Proton Magnetic Resonance Spectroscopy in Alzheimer Mouse Model

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Introduction

Proton magnetic resonance spectroscopy offers a non-invasive way to quantify many metabolites *in vivo*. Metabolite levels are sensitive to different *in vivo* pathologic processes at the molecular or cellular level; e.g. N-acetylaspartate (NAA) is a marker for neuronal number and health, and myo-inositol (mIns) is thought to be a marker for osmotic stress or astrogliosis. Transgenic mouse models have been developed for Alzheimer's disease (AD). Recently spectroscopic analyses of single transgenic amyloid precursor protein (APP) mice brains showed altered neurochemical profiles[1]. Mice that are doubly transgenic for human APP and presenilin mutations (APP-PS1)[2] develop "human-like" plaques at a younger age than APP mice. The purpose of this study was to characterize the neurochemical profile in APP-PS1 AD mice using high resolution single voxel ^{1}H NMR compared to that of wild-type mice, and to determine the changes in the neurochemical profile with age.

Methods

Twenty seven APP-PS1 and thirty B6SLJ wild type mice spanning the age from 66 to 904 days were used in the study. Some of the mice were scanned twice. Mice were anesthetized using 1.0% - 1.5% isoflurane and O_2/NO_2. Body temperature was maintained at 37°C by warm water circulation, and physiological monitoring was used for temperature, respiration and ECG.

In vivo ^{1}H NMR spectra were obtained using a previously described LASER[3] sequence at 9.4 T (31 cm horizontal bore magnet equipped with Varian INOVA console) from an 18 μL voxel placed in the cortex and hippocampus. Each individual FID was stored separately and then frequency and phase corrected based on the Cr/PCr signal at 3.03 ppm prior to summation. Small residual eddy currents effects were corrected using a reference water signal.

The spectra obtained were analyzed using LCModel which calculates the best fit of the experimental spectrum as a linear combination of model spectra (simulated spectra based on the known chemical shifts and J-couplings of metabolites). The spectra of eighteen metabolites and the experimentally measured spectrum of macromolecules were included in the basis set. The quantification was obtained using the Cr/PCr resonance as an internal standard.

Results and Discussion

A RARE image of a mouse brain with the placement of the voxel and the spectra, both representative of the quality consistently achieved in this study, are shown in Fig. 1. Figs. 1b and 1c show the spectra obtained from the brain of two and thirty month old APP-PS1 mice, respectively. Amyloid plaques are just starting to appear at 2 months of age whereas 30 month old mice have abundant plaques[4]. These spectra reveal an expected decrease in concentration of NAA and Glu relative to Cr/PCr resonance. The most strikingly consistent finding across all animals, however, was a dramatic increase in the concentration of mIns with age in AD mice (filled squares is Fig. 2). In contrast, wild-type animals (open squares in Fig. 2) displayed no change in mIns during the lifetime of the animals. Data averaged from all animals also demonstrated decreases in the concentration of both NAA and Glu (not shown) for both wild-type and APP-PS1 mice across age.

In conclusion, the age dependent neurochemical changes observed in doubly transgenic Alzheimer's mice match expectations based on prior *in vivo* human MRS studies. The most consistent and profound finding observed was an increase in mIns across age in AD. Activated microphages accumulate at the periphery of amyloid plaques both in human AD and in transgenic AD mice. Some believe that microglial activation may be responsible for at least some of the neuronal damage which characterizes AD. In theory, MRS could be used to measure the specific feature of AD pathology longitudinally and could serve as an *in vivo* biomarker of the efficacy of therapeutic measures designed to reduce neuronal damage mediated by microglial activation.

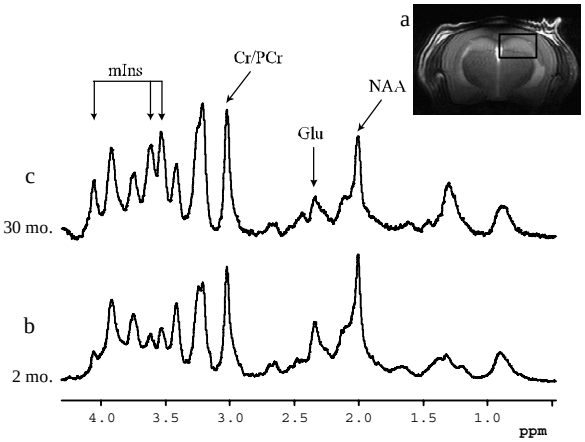

Figure 1. (a) RARE image of mouse brain (TR = 4 s, TE = 15 ms, echo train length = 8, matrix = 256 x 128, slick thickness = 1 mm, eleven slices). ^{1}H spectra obtained with LASER sequence (TR = 3 s, TE = 28 ms, 64 scans) from 18 μL voxel from the brain of (b) 2 month old and (c) 30 month old APP-PS1 mice. The spectra are shown with similar linewidths.
NAA = N-acetylaspartate, Glu = glutamate, mIns = myo-inositol, Cr/PCr = creatine/phosphocreatine,

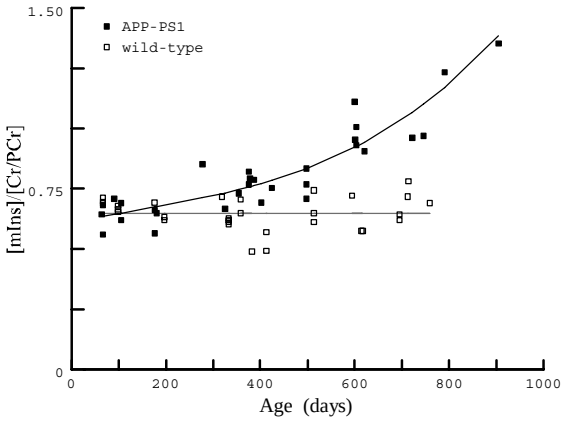

Figure 2. Ratio of concentration of mIns to Cr/PCr versus age of the mice. The bold line is the third order polynomial fitted through the points obtained for APP-PS1 mice, and light-gray line is the linear fit through the points obtained for wild-type mice.

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