Functional Vitamin E Deficiency in *ApoE4* Patients with Alzheimer's Disease

Emilie Mas^{a,c} Anne Marie Dupuy^{a, b} Sylvaine Artero^b Florence Portet^{b, c}
Jean Paul Cristol^a Karen Ritchie^b Jacques Touchon^{b, c}

^aDepartment of Biochemistry, Lapeyronie Hospital, ^bINSERM, Unit E361, Epidemiology of Neurodegenerative Pathologies of the Nervous System, ^cDepartment of Neurology, Gui de Chauliac Hospital, Montpellier, France

Key Words

Vitamin E · Apolipoprotein E · Alzheimer's disease

Abstract

Oxidative stress has been implicated in the development of Alzheimer's disease (AD). Consequently, antioxidant therapies including Vitamin E (VitE) supplementation for both prevention and treatment of neurodegenerative diseases currently appears to be a promising avenue of research. The aim of the present study was to examine the relationship between AD and the *ApoE* phenotype, lipid parameters and VitE levels in a large cohort of elderly subjects. No absolute deficit was observed in plasma VitE levels. However in AD, *ApoE4* is not associated with an increase in total cholesterol (TC) and VitE levels. Moreover, our results suggest that oxidative stress-induced injury and protection by VitE in AD are related to the *ApoE* phenotype. Our study strongly supports the hypothesis of an impairment of lipophilic antioxidant delivery to neuronal cells in AD leading to a tissular antioxidant deficiency which could facilitate oxidative stress.

Introduction

There is now increasing evidence that brain tissue is exposed to oxidative stress during the course of Alzheimer's disease (AD). Brain cells and brain cell metabolism may be affected at all levels; nucleic acid, proteins, lipids and carbohydrates may all be modified by oxidative processes. The brain is physiologically particularly vulnerable to oxidative insult as it contains a high proportion of polyunsaturated fatty acids (20:4, n-6; 22:6, n-3) which are a selective target for free radicals, and its metabolism requires substantial quantities of oxygen. Moreover both Fe and ascorbate, which together may enhance oxidant production and lipid peroxidation, are found in large quantities in brain tissue. This high oxidative insult is further enhanced in the presence of neurodegenerative disorders, especially AD [1–5]. The presence of amyloid β peptide (A β) in amyloid plaques increases reactive oxygen species production by exacerbating NADPH oxidase activity in microglia [6, 7], by impairing mitochondrial function and energy supply and by initiating local inflammation. Despite this increased free radical production, the brain is not highly enriched in antioxidant defenses [7–9]. Furthermore, ageing and neurodegenerative

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J.P. Cristol
Department of Biochemistry, Hôpital Lapeyronie
191 Avenue du Doyen Gaston Giraud
FR–34295 Montpellier Cédex 5 (France)
Tel. +33 467 338 345, Fax +33 467 338 393, E-Mail jp-cristol@chu-montpellier.fr

disease decrease the efficacy of antioxidant defense systems leading to increased neuronal vulnerability to exogenous insult [3]. In addition, A β could alter antioxidant defense mechanisms increasing reactive oxygen species production [10–12]. A β may thus be considered to cause oxidative stress leading to an increase in lipid peroxidation, protein oxidation and DNA oxidation [13–15]. It has been shown that all A β -induced changes observed in vitro could be inhibited by VitE or other antioxidants [14]. Consequently, antioxidant therapies for the prevention and treatment of neurodegenerative diseases appear theoretically promising. However, it has been observed in vivo that while VitE metabolism is directly associated with lipid metabolism including absorption, transport, delivery to cells and degradation, the major plasma apolipoproteins (Apo), Apo A and B, are absent in brain tissue. In the brain, only *ApoE* is present, which has a crucial function in lipid redistribution and cholesterol homeostasis [16].

