

Monitoring Therapy in a Triple Transgene Model of Alzheimer's Disease using MRS, Histology and Behavioral Correlations

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Introduction – Alzheimer's disease is the most common neurodegenerative disorder in the developed world. Treatment with non-steroidal anti-inflammatory drugs has shown some promise in preventing symptoms of AD. MRS has shown promise in evaluation of AD pathology in both humans (1) and mice (2-4). We evaluated the ability of MRS to provide quantitative information on disease pathology and neuroprotection in a triple transgene model of AD (APP_{Swe}, PS1M146V, and tauP301L). We also collected behavioral data using a radial arm water maze and histology related to both A-beta and tau pathology. in the same mice.

Methods – Eight 3xTg-AD mice were treated with tarenflurbil (donated by Myriad Pharmaceuticals) at a dose of 10 mg/kg/day from age five through seven months (orally in food chow). Ten untreated littermates were also used as control as well as six WT animals. After 2 months, at seven months of age, mice went under radial arm water maze testing (RAWM). Mice were euthanized and the brains immediately removed and brain sections were cut in half with one hemi-section fixed for use in immunohistochemical analysis and one frozen to obtain tissue punches for MRS with a diameter of 1mm in amygdala and 2 mm in hippocampus. We used high resolution magic angle spinning (HRMAS) on a Bruker 14T magnet. HRMAS measurements are performed using a sample spinning rate, of 2.5 kHz and a T2-filtered Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence [90-(t-180)_n acquisition]. HRMAS was used because it allows for small punches (1mm diameter) that would be difficult to obtain in vivo or using extracts. This reduces partial volume averaging and allows for better sensitivity to neurochemical deficits. Spectra were collected with Data were analyzed using the Chenomx (Edmonton, Alberta) package using the total chemical shift profile of each molecule.

Results – At seven months of age there were no significant differences between the WT, AD or treated AD for any of the MRS measurements except for an increase in glutamine in the regular diet animals (12 percent increase; $p < 0.01$). Flurbiprofen had a significant positive protective effect on learning in the radial arm water maze. There was a significant correlation between glutamate values and learning on the water maze ($R = 0.7$, $p < 0.01$). We examined a series of pathological markers of A-beta and tau pathology. The only significant effect of treatment was on PHF (paired helical protein) that is a major component of neurofibrillary tangles and is composed of microtubule-associated protein tau in a hyperphosphorylated state. PHF-1 correlated with both glutamine and with learning on the radial arm water maze. These data are shown in Fig. 1 below.

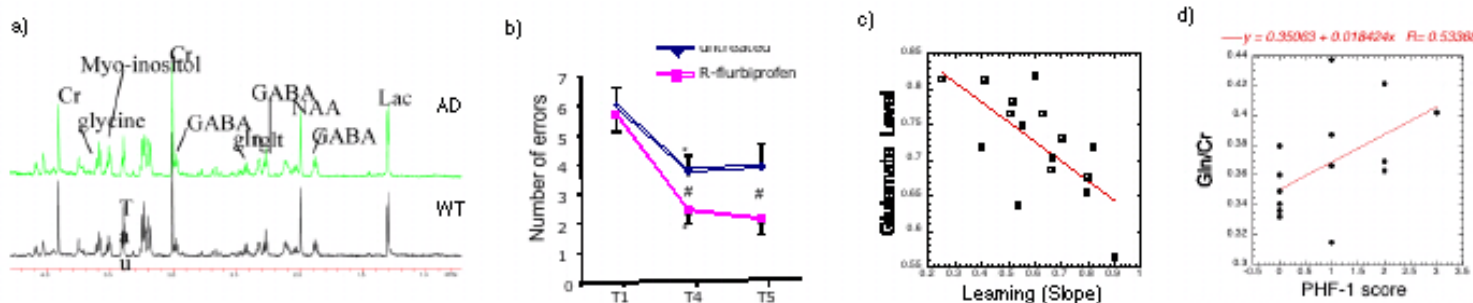


Figure 1 – A) Spectra of AD and WT mice showing the general pattern of metabolite changes noted. Only increased glutamine was significant at this age. B) Effect of flurbiprofen on learning in water maze showing significant effect on trials four and five. C) Inverse correlation between glutamate and learning on the water maze task noted in all animals ($R=0.7$; $p<0.01$). D) Correlation between glutamine and the number of neurons positive for PHF. The correlation was significant ($p<0.05$); there was a significant, but weaker correlation with glutamate and no other metabolites.

Discussion – These results demonstrate that MRS can play a valuable role in evaluation of potential therapeutics in AD. The early changes in glutamine and its relation to neurofibrillary tangles is suggestive of the importance of these changes and of gln being a marker for these changes. Our previous results showed a positive correlation between NAA and plaque burden. The correlation of glutamate with learning across all animals is of great interest as there is evidence of such correlations in human cognitive tasks and MRS measures of glutamate. In vivo studies are currently in progress, as are studies of older mice with more severe pathology.

References – 1) Shonk et al. (1995) *Radiol* **195**:65-72. 2) Dedeoglu et al. (2004) *Brain Res.* **1012**:60-65. 3) von Kienlin et al. (2004) *Neurobiol Dis.* **18**:32-39. 4) Marjanska et al. (2005) *PNAS* **102**:11906-11910.