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The effect of acetyl-L-carnitine and R-α-lipoic acid treatment in ApoE4 mouse as a model of human Alzheimer's disease

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ABSTRACT

We measured age-dependent effects of human ApoE4 on cerebral blood flow (CBF) using ApoE4 transgenic mice compared to age-matched wild-type (WT) mice by use of [^{14}C] iodoantipyrene autoradiography. ApoE4 associated factors reduce CBF gradually to create brain hypoperfusion when compared to WT, and the differences in CBF are greatest as animals age from 6-weeks to 12-months. Transmission electron microscopy with colloidal gold immunocytochemistry showed structural damage in young and aged microvessel endothelium of ApoE4 animals extended to the cytoplasm of perivascular cells, perivascular nerve terminals and hippocampal neurons and glial cells. These abnormalities coexist with mitochondrial structural alteration and mitochondrial DNA overproliferation and/or deletion in all brain cellular compartments. Spatial memory and temporal memory tests showed a trend in improving cognitive function in ApoE4 mice fed selective mitochondrial antioxidants acetyl-l-carnitine and R-α-lipoic acid. Our findings indicate that ApoE4 genotype-induced mitochondrial changes and associated structural damage may explain age-dependent pathology seen in AD, indicating potential for novel treatment strategies in the near future.

1. Introduction

A growing body of evidence suggests a common etiology for Alzheimer's disease (AD) and cardiovascular disease [1–5]. The E4 isoform of apolipoprotein E (ApoE) is involved in cardiovascular and cerebrovascular disorders and is the most prevalent risk factor for late onset or sporadic AD. ApoE facilitates transportation and metabolism of cholesterol and triglyceride in cells throughout the body [6,7], promotes the normal metabolism of cholesterol by the liver, and aids in building and repairing neuronal processes in the brain as well as in the periphery [6–9]. The genotype appears to be a determinant of brain amyloid-β (Aβ) burden in AD patients [10].

ApoE4 transgenic mice are appropriate models for studying the pathogenesis and preclinical treatment of ApoE-related cognitive deficits associated with late onset AD [11]. They express human ApoE4 in glia and/or neurons in the brain depending on the promoter driving expression and exhibit accountable cognitive impairments and cerebrovascular and neuronal pathology [12–21].

An important factor in the pathogenesis of AD is hypoperfusion-induced oxidative stress, which is caused by disturbed cerebral blood flow (CBF) [22]. AD patients exhibit decreased oxygen levels in the vasculature [23–25]. Many studies finding chronic cerebral hypoperfusion in mild cognitive impairment (MCI) and AD have concluded that it is an initiator of the reduced supply of oxygen [8,24–30]. This suggests that low blood flow is a prominent feature of the brain during chronic hypoxia/hypoperfusion and possibly an initiating factor during the development of AD [2,27–29,31,32].

The AD brain is characterized by the impairment of energy metabolism, indicating mitochondrial dysfunction [22,29,33,34]. These metabolic defects are present before AD symptoms develop in ApoE ε4 homozygotic patients [35,36]. In addition, it has been well documented that reduced resting global CBF is associated with cardiovascular diseases such as atherosclerosis, post-ischemic insult and heart failure (HF). A study by Alves and coworkers suggests that coexistence of blood flow reductions in HF patients with the functional deficit in these regions is relevant to the pathophysiology of the cognitive impairments presented by HF patients [37]. De la Torre proposed that advanced aging, along with a comorbid condition such as a vascular risk factor that further decreases cerebral perfusion, promotes a critically attained threshold of cerebral hypoperfusion [1]. Studying the effect of aging as a main reason for chronic brain hypoperfusion (CBH) in oxidative stress

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induced cerebrovascular lesions and their relationship to MCI and AD could uncover the ultimate pathogenic mechanisms that lead to AD. We have previously shown that atherosclerotic lesions are associated with mitochondrial DNA deletions in brain microvessel endothelium and amyloid angiopathy in human AD [23], aged transgenic mice over-expressing amyloid beta precursor protein (AβPP) [34,38], and two-vessel occlusion rat models of CBH [39]. These studies suggest that cerebrovascular pathology may play a crucial role in predisposition to stroke and possibly MCI and AD [27–29].

Our previous studies found that treating aged rats with the selective mitochondrial antioxidants acetyl-L-carnitine (ALCAR) and R-alpha-lipoic acid (LA) restores cognitive performance and abolishes oxidative stress induced structural changes in brain parenchymal cells (neurons, vascular wall cells and glia) [40,41]. The effect of aging on CBF and brain parenchymal cell ultrastructure and the potential for treating these abnormalities by using selective mitochondrial antioxidants have not yet been fully explored. In the present study, we used the vascular dementia paradigm in ApoE4 mice to analyze the effects of the selective mitochondrial antioxidants treatment with ALCAR and LA on CBF, neuropathology, brain and vessel ultrastructural abnormalities, and behavior.

