Experimental Gerontology 111 (2018) 118–121

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Changes in regional cerebral blood flow associated with a 45 day course of the ketogenic agent, caprylidene, in patients with mild to moderate Alzheimer's disease: Results of a randomized, double-blinded, pilot study

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ARTICLE INFO

Section Editor: Holly M. Brown-Borg

Keywords:
Caprylidene
Alzheimer's disease
Dementia
PET
Ketogenic diet
Cerebral blood flow

ABSTRACT

Background: Caprylidene is a ketogen that, when metabolized, produces the ketones beta-hydroxybutyrate and acetoacetate, which can cross the blood brain barrier. It has been hypothesized that ketone bodies can be used as an alternate energy source by neurons with impaired glucose utilization. Caprylidene has been shown to improve cognition in patients with mild-to-moderate Alzheimer's disease (AD) who lacked an AD-predisposing allele (ε4) of the gene for apolipoprotein E. In this pilot study, we examined the effects of caprylidene on regional cerebral blood flow (rCBF) in patients with mild to moderate AD.

Methods: Sixteen subjects with mild-to-moderate AD, based on NINCDS-ADRDA criteria, were enrolled in a double-blinded, placebo-controlled, randomized clinical trial. Fourteen subjects received treatment with caprylidene, and 2 subjects were given placebo. Subjects received 4 ^{15}O-water PET scans over the course of the study to assess rCBF: once before receiving a standard caprylidene or placebo dose and 90 min after the dose, on the first day and after 45 days of daily caprylidene or placebo consumption. The scans were examined by standardized volumes of interest (sVOI) and voxel-based statistical parametric mapping (spm) methods of analysis.

Results: Subjects lacking an ε4 allele had significantly elevated rCBF in the left superior lateral temporal cortex by sVOI analysis after adopting a caprylidene diet for 45 days ($p = 0.04$), which was further corroborated by spm. The anterior cerebellum, left inferior temporal cortex, and hypothalamus were also found by spm to be regions of long-term increase in rCBF in these subjects. In contrast, patients who possessed the ε4 allele did not display these changes in rCBF.

Conclusion: Daily ingestion of caprylidene over 45 days was associated with increased blood flow in specific brain regions in patients lacking an apolipoprotein ε4 allele.

1. Introduction

Alzheimer's disease (AD) is considered to be the most common cause of neurodegenerative dementia, currently affecting about 5.7 million Americans (Alzheimer's Association 2018). The number of patients with AD is expected to increase dramatically, to approximately 13.8 million by 2050 (Alzheimer's Association 2018). AD is characterized by extracellular neuronal accumulation of beta-amyloid plaques and intracellular neuronal accumulation of tau protein in neurofibrillary tangles (Caselli et al. 2017). These accumulations are associated with diminished synaptic communication and neuronal death, resulting in impairments in memory, thinking, and daily functioning. AD is associated with a progressive course of cognitive and functional decline, ultimately resulting in death, often secondary to pneumonia as the

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https://doi.org/10.1016/j.exger.2018.07.009
Received 11 December 2017; Received in revised form 14 June 2018; Accepted 10 July 2018
Available online 10 July 2018
0531-5565/

presumed cause (Brunnström and Englund 2009). Despite worldwide efforts to find a cure for AD, current pharmacological treatments are limited and can only temporarily improve cognition and function, or delay their decline (Eleti 2016). Though the underlying causes of AD occurring after 65 years old remain to be well-established, the strongest genetic risk factor that has been identified in this age group is carriage of the ε4 allele of the gene for apolipoprotein E (APOE4).

Caprylidene is a medium-chain triglyceride of caprylic acid that results in the production of ketone bodies, including beta-hydroxybutyrate and acetoacetate, after oxidation by the liver (Costantini et al., 2008). These ketone bodies can cross the blood-brain barrier and may be used as an alternate source of energy by neurons that exhibit impaired glucose utilization. In turn, the increased energy may improve neuronal survival and improve cognitive function (Cunnane et al. 2016; Sharma et al. 2014). Clinical trials have demonstrated highly elevated serum beta-hydroxybutyrate levels following caprylidene supplementation (Reger et al. 2004), and have found a significant difference in ADAS-Cog scores between active and placebo groups in caprylidene, previously formulated as Axona® (Accera, Inc., Boulder, CO), with the difference mainly driven by a decline in scores in the placebo group (Henderson et al. 2009). However, the exact neurobiological effects of caprylidene remain to be established.

The primary objective of this study was to evaluate acute and long-term effects of caprylidene on regional cerebral blood flow (rCBF) in mild-to-moderate AD subjects. An additional objective was to examine any differences in effects of caprylidene on rCBF between APOE4-positive and APOE4-negative subjects.

2. Material and methods

2.1. Patients

Sixteen subjects with mild-to-moderate AD were enrolled in a double-blinded, placebo-controlled, randomized, clinical trial to assess neurobiological effects of caprylidene. Eligible subjects were between ages 50–90 who had MMSE scores in the range of 10–28 and a diagnosis of probable AD based on NINCDS-ADRDA criteria (McKhann et al., 1984). The subjects were randomized with 14 subjects receiving treatment with caprylidene, and two subjects receiving placebo, as further detailed below.

