Preliminary characterization of Apolipoprotein E targeted replacement mice using MRI techniques

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Introduction

The ε4 allele of the apolipoprotein E (apoE) gene is associated with increased risk of Alzheimer's disease (AD). The three common apoE isoforms (ε2, ε3 or ε4) differ by only a single amino acid which has significant effects on the tertiary structure and function of the protein. Even in populations with a low apoE4 allele frequency, gene dosage of apoE4 increases the age-adjusted relative risk for developing AD. In the brain a major role for apoE is to maintain a constant supply of neuronal lipids that is necessary for normal brain function. By utilizing the mice model with targeted replacement (TR) of human apoE alleles (ε2, ε3 or ε4) in place of endogenous mouse apo E, simultaneous measurement of morphology, cerebral blood flow and metabolite concentration were carried out using MRI.

Methods

Twelve male TR homozygous apoE2, apoE3 and apoE4 mice were housed in plastic isolators with organic cellulose bedding (Taconic Farms, Germantown, NY). Animals received food and water *ad lib* and were maintained on a 12 hr/12 hr light/dark cycle, with testing during the light phase. Animal handling and MRI/MRS procedures were conducted under protocols that were approved by our institutional IACUC. MRI was conducted on a BioSpec 4.7T/40 system with an actively-decoupled linear volume coil for transmission and a quadrature surface coil for reception (Bruker BioSpin, Billerica, MA). Animals were anesthetized with isoflurane and the core body temperature was monitored and maintained at 37°C. Structural imaging was accomplished with a T2-weighted 3D RARE sequence covering a field of 16 × 16 × 19.2 mm^3 with 256 x 128 x 64 matrix points (TR = 2600 ms, TE = 100 ms, RARE factor = 16). A PRESS sequence was used to obtain spectroscopy on a 9 mm^3 voxel placed on the hippocampus with VAPOR water suppression and the following parameters: TE = 11 ms, TR = 3 s, 32 averages and 16 repetitions. Single-slice CASL measurement were performed using a 2.0 s continuous RF labeling pulse (60 mG), followed by a RARE image acquisition (TR=2.5 s, TE=8.0 ms, ETL=16). Whole brain and sub-region segmentation was performed by applying an atlas-based segmentation followed by manual correction. ANOVA with SNK pairwise multiple comparisons (SigmaStat, Point Richmond, CA) was used for statistical analysis. LCModel (2) was used for spectral analysis, and CASL images were processed in Matlab.

Results

No significant difference was observed in whole brain volume or any of the 4 segmented regions (hippocampus, ventricles, cortex and the cerebellum) between the 3 lines that were analyzed. Similarly, there was no significant difference in cerebral blood flow measurements amongst the three genotypes in the cortex or hippocampus. However, the apoE4 group shows a trend of lower cerebral blood flow compared to the apoE2 group in the cortex and the hippocampus. Spectroscopy measurements in the hippocampus showed a significant (p=0.005) apoE isoform-dependent increase (apoE4>E3>E2) in the levels of creatine (total) as well as a significant (p=0.02) apoE isoform-dependent decrease (apoE4<E3<E2) in the levels of glutamine. There were no significant changes observed in any of the other metabolites measured.

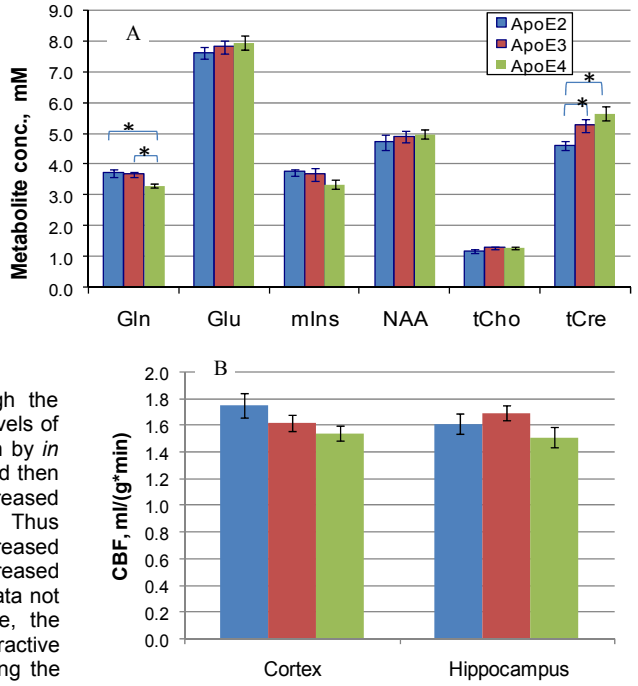

Figure1. A. Changes in metabolites measured by spectroscopy in the hippocampus. B. Cerebral blood flow values in the cortex and hippocampus. Green bars represent the ApoE4, red - ApoE3 and blue denote the ApoE2 group. Data are mean ± S.E.M

Discussion

Creatine, along with phosphocreatine (PCr), acts as an energy buffer through the creatine kinase reaction to maintain a constant supply of ATP. Thus, increased levels of creatine would indicate a decreased brain energy metabolism. It has been shown by *in vivo* ^{31}P studies in humans that PCr levels decrease initially with mild dementia and then increase as dementia worsens (3). Another clinical study has correlated decreased cognitive performance in healthy individuals to increased creatine levels (4). Thus increasing creatine levels is an early indication of declining cognition. The decreased trend of cerebral blood flow (CBF) is also supportive of decreased metabolism. Increased FDG uptake was also observed in the cortex and hippocampus of ApoE4 mice (data not shown), which may also be indicative of hypoxia. The changes in glutamine, the precursor of glutamate may also be indicative of a prologue to the hyperactive glutamatergic system, given the increasing trend of glutamate levels found among the different isoforms. The metabolic change precedes any structural or functional change. This change observed in the relatively young 9-10 month old mice, needs to be confirmed. In addition to behavioral studies, these measurements are being repeated in the same cohort of mice, now 15-16 months old.

References

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