ApoE is the most prolific apolipoprotein in the cerebrospinal fluid (CSF) and brain [17], and is synthesized in both oligodendrocytes and astrocytes [18]. It is recognized as the major lipid carrier protein in the brain and has been associated with growing neurons as well as neuron repair or synaptic remodeling [19]. *ApoE* presents a genetic polymorphism with three common alleles in the general population designated $\epsilon 2$, $\epsilon 3$, $\epsilon 4$ coding for protein *ApoE2*, *ApoE3* and *ApoE4*, respectively. The *ApoE4* genotype was recognized as a susceptibility gene for AD and could be involved in the biological chain of events leading to some neurodegenerative disorders. Several studies found that *E4* patients have increased numbers of neuritic plaques in the brain, as compared to *E3* carriers, suggesting that *ApoE4* facilitates A β aggregation and/or deposition. It has also been suggested that *ApoE4* impairs cholesterol transport in the brain [20, 21]. Finally *ApoE* isoform could interact with oxidative damage since the *ApoE4* genotype is associated with an increased level of peroxidation [22]. Moreover, the *ApoE4* allele is associated with the presence of 4-hydroxy-2 nonenal pyrrole adducts, a marker of lipid peroxidation, in neurofibrillary tangles in AD [23, 24]. By contrast, physiologic concentrations of *ApoE2* protect central nervous system cells against irreversible oxidative stress [25, 26]. Since VitE, as a principal antioxidant defense mechanism, is transported in plasma and delivered to tissue by lipoproteins, we hypothesized that a relative deficit in VitE delivery could account in part for the increase in oxidative stress observed in *ApoE4* AD patients. The aim of the present study was to evaluate in peripheral blood the relationship

between *ApoE* phenotype, lipid parameters and VitE levels in a cohort of elderly subjects in relation to the presence or absence of AD.

Materials and Methods

Subjects

A total of 464 patients (consecutive referrals) admitted to a neurology clinic specializing in memory disorders at the Gui de Chauliac (University Neurology Hospital in Montpellier France) were screened over a 2-year-period from 1996 to 1998. Subjects were aged between 65 and 98 years (mean $\pm$ SD: 74.1 $\pm$ 10.0 years). Subjective memory complaint was the principal reason for consultation. The diagnosis of AD was made according to a standardized neurological examination carried out by a neurologist based on DSM-IV and NINCDS-ADRDA criteria. One hundred patients were given a diagnosis of AD, and 186 were considered free of dementia and were included as controls.

One hundred and seventy-eight subjects were excluded: 62 presented a diagnosis of vascular dementia or mixed dementia, 50 were given a diagnosis of non-AD dementia (i.e. Lewy body dementia, frontotemporal dementia), 40 subjects were taking vitamin supplements and 26 hypocholesterolemic drugs. The protocol did not contain a food questionnaire.

Biochemical Parameters

All blood samples were taken after a fasting period of 12–14 h and transferred to vacutainer tubes containing 0.1% EDTA. Total cholesterol (TC) and triglyceride (TG) levels were determined by standard enzymatic methods (Biomérieux, France) using commercially available test kits on a Coulter CPA analyzer (Coultronics, France SA). Plasma VitE concentrations were measured by high performance liquid chromatography as described elsewhere [27] and using material from Waters chromatography (Millipore Waters, Les Ulis, France). Absolute level of plasma VitE were expressed as $\mu\text{mol/l}$. As lipophilic alpha-tocopherol is carried in lipoprotein, its concentration is highly dependent on the level of plasma lipid. Thus, it has been proposed that VitE evaluation in plasma requires lipid standardization, specially TC and TG. VitE concentration may thus be expressed as the ratio VitE/(TG + TC) in $\mu\text{mol/mmol}$ of lipids [28–30]. *ApoE* phenotype was determined by an isoelectrofocalization method [21]. Reading of phenotypes from gels were interpreted by two different biochemists.

Statistical Analysis

Statistical analyses were performed using the SPSS statistical software package. Data are presented as means and standard deviations (SD). The Mann-Whitney-Wilcoxon nonparametric test was used to compare differences in lipid and VitE levels among *ApoE* phenotypes. Significance was defined as $p < 0.05$.