2. Materials and methods

For the blood flow and ultrastructural studies, Glial Fibrillary Acidic Protein (GFAP)-ApoE4 transgenic and wild-type C57BL/6 control mice were obtained from the Jackson Mouse Colony (Jackson, FL, USA). The GFAP promoter drives the expression of ApoE cDNA in glia, primarily in astrocytes. The ApoE4 transgenic mice express no mouse ApoE and are on a C57BL/6 background. Animals were housed in 12 h dark/light conditions and had unlimited access to food and water. All experimental procedures were performed according to NIH and International Guidelines for the use of animals in research, and appropriate protocols were approved by the relevant institutional committees. Six week- and six- and twelve-month-old ($n=12$/group) ApoE4 and wild-type C57BL/6 control mice were used for the blood flow study.

2.1. Cerebral blood flow measurement

Age-dependent effects of ApoE4 on CBF were measured using [14C] iodoantipyrene autoradiography in conjunction with a mathematical algorithm [42]. Measurement of regional blood flow (rBF) was determined by a [14C] — iodoantipyrene (IAP, New England Nuclear) autoradiography technique modified for mice and described earlier by us [42,43]. This method measures local CBF by combining an intraperitoneal tracer with a single blood sampling from the heart. Mice were anesthetized by a halothane gas mixture (2% in 30/70% O_2 and NO_2), and then injected with IAP in normal saline (2.5 μCi in 200 μl) intraperitoneally. 60 s later the mice were frozen in liquid nitrogen and stored at −80 °C. Brains and hearts were dissected in a cryotome (−20 °C). Brains were thin-sectioned at the levels of atlas plate 13, 30 and 69 and then placed on glass slides. The slides were placed on Amersham Hyperfilm β-Max autographic film along with calibrated standards (Amersham [14C] Micro-scales, RPA 504 and RPA 511) and then exposed for 3 months [42]. The films were digitized using a BIOQUANT image analysis system (R & M Biometrics, INC) and background corrected. Optical densities were converted to nanocuries per gram using standard curves generated from the standards. The blood flow was calculated from the images and a reference blood sample using the equation below:

$$\text{Blood Flow}(ml/g/min) = \text{Tissue}(nCi/g) \div[(\text{reference blood}(nCi)*Time(min))]$$

Reference blood samples were prepared by pipetting 100 μl samples taken from the heart into a scintillation vial and then adding 15 ml of scintillation fluid (Aquasol, NEN Res. Products, DU PONT). After mixing, vials were counted on a γ-scintillation counter (dpm).

2.2. ALCAR and LA treatment

The Gladstone Institute, University of the California at San Francisco, provided Neuronal Specific Enolase (NSA)-ApoE4 transgenic mice for behavioral studies. These transgenic mice also are on a C57BL/6 background and have no mouse ApoE expression. Seven-month-old ApoE4 transgenic mice were randomly divided into two groups ($n=4$/group): control and treated (0.2% ALCAR in drinking water and 0.15% dexlipotam, a tris–salt of LA, which is equal to 0.1% LA) as described by our group elsewhere [40]. Wild-type C57BL/6 mice without treatment were used as controls. Mice were subjected to Morris water maze testing at age 12 months (following 5 months of treatment), again at 22 months (following 15 months of treatment) and to a Peak procedure test at age 13 months (after 6–7 months of treatment). At the end of the final cognitive tests all animals were perfusion fixed as described previously [31] for electron microscopic ultrastructural analysis, by using *in situ* hybridization techniques for mitochondrial DNA overproliferation determination, and deletion and immunogold decoration by using antibodies for protein immunoreactivity determination.

2.3. Morris water maze test of spatial memory

The Morris water maze task tests spatial memory by requiring mice to find a submerged platform in a pool of water using external visual cues as described previously [40,44,45]. The time required for an individual mouse to find the platform was measured using a digital camera and a computer system to record movement (Columbus Instruments, VideoMex-V). Trials (4 consecutive days, 4 trials/day) were with the same hidden platform location, but with varied start locations. On day 5, the platform was removed from the pool for a probe test, (60 s) and the time spent at the actual site where the platform was previously located was recorded. On day 6, the time required to reach a visible platform was measured to determine visual function and motor ability [40]. In the reversal test, the platform was moved to the opposite quadrant of the previous test (4 trials/day and 120 s/trial).

2.4. Peak procedure test of temporal memory

Temporal memory, as assessed by the peak procedure, measures the function of the internal clock, learning process, attention, and exploratory behavior [40,44,46,47]. Mice were tested in 18 identical boxes that contain a light source and a speaker (for delivering light or noise signals) and a lever that dispenses single food (45 mg) pellets when pressed (BioServ mix T101). Prior to the test, the food supply was decreased to 85% of the free-feeding amount. In this test the animal is rewarded with one pellet only if the lever is pressed within

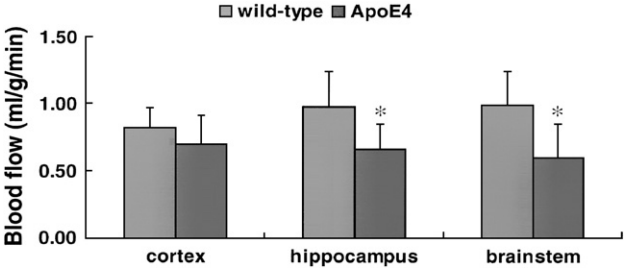

Fig. 1. Regional blood flow in 6-week-old mice. ApoE4 is associated with decreased regional blood flow.