Assessment of Experimental Cerebral Malaria Using Diffusion Tensor Imaging at Ultra-High Magnetic Field

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Target audience: basic scientists, physicians

Purpose: Cerebral malaria (CM), the most severe complication in the course of *Plasmodium falciparum* infection is lethal in 15-20 % of the cases with an estimated annual death toll of *ca.* one million. Currently, there is no treatment available against CM. A better understanding of its pathogenesis is crucial for the design of efficient adjunct therapies targeting this cerebral syndrome. Experimental CM induced by *Plasmodium berghei ANKA* (PbA) in certain strains of mice allows the investigation of CM pathogenesis. We previously demonstrated the preponderant role of ischemic edema in the fatal outcome of experimental CM[1-3]. The aim of this study was to define early MRI markers of experimental CM. For this purpose we investigated whether diffusion tensor imaging (DTI) at high magnetic field was able to provide information about alterations in brain structures during experimental CM.

Methods: Female CBA/J (n=8) and C57BL/6J (n=7) mice aged 8 weeks were infested by i.p. injection of *ca.* $2x10^6$ parasitized red blood cells (pRBC)[1]. Parasitemia was assessed on day 3 and 5 after pRBC inoculation, and mice were monitored daily for clinical signs. Infested mice died approximately 6-8 days after infestation. Mice were imaged before disease induction (controls) and from day 2 to day 6 after infestation. The animals were explored at 11.75T on a vertical spectrometer (Bruker AVANCE 500 WB, Germany) under isoflurane anesthesia (1-2%). Body temperature was maintained at approximately 37°C. Temperature and respiration were monitored during MRI exploration using a rectal thermometer and a pressure probe. The MRI protocol included axial T_2-weighted 2D fast spin echo sequence (RARE sequence) for morphological imaging (RARE factor = 8, TR = 5000 ms, effective TE = 36 ms, NA = 4, matrix 194 x 194, FOV=15x15 mm^2, 31 slices, slice thickness 0.5 mm). Two-dimensional DTI was performed with 12 directions, a diffusion gradient duration δ = 1.65 ms and a diffusion gradient separation time Δ = 10 ms resulting in a b-value of 1200 s/mm^2 in addition to the b = 0 s/mm^2 acquisition. A segmented EPI technique (TE = 15.7 ms, 12 interleaved segments, NA = 4, matrix 100 x 100) was used. DTI acquisitions were prospectively respiratory gated (TR ≥ 500 ms) using an MRI compatible monitoring and gating system (Small Animal Instruments Inc., NY). Five non-contiguous slices were acquired at approximately Bregma + 0 mm, + 1.75 mm, + 3.5 mm, - 1.75 mm, - 3.5 mm. Total acquisition time was 26 to 30 minutes depending on the respiration rate. DTI images were processed under ParaVision 5.1 software (Bruker, Germany), generating twenty-two DTI maps including S_0, FA, ADC, λ_1, λ_2 and λ_3. Post-processing was performed using ImageJ software. ROIs were positioned in different regions of white and grey matter. Non-parametric analysis was used and significance was set to $P<0.05$.

Results: Disease course was similar in both strains and was characterized by progressive weight loss and hypothermia. Severe signs linked to the development of the cerebral syndrome were overt from day 5 after infestation and consisted in ataxia, seizures, prostration, and ultimately coma and death. Progressive nerve crushing, focal white matter lesions, and brain swelling were observed on T_2-weighted images as previously reported[1,3]. DTI metrics were not different between the two strains of mice before disease induction. After infestation, FA maps showed progressive decrease of anisotropy in the olfactory bulbs, the anterior commissure, and the external capsule (Fig 1). The decrease in FA was already significant at an early stage of the disease, when the neurological signs were not yet detectable (day 2-4). Axial diffusivity (λ_1) was not altered, whereas radial diffusivity (λ_2 and λ_3) and ADC were significantly increased at the peak of the disease (day 6-7).

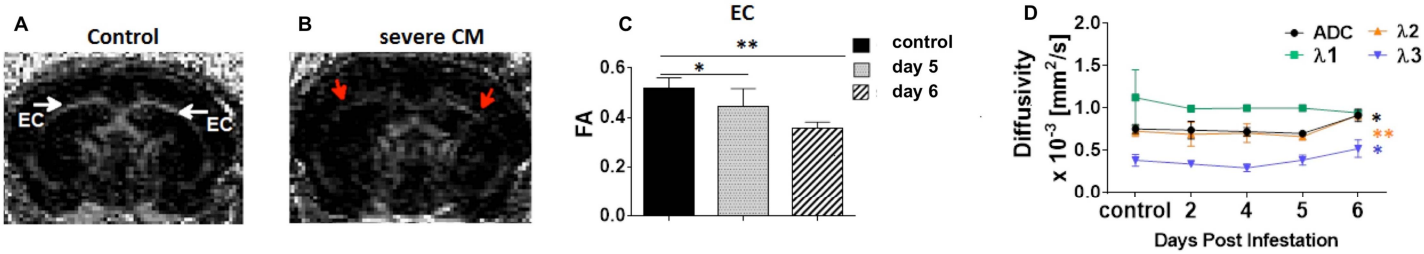

Fig.1: Changes in DTI metrics of the external capsule (C57BL/6J mice). A and B: FA maps of control and severe CM mice at Bregma - 1.75. C and D: DTI metrics in the external capsule. EC= external capsule.

Discussion: FA decrease during disease progression may indicate alterations in tissue organization. Indeed, reduced anisotropy in white matter has often been described in pathologies involving axon damage and demyelination such as multiple sclerosis and experimental autoimmune encephalomyelitis. The Increase in radial diffusivity (λ_2 and λ_3) in the absence of significant changes in axial diffusivity (λ_1) is evocative of myelin degradation without axonal loss[4]. The rise in ADC could also reflect myelin sheath alteration and cell loss. Detailed histological investigations are required to better understand the mechanisms by which white matter is altered in specific brain regions.

Conclusion: DTI allows the early detection of cerebral tissue microstructure alteration before the onset of neurological signs. Loss of tissue anisotropy is a new hallmark of the disease that may help improve our understanding of CM pathogenesis, and contribute to the diagnosis of CM in settings were MRI scanners are available.

References:

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