Results

General Characteristics of the Population

Of the original sample of 286 patients, 100 were given a diagnosis of AD. One hundred and eighty-six subjects were considered free of neurological disorder and were included as controls.

No significant difference in mean age was found between AD patients (73.52 ± 9.06 years) and controls (74.71 ± 10.88 years; table 1). In the control group 13.9% of subjects were E4 women and 8% E4 men. In the AD group 22% of subjects were E4 women and 12% E4 men. No significant difference in lipid parameters or VitE levels was observed between AD patients and the control group (table 1).

ApoE Isoforms and AD

In the AD group, the overall allele frequencies for E2, E3, E4 were 0.03, 0.76, 0.21, respectively, compared with 0.05, 0.82, 0.12 in the control group. A significant association was observed between the presence of the E4 allele and AD ($\chi^2 = 7.64$, $p < 0.01$; table 1). The odds ratio for AD in the presence of at least one E4 allele is thus estimated from the present series as 1.7 (95% CI: 1.1–2.3).

The Relationship between ApoE4 and Lipid Parameters Is Altered in AD

In the control group cholesterol levels were found to be significantly higher in subjects with an E4 allele (6.43 ± 1.3 mmol/l) as opposed to those without (5.85 ± 1.21 mmol/l) (Mann Whitney U test = 969, $p < 0.03$; table 2).

By contrast, within the AD group no significant relationship was found between ApoE4 and cholesterol levels

(E4 5.90 ± 0.72 mmol/l vs. non-E4 5.66 ± 1.18 mmol/l; table 2). AD subjects with E4 had lower mean cholesterol levels (5.90 ± 0.72 mmol/l) than controls with E4 (6.43 ± 1.3 mmol/l). This difference was significant ($p < 0.04$). The occurrence of AD did not impair cholesterol levels in non-E4 patients when adjusted for age and sex.

No significant relationship was observed between TG levels and the presence of an E4 allele in either the AD or the control group.

ApoE Isoforms and Plasma VitE Levels

In the control group, VitE level was significantly increased in E4 subjects (38.78 ± 10.47 μ mol/l) compared

Table 1. Sample characteristics and lipid parameters (mean $\pm$ SD) and VitE expressed as concentration (μ mol/l) or adjusted to TC and TG levels in AD patients versus controls.

	Control group	AD group
Subjects	186	100
Mean age	74.71 ± 10.88	73.52 ± 9.06
Females	59.67%	61%
TC, mmol/l	5.95 ± 1.25	5.73 ± 1.05
VitE, μ mol/l	33.51 ± 9.56	32.90 ± 10.12
VitE/TC + TG	4.54 ± 0.95	4.72 ± 0.95
ApoE allele frequency		
E2	0.05	0.03
E3	0.82	0.76
E4	0.12	0.21*

Values are expressed as mean $\pm$ SD. VitE = Vitamin E (23–34.8 μ mol/l); TC = total cholesterol (4.10–5.67 mmol/l); TG = triglycerides (0.60–1.70 mmol/l).

* $p < 0.001$ vs. E4 controls.

Table 2. Mean age, plasma lipid concentration, VitE levels and VitE adjusted to TC and TG levels in AD and control subjects according to ApoE phenotype

	Control group		AD group	
	with E4 allele	without E4 allele	with E4 allele	without E4 allele
Mean age	75.42 ± 9.49	74.63 ± 11.28	71.65 ± 8.92	72.98 ± 9.15
Subjects	41	145	34	66
TC, mmol/l	$6.43 \pm 1.3^{*}^{**}$	5.85 ± 1.21	5.9 ± 0.72	5.66 ± 1.18
VitE, μ mol/l	$38.78 \pm 10.47^{***}$	32.02 ± 8.88	33.91 ± 6.67	32.32 ± 11.55
VitE/TC + TG	4.74 ± 1.03	4.47 ± 0.92	4.79 ± 0.77	4.68 ± 1.03

Values are expressed as mean $\pm$ SD. * $p < 0.03$ vs. non-E4 controls; ** $p < 0.04$ vs. E4 AD; *** $p < 0.006$ vs. non-E4 controls.

to non-E4 subjects ($32 \pm 8.88 \mu\text{mol/l}$; Mann Whitney U test = 873, $p < 0.006$). This increase was not observed when VitE was normalized to TG and TC levels (table 2).