Participants underwent four ^{15}O-water PET scans, two scans on the first day (Day 1) and two scans after 45 days of daily caprylidene or placebo consumption (Day 45). On Day 1, each subject was scanned before receiving a 40-gram dose of caprylidene or a matching placebo formulation (Scan 1) and 90 minutes post-dose (Scan 2). On Day 45, subjects were scanned before receiving the last dose of caprylidene or placebo (Scan 3) and 90 min post-dose (Scan 4) (Fig. 1). Acute effects of a single dose of caprylidene on treatment-naïve participants (no prior history of caprylidene use before Day 1) and post-treatment (after 45 days of daily caprylidene supplementation) were tested by comparing Scan 1 versus Scan 2 and Scan 3 versus Scan 4, respectively. Long-term effects of a ketogenic diet were tested after 45 days of daily caprylidene supplementation through comparisons of the pre-dose scans (Scan 1 versus Scan 3) and the post-dose scans (Scan 2 versus Scan 4). Long-term changes in acute responses (Scan 2- Scan 1) versus (Scan 4-Scan 3) were also tested.

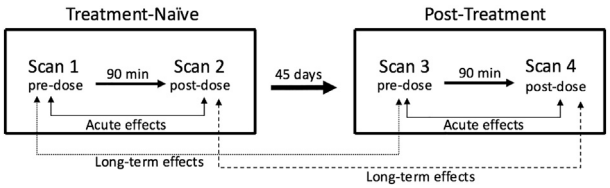

Fig. 1. All subjects underwent 4 PET scans (15O-H2O). Two scans were performed on the first day in treatment-naïve patients (no prior intake of caprylidene), with Scan 2 performed 90 min after a single dose of caprylidene. Two subsequent scans were performed post- treatment (after 45 days of caprylidene supplementation), with Scan 4 performed 90 min after a final dose of caprylidene. Scan 1 versus Scan 2 and Scan 3 versus Scan 4 were used to evaluate for acute changes, while Scan 1 versus Scan 3 and Scan 2 versus Scan 4 were used to evaluate for long-term changes.

Analyses were performed for all participants receiving caprylidene, as well as for subgroups stratified by APOE4 status. For the stratified analysis, subjects who carried at least one copy of APOE4 gene were grouped as APOE4-positive. Data from the placebo arm, comprised of only six scans and two subjects, were not used for any of the analyses reported here; rather, this group played a methodologic role to ensure rigorous double-blinding of participants and investigators, since no subject nor investigator knew before completion of the protocol whether a subject had been randomized by the pharmacy to active or placebo formulation.

2.2. Imaging protocol

PET scans were acquired dynamically, beginning immediately after intravenous administration of 555 MBq [15O] water, using an HR+ Siemens/CTI scanner. The data from the time the tracer bolus reached the brain (approximately 25 s after administration) to 120 s were then summed to derive the images. PET images were reconstructed using filtered back projection and transmission-based attenuation correction obtained with a rotating rod source.

2.3. Data analysis

The images were realigned, normalized and smoothed using the voxel-based statistical parametric mapping program, SPM12. The scans of subjects across all subjects receiving caprylidene were compared using paired *t*-tests voxel by voxel. Five comparisons paired by subject were selected for analysis: Scan 1 vs Scan 2, Scan 3 vs Scan 4, Scan 1 vs Scan 3, Scan 2 vs Scan 4 and (Scan 2 - Scan 1) vs (Scan 4 - Scan 3). These comparisons were also performed for each APOE4-based subgroup. Differences at the cluster level of analysis reaching $p < 0.05$, after statistical correction for multiple comparisons by false discovery rate (FDR) were considered significant. Cluster size was assessed as the number of contiguous voxels with intensity differences having p-value below 0.01 before statistical correction.

The rCBF data were also analyzed by quantifying 47 standardized volumes of interest (sVOI) using the FDA-cleared commercially available brain quantification software package, NeuroQ™ (Syntermed, Inc., Atlanta, GA), as derived by dividing the mean counts per second per pixel in each sVOI by the mean counts per second per pixel across all assessed regions in the brain (n = 240). Mean uptake ratios along with standard deviation of each sVOI in each scan were calculated, and paired *t*-tests were used to evaluate for differences in mean uptake ratios of each sVOI within APOE4-positive and negative subgroups as well as across all subjects undergoing caprylidene therapy. Comparisons identical to those performed by spm (i.e. Scan 1 vs Scan 2, Scan 3 vs Scan 4, Scan 1 vs Scan 3, Scan 2 vs Scan 4 and (Scan 2 - Scan 1) vs (Scan 4 - Scan 3) were performed. Differences with p-value of < 0.001 in each sVOI allowing for statistical correction for multiple comparisons were considered significant.

3. Results

3.1. Subject characteristics

In total, 54 PET scans were acquired from 16 subjects. Among the fourteen subjects receiving active caprylidene treatment, five of eight who were APOE4-positive and five of six who were APOE4-negative completed all four PET scans. The remaining four subjects completed two of the four planned PET scans. All available scans were analyzed in