High Resolution ^{1}H NMR Analysis of Cerebrospinal Fluid: Applications to Diseases Affecting the Brain

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Introduction

^{1}H NMR spectroscopy has been used extensively for the analysis of biofluids, including urine and plasma, in drug metabolism and toxicology studies.[1] Other biofluids, eg, synovial fluid, bile, digestive juices and cerebrospinal fluid (CSF) have been investigated to a much lesser extent. This is in part due to the more invasive nature of sample collection for these biofluids. CSF is in intimate contact with brain tissue and it would be expected to reflect the various metabolic changes that take place as a result of neurological disease. In this study, CSF samples from three patient groups, namely those with stroke, multiple sclerosis (MS) or cerebral malaria (CM) were examined using high resolution ^{1}H NMR spectroscopy.

Aims

CSF samples from the three named patient groups were analysed using NMR spectroscopy to answer the following questions: 1) To what extent do the NMR spectra of CSF vary between and within the patient groups? And, 2) what biochemical information can be obtained about these disease states?

Experimental

CSF (n=10 for each group) was collected by lumbar puncture for normal clinical analysis. CSF samples were frozen and stored at –20°C prior to NMR analysis. Whole CSF (400µl) was added to TSP/D_2O solution (350µl). The TSP/D_2O solution was used as a frequency lock, chemical shift reference and quantification standard. ^{1}H NMR spectra were obtained on a Varian Unity Plus NMR spectrometer (Varian Associates, Palo Alto, Ca) operating at 11.74T (500 MHz for protons). Spectra were obtained at 25°C (using 256 scans in 32k data points). The water resonance was attenuated by presaturation. Resonance peak areas for quantification were determined using the software SpecNMR (JEOL UK Ltd).

Results and Discussion

An advantage of using NMR spectroscopy to analyse biofluids is that no pre-selection of the metabolites to be studied is required.

The figure shows representative ^{1}H NMR spectra of CSF, from patients with stroke, MS and CM. The spectra from the three patient groups are significantly different from each showing that they possess different metabolic information. For example, CM shows the presence of the ketone bodies β-hydroxybutyrate ($P<0.005$) and acetoacetate ($P<0.0001$) despite normal levels of glucose.

In the case with stroke, high levels of a range of amino acids, such as threonine, valine and isoleucine can readily be observed. Also, elevated amounts of GABA are detected which may arise from cell damage. Stroke CSF also shows the presence of large amounts (up to 60 mmol/l) of lactate in some patients probably as a result of the induction of glycolysis after the ischaemic insult. With MS we observe generally lower levels of metabolites. We also observe the presence of choline-containing compounds, which may be produced as a result of myelin degradation, a characteristic of MS.

Conclusions

We have shown that the ^{1}H NMR spectra of CSF from stroke, MS and CM patients are indeed significantly different from each other. The metabolites observed are thus a function of brain pathology.

Acknowledgements

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Reference

1 Bell JD, Preece NE, Parkes HG, In NMR in Physiology and Medicine, Editor R.J. Gillies, Academic Press, (1994), Chapter 14, 221-236.

500 MHz ^{1}H NMR partial spectra of cerebrospinal fluid from patients with stroke, multiple sclerosis (MS) and cerebral malaria (CM)