

In Vitro Proton MRS of Cerebral Metabolites in a Mouse Model of Alzheimer's Disease

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Introduction

Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative disease associated with β -amyloid (A β) neuritic plaques, neurofibrillary tangles and neuronal and synaptic degeneration (Selkoe 2001; Blennow et al. 2006). AD is the leading cause of dementia among the elderly. It is characterised by progressive cognitive decline and memory loss, eventually leading to death. There is currently no definitive biomarker for AD.

Study

In this study the transgenic mouse TASTPM, which overexpresses both a mutant form of the amyloid precursor protein (hAPP695swe) and a mutant presenilin-1 variant (M146V) (Howlett et al. 2004) was investigated. TASTPM mice first show amyloid deposits at 3 months and cognitive impairment is seen from 6 months (Howlett et al. 2004). The aim of this study was to investigate measurable neurometabolic changes occurring over time in the brains of TASTPM mice compared to their base strain (C57/BL6). In order to accomplish this, chloroform-methanol extractions were performed on the brains and ¹H MRS was performed on the resulting extracts. A decrease in N-acetylaspartate (NAA), and increases in inositol and taurine have been observed in *in vivo* studies of different AD transgenic mice (Dedeoglu et al. 2004; Marjanska et al. 2005). A greater number of metabolites can be investigated *in vitro* and other metabolites which are of interest included succinate and glutamate, which are markers of energy usage and neurotransmitter metabolism.

Methods

Chloroform-methanol extractions were performed on frozen whole brains of both strains of mice at 3, 6, 9, 12, 15 and 18 months of age, there were between 5 and 9 mice in each group. ¹H MRS was performed at 25°C with a 9.4T Bruker Avance vertical bore magnet. Fully relaxed, one-dimensional spectra were acquired with water presaturation using a pulse-acquire sequence with the following parameters: repetition time = 6.4s, central frequency = 400MHz, sweep width = 8223.7Hz, number of data points = 32768, dwell time 60.8 μ s, flip angle 30°. Resonance assignments were based on published chemical shifts and coupling patterns of known compounds. Peak areas were integrated using NUTS NMR Utility Transform Software with baseline flattening around each integration region. Water suppressed spectra were used for integration due to the flatter baseline. Deuterated trimethylsilylpropionate was used as an internal standard. 2 way ANOVA was performed on the metabolite levels using Graphpad Prism. To reduce the likelihood of false positives, a significance level of $p < 0.005$ was set.

Results

Significant effects of age alone were identified for creatine ($p < 0.001$), glutamine ($p < 0.0001$) and total choline-containing compounds (the sum of glycerophosphocholine (GPC), phosphocholine and choline levels - $p < 0.0001$). Creatine was fairly stable and at similar levels in both groups until 15 months, where there was a marked increase in the TASTPM mice (Fig.1). Despite this change, there was neither an overall effect nor an interaction of genotype on creatine concentration. A significant effect of genotype alone was only identified for *myo*-inositol, which was generally higher in TASTPM mice at all timepoints. Though the difference appeared greater in the older age-groups there was no significant interaction with, or main effect of, age. Succinate, GPC and choline all showed significant effects of both age and genotype. Succinate concentration fell continuously from a lower starting point in the TASTPM mice, whereas it increased in wild type mice up until a fall at 15 months. Succinate was lower in TASTPM mice at all time points (Fig.1). Choline levels fluctuated over time, but this was much more pronounced in the TASTPM mice, with choline much lower than in the wild type at 6 – 12 months. GPC increased over time in both strains, but was slightly higher in TASTPM mice.

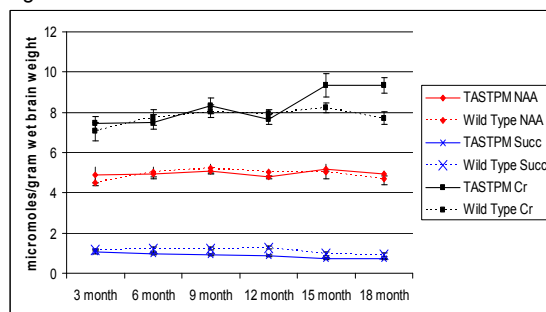


Fig.1 NAA, succinate and creatine levels in TASTPM and wild type mice. Values shown are mean \pm S.E.M.

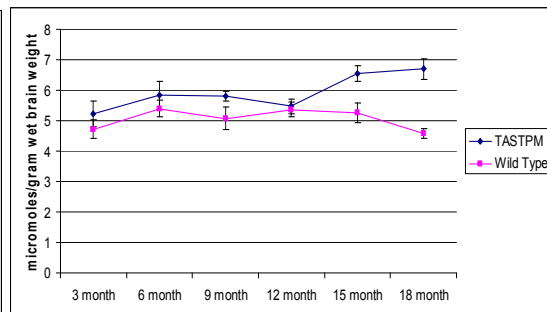


Fig.2 Myo-inositol levels in TASTPM and wild type mice. Values shown are mean \pm S.E.M.

Discussion

The decrease observed in succinate levels could indicate an impairment in neuronal energy production in the TASTPM mice, a similar effect has been seen in human AD sufferers (Drzezga et al. 2003). These metabolic deficits have been found to correlate with disease severity. The hypothesised changes in NAA, and taurine were not seen. The increased creatine signal in older TASTPM mice indicates care should be taken when using it as a reference metabolite *in vivo*. Had it been used in this case, a significant decrease in NAA levels would have been observed in older TASTPM mice. Myo-inositol showing a genotype only effect, with no interaction, could suggest that this is a marker of AD, without being related to disease progression.

- Blennow K. et al. (2004) *Brain Res* **1012**, 60-65.
Drzezga A. et al (2003) *Eur J Nucl Med Mol Imaging* **30**, 1104-1113.
Howlett D. R. et al (2004) *Brain Res* **1017**, 130-136.
Marjanska M. et al (2005) *Proc Natl Acad Sci U S A* **102**, 11906-11910.
Selkoe D. J. (2001) *Physiol Rev* **81**, 741-766.