal fluid.

Table 1. Demographic and Clinical Features of 78 Study Participants^a

	E/C/ALA (n = 28)	CoQ (n = 25)	Placebo (n = 25)
Age, y, mean (SD)	73.6 (9.1)	71.4 (8.4)	73.2 (9.5)
Women, %	46	44	48
Education, y, mean (SD)	14.6 (3.7)	15.0 (2.5)	15.8 (2.4)
BMI, mean (SD)	26.5 (4.1)	27.0 (4.2)	25.8 (4.1)
Acetylcholinesterase inhibitor use, %	88	84	100
Memantine use, %	44	40	50
Concomitant vitamin or supplement use, % ^b	52	64	43
MMSE score, mean (SD)	23.1 (3.9)	23.3 (4.4)	23.1 (3.4)
MMSE score change at 16 wk, mean (SD)	-2.8 (2.9) ^c	-1.0 (2.5)	-0.9 (2.5)
ADCS-ADL score, mean (SD)	61.1 (16.3)	65.2 (12.8)	65.4 (8.7)
ADCS-ADL score change at 16 wk, mean (SD)	-4.6 (7.9)	-2.4 (6.7)	-2.3 (5.9)

Abbreviations: ADCS-ADL, Alzheimer's Disease Cooperative Study Activities of Daily Living Scale; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CoQ, coenzyme Q; E/C/ALA, vitamin E plus vitamin C plus α -lipoic acid; MMSE, Mini-Mental State Examination.

^a Intention to treat. Demographic or clinical variables listed in Table 1 did not differ at baseline among the treatment arms.

^b Concomitant vitamins or supplements were allowed only if they contained vitamin E, vitamin C, α -lipoic acid, or CoQ in amounts less than defined thresholds, much lower than the doses used in the treatment arms of this trial.

^c *P* = .04, analysis of covariance, adjusted for multiple comparisons. In a logistic model that compared MMSE score changes in the E/C/ALA and placebo arms, and included age, sex, and baseline MMSE score as covariates, treatment arm was significant (*P* = .02).

of the treatment groups. Three serious adverse events were reported, in different subjects, and were judged to be unrelated to the study drug. One subject was hospitalized briefly after a pacemaker lead fell off. One subject fell and sustained a wrist fracture, which was treated. One subject developed dizziness and headache about 7 days before the

Table 2. CSF Biomarker Data

	Mean (SD)		
	E/C/ALA (n = 24)	CoQ (n = 20)	Placebo (n = 22)
A β 42 level at baseline, pg/mL	190 (43)	185 (54)	201 (57)
A β 42 level change	-8 (23)	-15 (31)	-6 (37)
Tau level at baseline, pg/mL	123 (60)	109 (64)	114 (84)
Tau level change	-23 (39)	-9 (21)	-12 (19)
P-tau ₁₈₁ level at baseline, pg/mL	68 (33)	70 (37)	58 (35)
P-tau ₁₈₁ level change	-6 (19)	-4 (17)	0.5 (19)
F2-isoprostane level at baseline, pg/mL	38 (15)	34 (15)	35 (12)
F2-isoprostane level change	-7 (10) ^a	0 (8)	0 (6)

Abbreviations: CoQ, coenzyme Q; CSF, cerebrospinal fluid; E/C/ALA, vitamin E plus vitamin C plus α -lipoic acid.

^aP = .04, analysis of covariance, adjusted for multiple comparisons.

week 16 visit. That subject successfully completed the week 16 lumbar puncture but was noted to have drowsiness, worsening mental abilities, and mild left hemiparesis about 5 days later. Brain magnetic resonance imaging revealed a tumor subsequently diagnosed as glioblastoma multiforme. There was no evidence of a tumor on a head computed tomography reviewed when this subject was screened, which was obtained about 18 months before the subject entered the study.

Clinical measures of cognitive abilities (MMSE) and function (ADCS-ADL) did not differ between groups at baseline. The MMSE scores showed a greater decline in the E/C/ALA group relative to placebo. There were no differences between groups in changes in ADCS-ADL total scores, or in scores for subscales of basic or instrumental ADL, although there were trends toward greater decline in the E/C/ALA group.

Cerebrospinal fluid levels of A β 42, tau, P-tau₁₈₁, and F2-isoprostanes were measurable in 66 subjects at baseline and week 16 (**Table 2**). Levels of each biomarker did not differ between the 3 groups at baseline. At 16 weeks, mean changes in each of the biomarkers were small. Changes in levels of A β 42, tau, and P-tau₁₈₁ did not differ between the 3 groups. Levels of F2-isoprostanes decreased in the E/C/ALA group from baseline but did not change in the CoQ or placebo groups. This was significant before and after adjustment for age and sex and represented a mean decrease of about 19%.

