Metabolic profiling of *post mortem* multiple sclerosis brain using high resolution ^{1}H NMR spectroscopy

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Introduction

MS is an inflammatory demyelinating disease of the central nervous system (CNS). MR spectroscopy (MRS) is being used to probe MS (1). Changes in the metabolic composition of the CNS of patients with MS include reduced levels of N-acetyl-aspartate (NAA) (1,2) and increased levels of myo-Inositol (Ins) (3,4). The pathological substrates of these changes are less clear (5). Whereas NAA has been mainly implicated as a marker of neuronal integrity (6), Ins may be related to glial activation and gliosis (7). In order to quantify the pathological changes associated with specific metabolite levels we applied high-field MRS and quantitative histology to post mortem brain from patients with MS.

Methods

Fresh *post mortem* MS brain slices of five women and four men (mean age 53 years; SD 9) who had MS for 31(SD 6) years were provided by the UK Multiple Sclerosis Tissue Bank. Samples weighing 74mg (SD 106) were obtained 40 hours (SD 8.5) post mortem from normal appearing white matter (NAWM) regions and from white matter lesions (WML) and snap frozen. The remainder of the tissue was fixed in 10% formalin. NMR: Dual phase chloroform-methanol-water extractions (8) were carried out. After extraction the aqueous fractions were freeze dried and prepared for NMR with the addition of 800 µl D_2O and 25 µl sodium trimethylsilylproprionate solution (1 mg/ml in D_2O). The samples were adjusted to pH 7. ^{1}H NMR spectra of the aqueous extracts were acquired on a Varian Unity Plus spectrometer (Varian Inc, Palo Alto, USA) operating at 500 MHz for ^{1}H. Spectra were collected using a sweep width of 6 kHz using 32k data points and 256 averages. The acquisition time for each spectrum was 2.73 s with a 45° pulse and a total relaxation delay of 5.23 s. The residual water resonance was attenuated using a gated irradiation pulse. Resonance assignments were determined using standards of the respective compound and published chemical shift values, (Figure). Pathology: From the regions where samples had been obtained for MRS tissue blocks were dissected and processed for embedding in paraffin. Sections were stained for Hematoxylin & Eosin, Luxol-Fast Blue (LFB) and glial fibrillary acidic protein (GFAP). Lesions were classified as either chronic active (CA) or inactive (CI). Transmittance (Tr) was obtained from LFB- and GFAP-stained slides to quantify myelin (Tr_{myelin}) and gliosis ($Tr_{gliosis}$). T-tests of patient means (averaged across tissue sample) and linear regression were used for analysis. Metabolite concentrations are given in nmol/g.

Results

The Table shows the concentrations in NAWM and WML of a range of metabolites. Significant differences were detected between NAWM and WML for Ins, Glycine and for $Tr_{gliosis}$. Trends were detected for an association of NAA, alanine and glutamine with myelin content and for glycine with gliosis. Histological data were available of NAWM in seven and of WML in three cases (nine lesions, four/9 CA and five/9 CI). No correlation was detected between myelin content and the severity gliosis (r= 0.491, p=0.2639).

Table

	NAWM	SD	lesions	SD	p
Lactate	14.8	7.3	4.2	8.4	0.349
Myo-Inositol	7.2	6.3	2.6	1.7	0.049
Creatine	5.4	4.9	0.9	0.5	0.136
Glycine	3.1	2.6	1.5	1.0	0.030
Aspartate	3.3	2.5	0.9	0.6	0.139
Glutamine	3.4	2.7	0.8	0.6	0.121
Glutamate	6.5	8.0	2.2	1.5	0.302
NAA	1.8	2.3	0.5	0.8	0.147
Alanine	2.3	2.2	1.0	0.5	0.111

Conclusion

Our preliminary data suggest that high-field MRS is a useful tool to identify metabolic changes due to lesions in post mortem MS brain. The combined use of high-field MRS and quantitative histology allows the accurate assessment of the pathology underlying these metabolic changes. The reduction of Ins in lesions compared to NAWM may be due to a higher number of glial cells in NAWM, hence leading to a larger contribution to the total Ins concentration. However, more cases need to be investigated to verify this finding. Further analysis will include quantification of glial cells and axons in NAWM and WML.

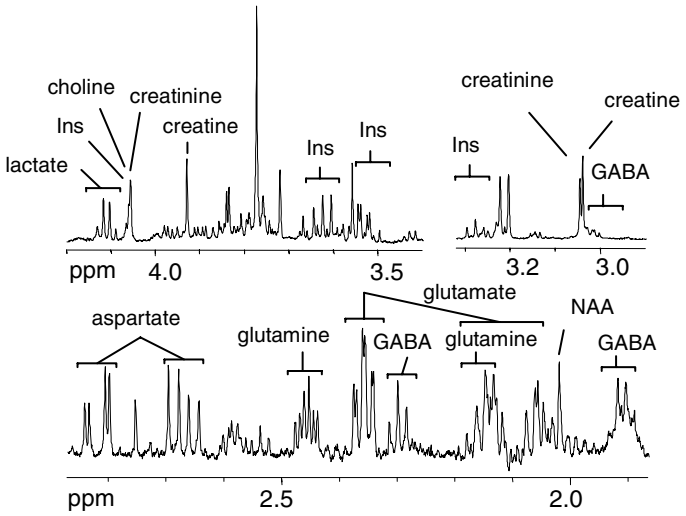

Figure Typical 500 MHz ^{1}H NMR spectrum of an aqueous extract of brain tissue. A number of peak assignments are displayed. Myo-inositol, Ins.

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