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Investigation of the mouse cerebellum using STIM and μ-PIXE spectrometric and FTIR spectroscopic mapping and imaging

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ABSTRACT

The cerebral biochemistry associated with the development of many neurological diseases remains poorly understood. In particular, incomplete understanding of the mechanisms through which vascular inflammation manifests in tissue damage and altered brain function is a significant hindrance to the development of improved patient therapies. To this extent, a combination of spectrometric/spectroscopic mapping/imaging methods with an inherent ability to provide a wealth of biochemical and physical information have been investigated to understand further the pathogenesis of brain disease.

In this study, proton-induced X-ray emission (PIXE) mapping was combined with scanning transmission ion microscopy (STIM) mapping and Fourier-transform infrared (FTIR) imaging of the same tissue sample to study directly the composition of the murine (mouse) cerebellum. The combination of the elemental, density and molecular information provided by these techniques enabled differentiation between four specific tissue types within the murine cerebellum (grey matter, white matter, molecular layer and micro blood vessels). The results presented are complementary, multi-technique measurements of the same tissue sample. They show elemental, density and molecular differences among the different tissue types.

1. Introduction

Investigation of biochemical changes, as well as the distribution of trace elements within diseased tissues, can provide significant new insights into the biochemical mechanisms of disease pathogenesis and, hence, may lead to new treatment strategies. Traditionally, histological methods, such as haematoxylin and eosin staining (H&E), followed by optical microscopy have been used to visualise changes in tissue structure. However, while optical microscopy methods are well-suited to an examination of disease-induced morphological changes within a tissue, they often do not provide insight into the complex biochemical alterations that occur during disease pathogenesis. Further, alterations in the biochemical composition of a tissue often occur prior to morphological manifestation. Therefore, mapping/imaging methods that provide complementary chemical, elemental and density information are sought.

The nuclear microprobe is an analytical instrument that can be used with a number of different analytical techniques for both quantitative elemental analysis and imaging, such as μ-PIXE (proton induced X-ray emission) and STIM (scanning transmission ion microscopy) studies [1,2]. Increasingly the nuclear microprobe has been recognised as an analytical tool for the analysis of tissue sections, in both environmental [3,4] and biomedical research [5,6]. The strength of μ-PIXE is its high sensitivity for essential metals in biological tissues, such as Fe, Zn and Cu. In a way that is complementary to μ-PIXE mapping, STIM studies provide physical information of the tissue density. A combination of μ-PIXE and STIM with a molecular imaging technique, such as FTIR (Fourier-transform infrared) spectroscopy, allows the biochemical, elemental and density composition of biological samples to be probed.

In this study, the combination of Fourier-transform infrared (FTIR) micro-spectroscopy, proton-induced X-ray emission (PIXE) spectrometry, and scanning transmission ion microscopy were investigated as a new method to study further the biochemistry associated with brain pathology. We present initial findings using the above techniques to identify and characterise the density and biochemical/elemental composition of physiologically distinct

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regions (grey matter, white matter, molecular layer and blood micro-vessels) within the brain cerebellum.

2. Experimental

Tissue sections were cut from mouse brains obtained from a larger study investigating the effects of cerebral malaria on cerebral metabolism, which had been approved by The University of Sydney Animal Ethics Committee. Before sectioning, the blood was drained from the head via retro-orbital bleeding, so that chemical alterations observed in the study directly related to the chemical composition of the brain tissue.

The mouse brain was obtained via rapid dissection, immediately embedded in optimal cutting temperature (OCT) medium, snap-frozen in a hexane slurry cooled with liquid nitrogen and stored at −80 °C. Thin sections (10-μm-thick) were cut using a cryotome at −14 °C and the sections were placed onto Si_3N_4 windows (Silson Ltd., Northampton, England). In order to avoid alteration of the elemental concentrations, no further fixation techniques were used on the samples. Prior to FTIR spectrometric analysis, the tissue sections were air-dried for a period of 2 min. Following FTIR spectroscopic analysis the sections were freeze-dried. Spectrometric and spectroscopic measurements (FTIR, PIXE and STIM) were carried out on the same tissue section in the order listed below. By allowing the sample to air dry during the FTIR analysis, some redistribution of elements is possible. However, comparing samples prepared in the manner described above with samples that were freeze dried immediately showed very limited or no redistribution between the specific tissue layers.

2.1. Fourier-Transform Infrared (FTIR) microscopy

For comparison with STIM, FTIR spectra were collected using a Bruker Vertex 80v FTIR spectrometer, which was coupled to a Hyperion 3000 microscope. The microscope was equipped with a liquid nitrogen cooled 64 × 64 pixel focal plane array (FPA) detector. Spectra were acquired with the co-addition of 512 scans at 4 cm^{-1} over the range 3600–900 cm^{-1}. Images were collected using a ×15 microscope objective and the sampling area was 170 × 170 μm (4096 spectra). A background image (4096 spectra) was collected from a blank Si_3N_4 window before the collection of each sample image.

