Investigating the Biochemical Mechanisms of Cerebral Malaria Using SR-FTIR Spectroscopic Mapping

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Cerebral malaria (CM), a devastating disease, kills greater than two million people each year in sub-Saharan Africa and parts of South East Asia (1). It is a complicated manifestation of *P. falciparum* infection, for which the biochemical mechanisms remain unresolved.

In this investigation, SR-FTIR mapping has been used in conjunction with a murine model for human CM to investigate disease-induced alterations to the biochemistry of the central nervous system (CNS). The observed alterations are manifestations of CM pathogenesis and consequently yield new information and significant insights into the biochemical mechanisms behind the disease.

Preliminary studies indicated that alterations in the biochemical profile of the three layers of the cerebellum (molecular, granular and inner white matter) allowed discrimination between healthy mice and those diseased with cerebral malaria (2,3). The aim of the experiment discussed here, was to examine a much larger area of the cerebellum of healthy and diseased mice to validate previous findings.

Experimental: Mice (n = 2) suffering from cerebral malaria, (induced by infection with *P. berghei ANKA*), were compared against healthy controls (n = 2). Brains from sacrificed mice were snap frozen in hexane at −70 °C and embedded in optimal cutting temperature (OCT) tissue embedding medium. Sagittal cross sections were obtained via cryosection at –20 °C and were fixed in 10% w/v formaldehyde onto infrared reflective slides (Kevley Technologies, Chesterland OH, USA). SR-FTIR maps were collected across representative areas of the cerebellum (~250 × 60 μm). Spectra were collected in the spectra range 4000–700 cm^{-1}, at a resolution of 4 cm^{-1}, with the co–addition of 32 scans. An aperture of 5 × 5 μm^2 and a stepsize of 20 μm was used at all times.

Results and Discussion: There are two spectral regions containing bands that are attributed to lipids. The C–H stretching region 3200–2800 cm^{-1}, is a complex region composed of a number of overlapping bands that arise from the various C–H groups present in both lipids and proteins. The second region contains an often weakly absorbing doublet at 1720 and 1740 cm^{-1}. These bands are assigned to lipid ester carbonyl stretches ($\nu(C=O)_{ester}$).

The lipid distribution within the various layers of the cerebellum tissue was investigated by producing functional group maps based on the ν(C–H) and the $\nu(C=O)_{ester}$ regions. The maps highlighted an increase in the lipid content within the inner layer of the cerebellum in mice suffering cerebral malaria, and supports previous findings (2,3).

A shift in the position of the two ν(C=O) bands (~1740 and 1720 cm^{-1}), and differences between the ratio of these bands were observed from the spectra collected within the inner layer of the cerebellum of diseased mice. These spectral changes are proposed to be due to an altered biochemical environment within the cerebellum of the malaria infected mice, such as lipid oxidation.

The amide I (1700–1600 cm^{-1}) and amide II (1600–1500 cm^{-1}) bands were used to create functional group maps, illustrating the distribution of proteins throughout the tissue layers of the cerebellum. An increase in the ratio of α-helix to β–sheet proteins within the granular layer of the cerebellum in diseased mice appear evident. The results identify alterations in the lipid and protein content of the inner and granular layers of the cerebellum in mice diseased with cerebral malaria. Further data analysis is being undertaken to study the nature of these changes and the possible biological mechanisms behind them.

Acknowledgements: We are grateful for financial support for this work that was provided by Australian Research Council (ARC) Discovery grants, the Australian Synchrotron Research Program (ASRP) grant for the access to NSRRC. The ASRP is funded by the Commonwealth of Australia under the Major National Research Facilities Program.

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