MRI Reveals BBB Breakdown and Alterations in Hippocampal T2 in Murine Cerebral Malaria

D. J. Stuckey[1], D. C. Anthony[2], I. M. Medana[3], J. P. Lowe[1], P. Styles[1], A. M. Blamire[1], N. R. Sibson[1]

[1]Experimental Neuroimaging Group, Department of Biochemistry, University Of Oxford, Oxford, United Kingdom, [2]Molecular Neuropathology Laboratory, School of Biological Sciences, University of Southampton, Southampton, United Kingdom, [3]Nuffield Department of Clinical Laboratory Sciences, Oxford-Wellcome Centre for Tropical Medicine, John Radcliffe Hospital, Oxford, United Kingdom

Introduction

Over one million people die from cerebral malaria (CM) every year[1]. Although CM is a serious health problem, the exact cause of death is not well understood, but it is believed to involve an inflammatory component[2] and sequestration of parasitized red blood cells (PRBCs) in the cerebral vasculature[3]. The majority of CM cases arise in developing countries where access to MR scanners is limited; hence little information about human CM has been gained by MR. A murine model of CM has been extensively studied. *Plasmodium berghei* ANKA infection of CBA mice results in CM-like symptoms seven days after infection and is fatal in 95% of cases. In this study we have used MRI to investigate the murine model of CM *in vivo* for the first time. This work represents a new and exciting approach to studying this devastating disease.

Methods

Two groups of animals were studied, (a) 6-8 week old male CBA mice injected i.p. with 1×10^6 PRBCs (n=6) and (b) control wild type age matched male CBA mice (n=4). Animals were imaged at the terminal stage of the disease (day 6 or 7 post-inoculation) in a vertical bore 9.4T magnet with a Varian Inova spectrometer. Animals were anaesthetised with 0.5-1.5% isoflurane in 70% N_2O: 30% O_2 and positioned in an Alderman-Grant resonator. Temperature and ECG were monitored throughout the experiment. Images (1.5mm slice thickness, 3 × 3cm field of view, 256 × 256 matrix size) were acquired at 1 and 3 mm posterior to Bregma. T_2 maps (TE=0.02, 0.04 and 0.06sec, TR=3sec) were acquired and regions of interest (hippocampus, cortex and striatum) were selected on a slice 3mm posterior to Bregma. T_1 weighted images (TR=0.5 sec, TE=0.02sec) were acquired pre- and 10 min post-gadolinium-DTPA (Gd) injection to assess blood brain barrier (BBB) breakdown. T_1 maps (TIR=0.025, 0.25, 0.5, 0.75, 2 and 5sec, TR=5sec) were acquired at a slice position 1 mm posterior to Bregma.

Results

T_1 weighted images acquired pre- and post-injection of Gd demonstrate a striking global increase in BBB permeability as well as more focal areas of intense BBB breakdown in the brains of terminal CM mice (see subtraction images in figure 1A and B). These changes were not seen in control mice as demonstrated by the lack of signal from the brain in the subtraction images (figure 1C and D). The T_2 relaxation time was significantly increased (p=0.02) in the hippocampus of CM mice compared to controls (figure 2), but no significant changes were identified in either the cortex or striatum. No significant changes on the T_1 maps were found.

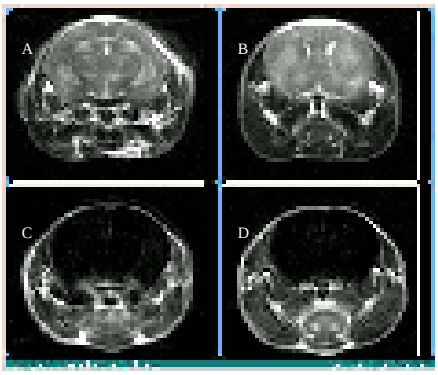

Figure 1 - BBB breakdown shown in images created by subtraction of pre-Gd from post-Gd T_1w images. A and B, images acquired at 1 and 3mm posterior to Bregma from a CM mouse, C and D, images from the same position acquired from a control mouse.

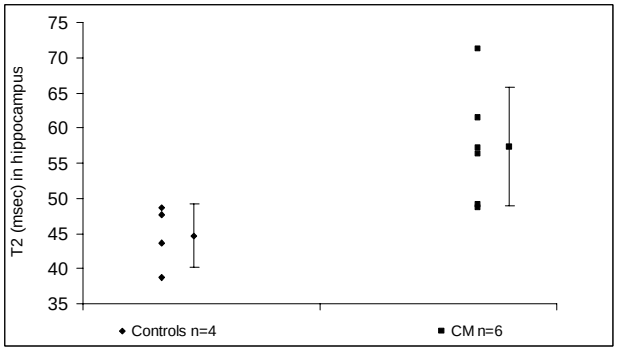

Figure 2 – Graph showing a significant (p=0.02) elevation in T_2 relaxation time in the hippocampus of terminal stage CM mice.

Discussion

Our finding of global BBB breakdown in this model of CM is in agreement with previous reports in which Evans blue dye was used to identify BBB breakdown[4]. In addition we have demonstrated that there are focal regions of greater BBB compromise. We have also identified significantly elevated T_2 in the hippocampus of CM mice in this study. This finding is in accord with one of the few MRI studies of human CM in which hyperintensities were observed in T_2 weighted images [5]. This study suggests that MRI will be a useful method for studying the pathology of murine CM, and may yield new information on the mechanisms of the disease and response to treatments.

[1] World Health Organisation. Malaria fact sheet **94** 1998.
[2] Grau GF *et al* (1994) *Parasitol Today*, **10**:410-412
[3] MacPherson *et al* (1985) *Am J Pathol*; **119**:385-401.
[4] Thumwood CM *et al* (1988) *Parasitology*, **96**:579-589
[5] Cordoliani *et al* (1998) *Am J Neuroradiol*, **19**:871-874.