

Integrated MRS and neuropathological analysis of Alzheimer mouse brain in response to anti-inflammatory treatment

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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 double transgene model of AD (APPxPS1). We also collected data on plaque areas, and A β peptides (1-40 and 1-42) in the same mice subjected to MRS.

Methods – We examined mice at both six months of age and 18 months of age. Mice overexpressing human APP_{Tg2576} were crossbred with homozygous transgenic female mice expressing mutant human presenilin-1 (PS-1_{M146V}) to generate double transgenic mice heterozygous for APP and PS-1 (APPxPS1). Littermates were used as control wild types (WT). Some of the mice were fed standard mouse chow supplemented with either ibuprofen (375ppm) or celecoxib (120 ppm). Data were collected from frontal cortex in the mice with one hemisphere being used for histology/ELISA and the other hemisphere plunged into liquid N₂ then extracted with PCA. Brain extracts were run on Bruker 600 MHz spectrometer with formate as an internal reference for quantification. Spectral Data were analyzed using integration of metabolites spectra. Metabolite levels were compared using ANOVA factor analysis. We further examined the ability of neural network classifiers to group the data based upon different metabolite factors using averaged WT training set and between 80-200k iterations to reach convergence.

Results – At six months of age there were no significant differences between the WT, AD or treated AD for any of the MRS measurements. Among the histological/biochemical measures we found a significant increase in A β measured by ELISA as well as plaque burden. Ibuprofen significantly decreased the A β (42/40) ratio. At eighteen months of age numerous differences were found in the MRS data (n=10 per group). There were significant increases in myo-inositol (22 \pm 6%), scyllo-inositol (29 \pm 10%) and glutamine (23 \pm 5%) as well as significant decreases in NAA (-29 \pm 3%) and glutamate (-11 \pm 3%). Interestingly, the neuroprotective effects of ibuprofen and celecoxib were different. Celecoxib provided significant protection against NAA loss only, whereas ibuprofen did not. Conversely ibuprofen prevented the glutamate decrease but celecoxib did not. Neither drug prevented the increase in myo or scyllo-inositols. Interestingly, both ibuprofen and celecoxib significantly raised alanine levels compared to either normal AD or WT animals. Classification of the metabolite results based upon neural network classifiers showed sorting of the animals into either AD or WT was best achieved using fewer, rather than more, metabolites. We used individual metabolites as well as ratios of myo-inositol/NAA and (myo-inos+gln)/(NAA+glt). The best classifiers were the latter two ratios, both of which were 100% correct for AD and 70% correct for the WT animals. There were significant inverse correlations between glutamate and glutamine and NAA and myo-inositol. The only significant correlation between the MRS data and the histopathological data (excluding the WT animals) was a highly significant correlation between NAA and plaque burden as measured by the percent of the cortex covered in plaques but no correlation with myo-inos (R = 0.17, NS). These data are shown in Fig. 1 below.

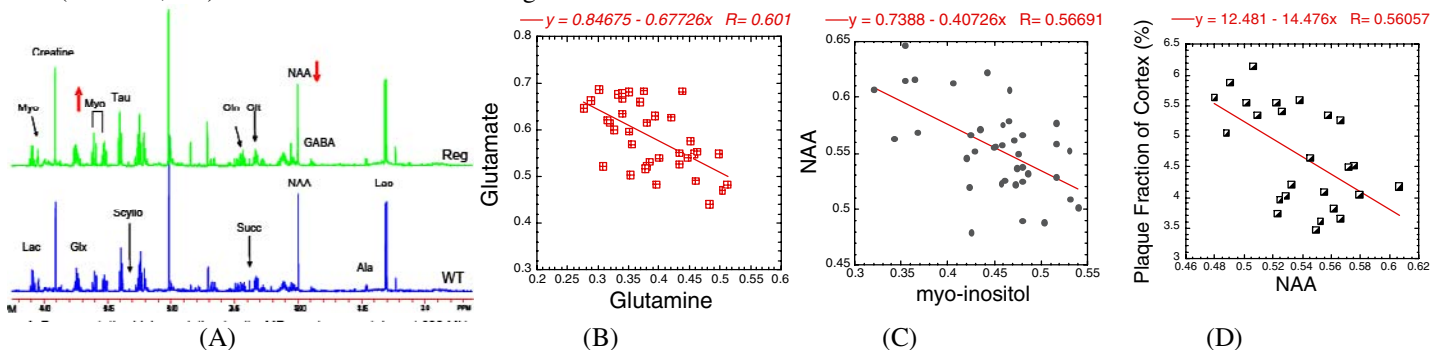


Figure 1 – A: Spectra of AD and WT mice showing the general pattern of metabolite changes noted. B: Inverse correlation between glutamate and glutamine noted in all animals. C: Inverse correlation between NAA and myo-inos. C: Inverse correlation between plaque area (as a fraction of the cortical area) and NAA levels in the AD mice only. All correlations were significant at $p < 0.01$.

Discussion – These results demonstrate that MRS can play a valuable role in evaluation of potential therapeutics in AD. The lack of correlation between MRS and ELISA measurements of A β peptides indicates the limitations of the MRS only approach. The differences between NAA and myo-inositol with respect to plaque burden suggest these two metabolites reflect different processes. In vivo studies are currently in progress.

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.