Metabolic Profiling to Characterise Brain Tissues from a New Animal Model of Neurodegeneration with Lewy Body Pathology

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INTRODUCTION

Background: A variety of animal models of neurodegenerative diseases have been developed to investigate different aspects of neuronal loss in a controlled environment. The cellular mechanisms leading to neurodegeneration are largely unknown. To characterise the underlying metabolic alterations that may lead to or result from neurodegeneration, nuclear magnetic resonance (NMR)-based metabolic profiling techniques have recently been applied to mammalian brain tissue[e.g.1,2,3]. This approach affords the characterisation of a large collection of small molecules in a biological system, such as concentration alterations of metabolites in a (patho)physiological context[4] or indeed the identification of new molecules as potential biomarkers. The potential of this method is that it can (and could in the research cited above) (a) confirm previously suggested metabolite changes, such as a concentration decrease of the neuronal marker N-acetyl-aspartate (NAA) in neurodegeneration[5], and (b), perhaps more importantly, highlight metabolic perturbations which may have been overlooked in targeted analyses[6].
Recently, a unique model of neurodegeneration has been developed, based on the functional reduction of the ubiquitin system activity by selective depletion of the 26S proteasome: in neurons in the mouse brain, this causes accumulation of ubiquitylated proteins in affected neurons, leading to early neuropathological changes, such as pyknotic nuclei.
Aim: Using four-week old, 26S proteasome depleted mice and applying nuclear magnetic resonance (NMR) spectroscopy-based metabolic profiling techniques, the present pilot study aimed to evaluate the ability to characterise metabolic changes that may underlie early neurodegenerative processes.

EXPERIMENTAL

Animal Tissue: Cortex and hippocampus tissues were collected from decapitated four-month old, 26S proteasome depleted (Psmc1fl/fl;CaMKIIα–Cre[7]) and control mice (n=3 per group) and extracted with chloroform and methanol[8]. **NMR:** Spectra of the aqueous extract (Fig. 1) were recorded with a Bruker Advance system at 300K: 500 MHz, NOESY pulse sequence with water irradiation; TD=32k, NS=512, RD=2s, aqt=2.55s. **Evaluation:** All spectra were processed using Bruker Topspin 2.1 and bucketed with Amix 3.9.1. Amix was also used to quantify specific resonances (2.5 times higher than noise) by addition of data points in the selected area. All data were normalised to the total spectral area. Bucketed data were mean-centred and principal component analysis (PCA) was performed using the PLS toolbox 5.2.2 in Matlab 7.8.0. In Matlab an unpaired t-test was also conducted for univariate group comparison of specific metabolites.

RESULTS

PCA demonstrated distinct metabolic profiles according to brain area and genetic status (Fig. 3). Brain area accounted for the greatest variation in the data. The PCA loadings indicated that NAA, taurine, aspartate and glutamate were more abundant, whereas lactate and glycine concentrations were lower in the cortex than in the hippocampus. Given the strong metabolic influence of the brain area, differences due to genetic modification were evaluated for both regions separately. In the cortex concentrations of NAA* (Fig.2), taurine* and creatine were lower, and those of total choline-containing compounds* and lactate were higher in the mutants compared to controls. Similarly, in the hippocampus, NAA[a], taurine, creatine* and γ-amino-butyric acid were less abundant, and total-choline containing compounds*, lactate and acetate levels were elevated in the genetically modified mice. [* indicates statistical significance in the t-test, defined as $p<0.05$; [a] indicates a trend to significance in the t-test, defined as $p<0.1$.]

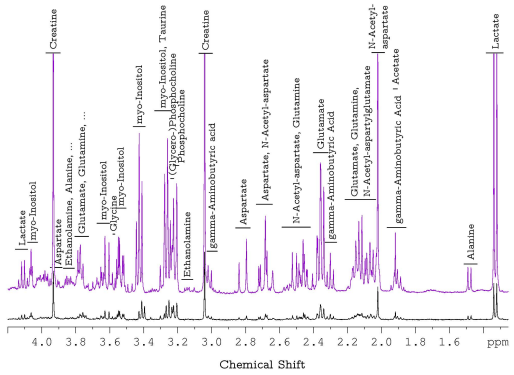

Fig. 1. Assigned representative up-field NMR spectra of a control mouse cortex (above; purple) and hippocampus (below, black) recorded at 400 MHz and referenced to trimethylsilylpropionic acid (TSP, δ0.00 ppm).

DISCUSSION and CONCLUSION

The analysed brain tissues were taken from animals at an age when pyknotic nuclei but no extensive neuronal loss had yet occurred which models early stages of dementia with Lewy bodies[7]. In the cortex, the area that was primarily targeted in this animal model of neurodegeneration, discrimination between the two genotypes was more pronounced than in the hippocampus (Fig. 3). Against the strong *in vivo* and *in vitro* evidence for NAA loss to indicate neuronal loss as a major hallmark of neurodegeneration[9], the observed strong NAA decrease in mutant tissue (Fig. 2) can be seen as proof of principle demonstrating that metabolic tissue profiling allows assessing the extent of impaired neuronal integrity[5]. An increase in choline-containing compounds, as found here, had been associated with cell membrane remodelling[10] and has even been considered as non-specific neuronal marker[11]. Taurine levels were decreased in both brain areas in mutant tissue which interestingly had previously been found in a neurotoxic animal model of Parkinson's disease[1]. In contrast, animal models of Alzheimer's and Huntington's disease demonstrated elevated taurine levels which had been attributed to gliosis reactive to neuronal loss[5,12]. This points to a significant difference between the animal models and possibly diseases as in this study myo-inositol changes – a sensitive glial cell marker[1] – and was not observed. Moreover, gliosis was not shown to be present at four weeks in this animal model[7].

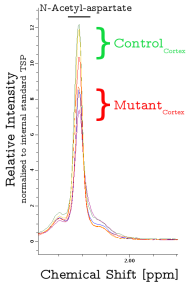

Fig. 2. Detail of NMR spectra (δ2.02 ppm; assigned to NAA) obtained from mouse cortices: signals differed with genotype.

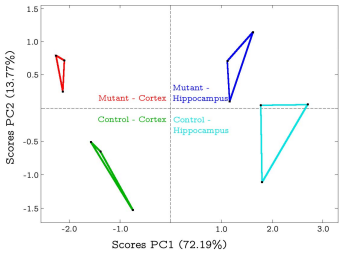

Fig. 3. PCA scores plot demonstrating clear metabolic differences due to brain area (along PC1) and genetic modification (along PC2).

The results presented here demonstrate clearly the ability of NMR-based metabolic profiling techniques to aid in the characterisation of early neurodegeneration. Next, we plan to characterise the dynamic changes in more detail as basis for *in vivo* intervention studies. Tissue-based metabolic profiling studies are a useful adjunct to *in vivo* spectroscopic studies for its higher sensitivity, easier access and to inform *in vivo* study designs regarding appropriate regions and time points.

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