Mouse MRI and MR angiography at 9.4T to study the role of PKC Θ protein in neurological complication of malaria

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Introduction

Malaria is one of the most important public health problems. It is the most severe neurological complication in children and young adults infected with *Plasmodium falciparum* and is a deadly complication which annually kills more than 1 million people. It involves intravascular changes with sequestration of parasitized erythrocytes and host cells in cerebral micro vessels [1]. An animal model infected by Plasmodium berghei ANKA (PbA) [2] has been established to study the pathology. The susceptible mice developed a neurological syndrome. In this model, T-cell activation is required for the development of *Plasmodium berghei* ANKA (*Pb*A)-induced experimental cerebral malaria (ECM) [3]. In order to characterize the T cell activation pathway involved, we wanted to adress the role of protein kinase C-Theta (PKC-θ) from ECM development upon blood stage infection with PbA [4,5]. In time of flight MRA, gradient echo flow-compensated sequences are optimized to favour the vascular signal compared to surrounding tissues by saturating the signal of stationary tissue with very short TR. The longitudinal magnetization of these tissues has no time to grow again and signal weaks favouring the inflow effect of circulating blood entering the slice. As circulating blood is not saturated, the longitudinal magnetization is maximum. The signal from the bloodstream is very high compared to that of the saturated tissues. In this study, MRA and T2-weighted MRI were used to verify the lack of ischemia and microvascular pathology in PKC-θ PbA infected mice

Material and method

12 mice were included in this study. They were divided in three groups: Control, C57BL6 infected mice with Plasmodium Berghei ANKA (PbA) strain and PKCθ KO PbA mice. The last group was infected but did not develop malaria symptoms (unlike C57BL6 infected mice). A cloned line of *Plasmodium berghei* ANKA (PbA) transfected with GFP was obtained from Dr. A Waters [6]. Mice were infected by intraperitoneal injection of 10^5 parasitized erythrocytes as described before (Rudin, Eugster et al. 1997). Mice were observed daily for clinical neurological signs of ECM culminating in ataxia, paralysis and coma.

MR experiments were performed 6 days post infection on a 9.4 T horizontal magnet (94/20 USR Bruker Biospec, Wissembourg, France) with a 35mm diameter birdcage coil. During the MR experiments, mice were placed in a custom-built cradle to immobilize their head. They were anesthetized during MR experiment with 1.5% isoflurane and a mixture O_2/N_2O (1:1) with an output of 0.7L / min. Respiratory motion was monitored during all the experiment using an air pillow. Mice body temperature was maintained constant with a warm water circulation.

- Sagittal T2 weighted images were obtained using a MSME sequence (TR/TE = 4000/46 ms) with 156*156*1000 µm resolution to find anatomical landmarks to position the slices
- Axial T2 weighted images were obtained using a MSME sequence (TR/TE = 4000/46 ms) with 66*66*500 µm resolution.
- Axial MR angiographic images were obtained using a Flash sequence (TR/TE = 30/5 ms). Those images were then reconstructed by generating maximum intensity projection MIP images (Paravision PV4.3.2

Results

T2-axial and MRA images are respectively presented below on figure1 and 2:

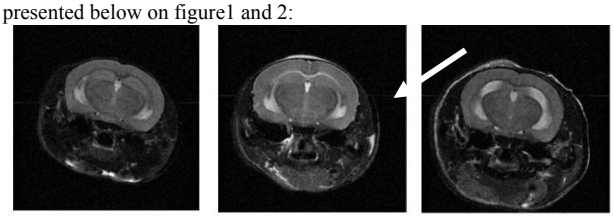

Fig.1: T2-weighted axial MR images for control (A), PbA infected (B) and PKCθ deficient PbA infected mice (C)

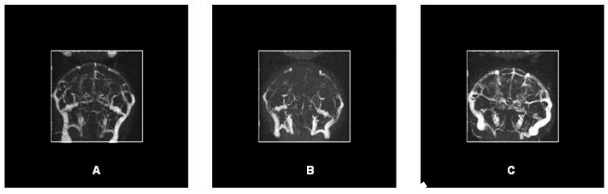

Fig.2: MRA reconstructed images for control (A), PbA infected (B) and PKCθ deficient PbA infected mice (C)

MRI and MRA images of uninfected (A) and infected mice (B and C) are shown in *Figure 1 and 2*, respectively. Distinct anomaly (edema formation) is visible on PbA infected mice T2-weighted images. It appears as a bilateral hyper intense signal in corpus callosum-external capsule region as indicated by a white arrow (Fig 1B). This is not the case for PKCθ PbA mice (Fig. 1C) which present similar T2-weighed image as Control mice (Fig.1A)

Vascular blood flow perturbation (loss of vascularization) is present on PbA infected mice MRA images (Fig. 2B) as indicated by a white arrow. This is not the case for PKCθ PbA mice (Fig. 2C) which present similar MRA images as Control mice (Fig.2A)

Conclusion

Here we addressed the role of PKC-θ in the development of ECM after *Pb*A infection. Using PKC-θ deficient mice we identify PKC-θ as an obligatory pathway for the development of ECM upon PbA. PKC-θ deficient mice were protected from ECM development upon blood stage infection with PbA. MRI and MRA confirmed the lack of ischemia, microvascular pathology and brain morphologic changes in those mice.

References

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