In the AD group, no significant difference in VitE concentration was observed between E4 or non-E4 subjects, even after normalization according to lipid levels (table 2). AD subjects with E4 were observed to have lower mean VitE ($33.91 \pm 6.67 \mu\text{mol/l}$) than controls with E4 ($38.78 \pm 10.47 \mu\text{mol/l}$).

Discussion

Our results show that AD is associated with a modification in the relationship between the *ApoE4* isoform and lipid metabolism as well as lipophilic antioxidant transport. The well-established *ApoE4* raising effect on cholesterol is not observed in AD patients. Moreover, in controls this rise in cholesterol is associated with an increase in lipophilic VitE levels. Interestingly this increase in VitE disappears in *ApoE4* AD patients.

It is now well established from experimental data and clinical findings that oxidative stress is involved in the course of AD. Thus, antioxidant supplementation has been proposed as a preventive approach or as a potential therapeutic adjuvant. Among antioxidants, lipophilic VitE could prevent in vitro oxidative insult in neuronal cells [14]. VitE decreases cellular death in rat hippocampal cell cultures induced by A β and attenuates toxicity in neuroblastoma cells induced by excitatory amino acid [31]. Decreased plasma VitE levels in AD patients have been observed in numerous studies [32–37]. On the other hand, Riviere et al. [38] did not detect a significant difference in plasma VitE concentrations between controls and AD patients. Controversial data have also been reported in CSF. Both Jimenez-Jimenez et al. [34] and Schippling et al. [39] found lower VitE concentrations in patients with AD, but no difference between patients with AD and controls was found by Metcalfe et al. [40]. In our study, we did not observe a difference in VitE levels between AD patients ($32.90 \pm 10.12 \mu\text{mol/l}$; $n = 100$) and controls ($33.51 \pm 9.56 \mu\text{mol/l}$; $n = 186$). These conflicting reports on antioxidant defense mechanisms in peripheral blood could be linked to AD heterogeneity, including *ApoE* polymorphism, evolution of the disease and nutritional status.

Since transport and delivery of lipophilic antioxidants in tissue are clearly linked to lipid metabolism, the study of VitE levels according to *ApoE* polymorphism is of par-

ticular interest. *ApoE* is known to play a crucial role in the delivery of lipids to tissue via the ApoB/E receptor, especially in the brain, where ApoB lipoprotein is not expressed [19].

In our study, the *ApoE4* isoform in controls is clearly associated with increased plasma levels of cholesterol and VitE. This *ApoE4* raising effect observed in VitE disappears after lipid standardization, stressing the clear relationship between lipid and VitE metabolism.

ApoE could influence lipid and VitE bioavailability by different routes [41]. Firstly, in the circulation, *ApoE4* regulates the hepatic lipase activity which is involved in the conversion of very-low-density lipoprotein (VLDL) to low-density lipoprotein (LDL). As a result, a greater conversion of VLDL to LDL is observed in *ApoE4* carriers. On the other hand, *ApoE* is known to regulate the cell delivery of cholesterol and phospholipids via the Apo B/E receptor which exhibits different affinities for the three common *ApoE* isoforms (*ApoE2* < *ApoE3* < *ApoE4*). Due to its high affinity, *ApoE4*-containing lipoproteins (VLDL, chylomicron remnants and intermediate density lipoproteins) are removed faster from plasma than those containing *ApoE3* and *ApoE2*. Cholesterol input from these lipoproteins induces a downregulation of the LDL receptor and thus higher concentration of circulating cholesterol.

