High resolution ^{1}H NMR spectroscopic based metabolomic urine analysis of EAE, an experimental murine model of multiple sclerosis

H. G. Parkes[1], S. Romero Shorter[2], P-W. So[3], D. Baker[4], G. Giovannoni[4], G. Pryce[4], and K. Schmierer[1]

[1]Department of Neuroinflammation, Institute of Neurology, University College London, London, United Kingdom, [2]Department of Chemistry, Birkbeck College, London, United Kingdom, [3]Biological Imaging Centre, Imaging Sciences Department, MRC Clinical Sciences Centre, Hammersmith Hospital, Imperial College London, London, United Kingdom, [4]Institute of Cell and Molecular Science, Queen Mary University of London, London, United Kingdom

Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS). Diagnosis of MS is made on the basis of symptoms and assisted by clinical investigations, including MRI, analysis of cerebrospinal fluid (CSF) and evoked potentials (EP). However, MRI is expensive and in high demand; CSF requires invasive lumbar sampling and EP is non-specific and so, such a battery of test is often inconclusive. In addition, MRI findings only correlate modestly with disability. Thus, alternative methods of monitoring disease progression and effects of therapeutic treatments are required. Metabolomic analysis of ^{1}H NMR spectra of urine has been used to assess the onset, progression and recovery processes during induction of toxicity by compounds [Holmes *et al*., 1992]. In this study, we have used such methods to study urine samples obtained from a murine model of experimental autoimmune encephalitis (EAE). EAE exhibits a similar disease progression to clinical MS, involving acute, chronic, relapse and remission phases.

Methods and Materials

EAE was induced in female Biozzi ABH mice by injection of complete Freund's adjuvant containing freeze-dried homogenate (1mg/animal, 150µl) into two sites on the flank at days 0 and 7. Animals were kept in plastic cages and fed *ad libitum* on a standard mouse diet. Control urines and urines (9 – 220µl) collected from animals with acute, chronic, remission and relapse pathological types of EAE were freeze-dried and then reconstituted in 800µl D_2O containing TSP (10µl, 1mg/ml) and placed in 5mm NMR tubes. ^{1}H NMR spectra were acquired on a Varian Unity Plus 500MHz NMR spectrometer and 32k data points collected over a 6kHz spectral width, a 45^o pulse width and 256-1024 averages. The residual water resonance was attenuated by a gated irradiation at the appropriate frequency. NMR spectra were processed using MestreC software (Santiago de Compostela University, Spain) and resonances assigned with the aid of Chenomx NMR Suite 4.6 (Chenomx Inc, Edmonton, Canada). Specific metabolite concentrations were obtained by integration of selected spectral peaks and comparing intensities with TSP: significant differences between levels of metabolites in different groups were assessed using ANOVA and post-Dunnetts testing. For metabolomics, spectra were bucketed (δ0.04ppm widths) and principal components analysis (PCA) carried out using SIMCA (Umetrics Ltd, Windsor, UK).

Figure 1. Typical 500MHz ^{1}H NMR spectrum of control mouse urine. (TMAO, trimethylamine-N-oxide)

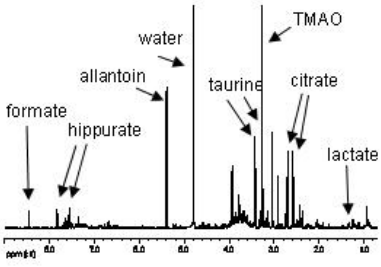
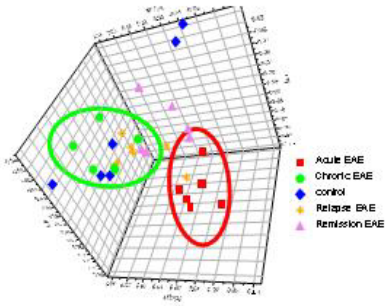


Figure 2. Three-dimensional PCA plot showing clear discrimination between acute (red) and chronic (green) EAE groups.



Results and Discussions

Various metabolites were detected by ^{1}H NMR spectroscopy (Figure 1). By metabolomic analysis, a significant separation was observed between the acute EAE group and both the chronic EAE and control groups (Figure 2). Some separation was observed between the control and chronic EAE groups but to a much lesser extent. Discrimination of the remission and relapse EAE groups from the other groups were unclear in the PCA plot. However, the PCA plot does suggest possible differences between the individual groups. Indeed, subsequent metabolite quantification showed hippurate was significantly reduced in the urines of all EAE animals ($P<0.001$); both citrate and TMAO were decreased in acute, remission and relapse EAE ($P<0.01$); allantoin was decreased in both remission and relapse EAE ($P<0.01$) but taurine was only decreased in remission ($P<0.01$) and creatine was only significantly decreased in acute EAE ($P<0.001$).

By using multivariate (parametric and non-parametric) testing the multidimensionality of the data set can be reduced to a single point based on its two or more principal components, allowing ready comparison of large data sets. Indeed, the acute and chronic EAE groups mapped significantly differently in the PCA plot although discrimination between other groups were not readily observed in the three dimensional PCA plot. Quantification of certain metabolites suggested significant differences between urines from control and EAE, and even between different pathological types of EAE such that certain metabolites may act as biomarkers for particular types of EAE.

Conclusions

In conclusion, ^{1}H NMR spectroscopy may be able to generate biomarkers of the different pathologies of EAE which may be translated to the clinical MS arena. Thus, its ready addition to the battery of tests for MS may lead to increased sensitivity and specificity for the diagnosis and classification of different pathologies of MS.

References

Holmes *et al*., (1992), NMR Biomed., 5(6): 368-72.

Acknowledgements

The authors would like to thank Prof. David Gadian (UCL) for access to NMR spectroscopy facilities.