2.2. PIXE-Spectrometric analyses

The μ-PIXE analyses were performed using the ANSTO high-energy heavy-ion microprobe (HIMP) [7]. Analysis was performed with a 3 MeV proton beam and a spot size of between 3–5 μm. X-ray fluorescence spectra were collected using a high purity Ge detector with an active area of 100 mm^2. In order to reduce low energy X-rays and to prevent scattered protons from entering the detector, a 100-μm Mylar foil was placed in front of the detector.

The μ-PIXE data were analysed using GeoPIXE software [8,9] and elemental maps were extracted from the data. These elemental maps were used to calculate average elemental concentrations in the different tissue types by integration over a larger region.

2.3. Scanning transmission ion microscopy (STIM) analyses

STIM analyses were performed using a 3 MeV He beam, as well as 5.9 MeV Be beam, with spot sizes less than 1 μm. With this technique density or structural variation was measured in the brain sections. Beside 3 MeV He, the heavier beam of Be was used on some samples to improve the contrast between different tissue regions. The transmitted ions were measured with a surface barrier detector at 0° [5], which enabled very high resolution imaging of the samples, with approximately 1 μm resolution.

3. Results and discussion

While studies on a single section are reported here, the results are typical of a much bigger sample set of different sections from the same or different control mice. Fig. 1 shows an optical micrograph, typical of a tissue section from a control mouse brain, mounted on a Si_3N_4 window. In the micrograph, the different tissue layers within the cerebellum (grey matter, white matter and molecular layer) are clearly distinguished. As indicated in the Fig. 1, the layers are distinguished both by their different colour intensity and internal morphology. Fig. 2 displays the optical image

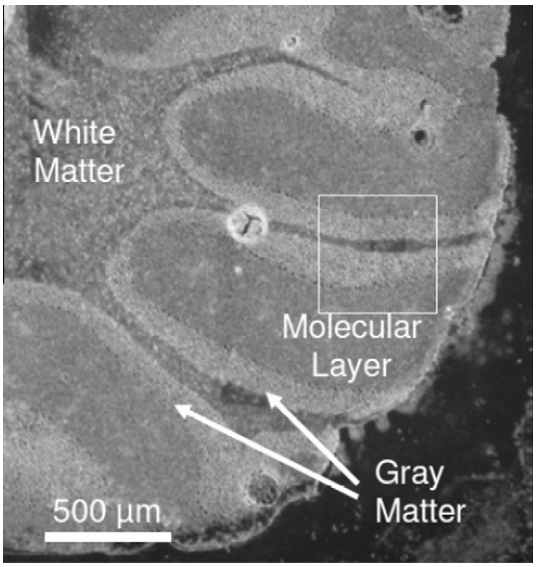

Fig. 1. Optical micrograph of section of mouse brain cerebellum. The image clearly shows individual lobes of the brain cerebellum, as well as different tissues such as the molecular layer (ML), white matter (WM) and grey matter (GM).

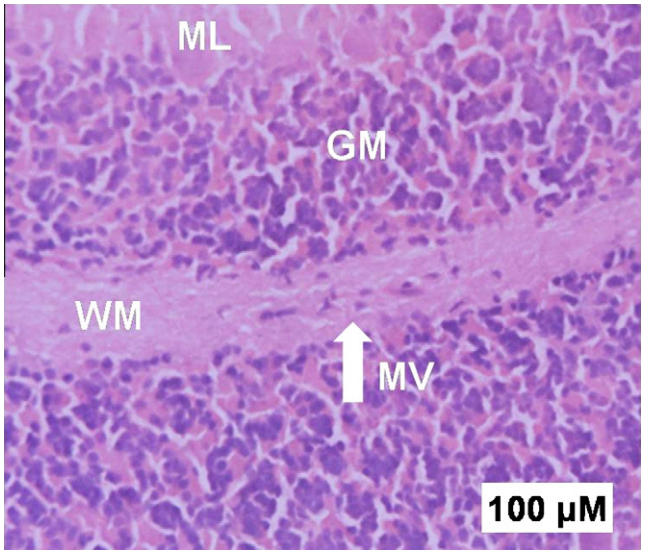

Fig. 2. Micrograph (x40 objective) of brain section taken from the adjacent section to that presented in Fig. 1. The section was stained (H&E) to identify the specific tissue layers (WM = white matter, GM = grey matter, ML = molecular layer, MV = blood micro-vessel).