Correlations were examined between clinical scores (MMSE and ADCS-ADL) and CSF biomarker indices (levels of tau, P-tau, A β 42, and F2-isoprostanes), analyzing baseline and 16-week change data. Significant correlations were found among all subjects for baseline MMSE

scores vs ADCS-ADL scores ($R = 0.42$) and vs CSF total tau level ($R = -0.32$). No other correlations were significant for baseline or change data, among all subjects or within specific treatment arms.

COMMENT

Basic and observational studies provide strong support for antioxidant treatment in AD. Oxidative stress is a widespread cellular process and lacks a typical treatment target such as a receptor or a single major metabolic pathway. This is a challenge to designing and measuring treatment interventions. Approaches to combat oxidative stress typically involve augmenting antioxidant defenses, eg, with nutritional supplements or vitamins. Key questions include what doses to use, whether defenses are more critical in relation to mitochondrial or cytosolic oxidative stress, and whether a combination of antioxidants is more effective than a single agent at quenching oxidative reactions. A related question, whether a diet high in naturally occurring antioxidants may reduce the risk of developing AD, is difficult to address in an intervention study.

The present study examined the effects of a high dose of CoQ, a naturally occurring antioxidant that may protect mitochondria from reactive oxidative species generated during oxidative metabolism. Oral administration can achieve brain antioxidant effects in animal models.^{19,31} A phase 2 trial in Parkinson disease suggested that 400 mg of CoQ 3 times/d resulted in slowing of motor progression,³² and a phase 3 trial is currently in progress. A phase 2 trial in amyotrophic lateral sclerosis used a high-dose range of CoQ (1800 and 2700 mg/d) but found no difference in clinical progression over 9 months of treatment compared with placebo.³³ Although our study found that CoQ was safe and well tolerated in patients with AD, the absence of a biomarker signal in CSF suggests that CoQ, at the dose tested, does not improve indices of oxidative stress or neurodegeneration. These results do not support further clinical trial development of CoQ in AD. Idebenone, a benzoquinone derivative structurally related to CoQ, has been studied widely in AD and other disorders. A placebo-controlled 12-month clinical trial that assessed 3 different doses of idebenone in mild to moderate AD found no differences between treatment and placebo.³⁴

Doses for the E/C/ALA combination were selected based on expert opinion and clinical trials for other indications. A similar combination of E/C/ALA improved indices of skeletal muscle metabolism in elderly subjects.³⁵ Although prior studies of vitamin E in AD and other neurodegenerative disorders have used high doses, typically 2000 IU/d, we limited vitamin E to 800 mg/d because of a meta-analysis of clinical trials and prevention studies that raised the possibility that more than 800 IU/d of vitamin E may be associated with increased all-cause mortality.³⁶

Increased decline on the MMSE and a trend in this direction on the ADCS-ADL in the E/C/ALA group raises a concern that this combination could adversely affect cognition in AD. The lack of correlation of changes in these measures with changes in CSF biomarkers suggests that the cognitive changes may not be due to worsening of AD-related pathol-

ogy. Although a mechanism is uncertain, this cognitive finding raises a caution and will need to be carefully monitored if longer-term studies are planned.

The combination of E/C/ALA did not affect CSF biomarkers related to A β , tau, or P-tau. The half-life of A β 42 in CSF is extremely short, measured in hours.³⁷ Tau and P-tau persist for a few weeks in CSF after acute stroke.³⁸ Our clinical trial was therefore long enough to be able to detect a potential anti-AD effect on these biomarkers. Our findings suggest that this antioxidant combination did not influence pathways related to amyloid and tau pathology. However, E/C/ALA did result in a significant decrease in CSF levels of F2-isoprostanes, consistent with antioxidant effects in the brain. These findings are analogous to the transgenic mouse model study cited earlier, in which vitamin E plus vitamin C decreased neuroprostane levels in the brain, without affecting measures of A β , plaques, or neuritic changes; in this model, there was improvement of performance on spatial memory testing.¹¹

It is unclear whether the relatively small reduction in CSF F2-isoprostane level seen in this study may lead to clinical benefits in AD. The more rapid MMSE score decline raises a caution and indicates that cognitive performance would need to be assessed if a longer-term clinical trial of this antioxidant combination is considered.

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