Taken together, these data explain the *ApoE4* raising effect on cholesterol. As LDL is the principal transport vehicle of VitE in the circulation, *ApoE* genotype could directly influence both plasma VitE level and cell delivery. As expected, we observed an increase in absolute plasma VitE level in *ApoE4* carriers which disappears after lipid standardization. Since *ApoE4* decreases cell uptake in both TC and VitE, this increased plasma level could be associated with retention of VitE in plasma lipoproteins and VitE deficiency in peripheral tissue. This mechanism observed in peripheral cell could be further extended to neuronal cell, especially as *ApoE* expression is decreased in brain tissue from *ApoE4* carriers.

Interestingly, a clear relationship between *ApoE* isoforms and brain lipid transport has recently been observed [16, 19]. Impairment in cholesterol transport and recycling from astrocytes to neurons could be involved in neuronal degeneration and could account in part for the decrease in cholesterol content previously reported in brain tissue from AD patients [16, 42]. Dysregulation of cholesterol levels by *ApoE* phenotype in the presence of AD has been reported by several groups [43–49], including our research group [21]. The lack of increase in plasma cholesterol level and low cholesterol uptake by cells could account in part for the decrease in cholesterol contained

in the AD brain [47]. However, few studies have been conducted on the relationship between *ApoE* polymorphisms and lipophilic antioxidants. Fernandes et al. [50] did not observe any association between the presence of an *ApoE4* allele and plasma or erythrocyte VitE levels in either controls or AD patients. This *ApoE4*-related raising effect on lipids and lipophilic antioxidant was not seen in our AD patients. Here again the decrease in absolute plasma VitE level could compare with *ApoE4*-induced low delivery to neuronal tissue leading to antioxidant deficiency and oxidative stress in cells. By contrast, it has been suggested that the AD protective effect of *ApoE2* may be associated with a VitE enrichment of the central nervous system leading to increased VitE efficiency [51]. From this clinical study, it is difficult to speculate on the mechanism of both CT and lipophilic antioxidant decrease in AD which could be related to specific metabolic alteration or to dietary disorder. However, the lack of hypocholesterolemia and hypovitE in non-E4 does not support the nutritional hypothesis. The observations that AD modifies absolute values of both CT and VitE in *ApoE4* carriers leading to a possible cell antioxidant deficiency, could be a potential interest in prevention, suggesting a more effective role for such strategies in *ApoE4* subjects. Convergent epidemiologic studies suggest that antioxidant supplementation could have a role in preventing or slowing AD and cognitive decline [52, 53]. The Rotterdam Study [54] reported that high dietary intake of VitE decreased the risk of subsequent development of AD. Similarly, none of the VitE users (n = 27) in a group of 633 patients over 65 years developed AD during 4.3-year follow-up [55]. A new study suggests that higher intakes of VitE are associated with a reduced inci-

dence of AD [56]. It has also been suggested that the combination of vitamin C and E supplements could prevent cognitive decline [57] or significantly decrease the risk for AD in elderly populations. In addition, VitE (associated with vitamin C) significantly reduces CSF F2 isoprostanes in patients with mild AD [58], stressing the important role of VitE in neuronal cell protection. However, recent studies showed that VitE alone have no effect during the development of AD or in mild cognitive impairment [59–61]. Antioxidant capacity is achieved by a complex collaboration between major antioxidants, vitamins and enzymes in which VitE could play a pivotal role. VitE prevents lipid peroxidation leading to VitE radical. By contrast, in the absence of a suitable electron donor, such as vitamin C, in the cycling cascade of free radical equivalents, the VitE antioxidant can become a pro-oxidant. By approaching the interface of lipid membranes the hydrophilic vitamin C can recycle the lipophilic VitE radical. In fact, the redundancy of the antioxidant system might ensure antioxidant defense that should be achieved by supplementation of antioxidant cocktails [62].

Previous studies in vitro and clinical findings have reported that *ApoE4* isoform could enhance oxidative injury in the brain during the course of AD. Our study strongly supports the hypothesis of an impairment of lipophilic antioxidant delivery to neuronal cells leading to tissue deficiency. Taken together, these data suggest that antioxidant supplementation or antioxidant-rich diet may constitute an important therapeutic intervention strategy in *ApoE4* subjects in order to prevent oxidative injury. This hypothesis is currently being investigated in clinical trials.

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