Infection with Plasmodium berghei ANKA leads to brain damage in mice resistant to cerebral malaria

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Introduction

Malaria is a major cause of morbidity and mortality with an annual death toll exceeding one million. Severe malaria is a complex multisystem disorder including complications such as cerebral malaria, anemia, acidosis, jaundice, respiratory distress, renal insufficiency, coagulation anomalies and hyperparasitemia. We previously performed the first characterization of the experimental cerebral syndrome using in MRI and MRS techniques and demonstrated the coexistence of inflammatory and ischemic lesions and proved the preponderant role of edema in the fatal outcome of experimental cerebral malaria (CM) (1). The purpose of the current study was to examine the cerebral effects of Plasmodium berghei *ANKA* (PbA) infection in a mouse strain resistant to CM. We tested the hypothesis that PbA infection could elicit brain injury, despite the absence of experimental CM, and by mechanisms different from those involved in CM.

Material and methods

Mice resistant to cerebral malaria (BALB/c strain) were infected with Plasmodium berghei *ANKA* and explored 8 and 15 days later by using in vivo brain magnetic resonance imaging and spectroscopy. The mice were explored on a 4.7 T horizontal Bruker 47/30 AVANCE Biospec MR system (Bruker, Ettlingen, Germany), except for angiography which was performed on a on a 11.75 T vertical Bruker AVANCE 500WB wide-bore MR system. Besides angiography, the MRI protocol included, multi-slice T_2 and T_2*-weighted images as well as diffusion and pulsed arterial spin labeling perfusion MRI of brain. T_1-weighted images were acquired before and after the injection of Gd-DTPA. *In vivo* metabolic explorations included ^{1}H MRS (PRESS sequence with TE=16 ms and TE=135 ms) and ^{31}P MRS. ^{1}H-MRS of brain extracts was performed on a vertical Bruker AVANCE 500WB wide-bore MR system operating at 11.75 T.

Results

The infected mice did not show the lesional and metabolic hallmarks of cerebral malaria. However, we detected brain dysfunction related to anemia, parasite metabolism and hepatic damage in hyperparasitized mice (HP) when compared to controls and to infected mice with low parasitemia (NCM). Indeed, we found a significant increase in cerebral blood flow (Figure 1) and a decline in cerebral choline in vivo (Table 1). In vitro, we measured a reduction in glycerophosphocholine, in *myo*-inositol, and an increase in glutamine.

Figure 1: Quantitative perfusion maps of mouse brain

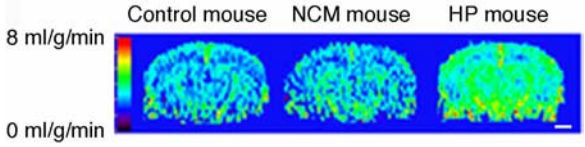

Table 1: Brain metabolite analysis determined by in vivo ^{1}H-MRS from control, NCM and HP mice

TE = 16 ms	Control mice (n = 9)	NCM mice (n = 7)	HP mice (n = 10)	post hoc test
NAA/S	0.27 ± 0.05	0.27 ± 0.05	0.28 ± 0.04	
tCr/S	0.18 ± 0.02	0.18 ± 0.03	0.19 ± 0.03	
Cho/S *	0.12 ± 0.02	0.13 ± 0.02	0.11 ± 0.01	CTL vs HP*;NCM vs HP*
Glx/S	0.18 ± 0.05	0.16 ± 0.05	0.18 ± 0.05	
taurine/S	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	
myo-inositol/S	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	

Statistics: Kruskal-Wallis analysis followed by Schéffé's test. Values are reported as means ± SD. Values of $P < 0.05$ were considered significant.

S is the sum of all metabolite signal areas.

Discussion

Increased blood flow is a compensatory mechanism allowing to temporarily maintain oxygen supply to brain despite anemia. The brain choline decrease appears related to hyperparasitemia as choline consumption is necessary for the parasite to proliferate. Finally, glutamine and *myo*-inositol anomalies together indicate hepatic encephalopathy, a finding in agreement with the liver damage detected in these mice by using biochemical and histological analyses. Our results highlight the vulnerability of brain to malaria infection even in the absence of cerebral malaria.

(1) Penet MF, Viola A, Confort-Gouny S, Le Fur Y, et al. J Neurosci. 2005;25(32):7352-8.