Temporal and Regional Evolution of Aquaporin 4 Expression and Magnetic Resonance Imaging in a Rat Pup Model of Neonatal Stroke

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Introduction

Edema formation can be observed using magnetic resonance imaging (MRI) in patients with stroke. Recent studies have shown that aquaporin-4 (AQP4), a water channel, is induced early after stroke and potentially could have a dual role in the development of brain edema[1]. We studied whether induction of AQP4 correlated with edema formation in a rat pup filament stroke model using high field (11.7-Tesla) MRI followed by immunohistochemical investigation of AQP4 protein expression.

Methods

Neonatal Ischemia: Neonatal ischemia was induced in 10 day old rat pups as previously published [2]. The transient filament middle cerebral artery occlusion model (tfMCAO) was performed and the MCAO was occluded for 1.5 h. Serial MRI was performed immediately after the onset of stroke until 28 days after injury. Neuroimaging and Analysis: All 10-day old tfMCAO (n=7) and sham (n=6) rat pups were imaged using diffusion weighted imaging (DWI) and T2WI to monitor ischemia and lesion progression in an 11.7 T scanner (Bruker, Bruker Biospin). Analysis consisted for 3D volumetric reconstructions of whole brain and ischemic tissue volume and location using Amira (Mercury Computer Systems) software. At 28 days animals were sacrificed and standard histological stains and AQP4 immunohistochemistry were used evaluate lesion size, location of AQP4 staining and correlation with imaging results.

Results

At 24h, we observed increased T2 values and decreased apparent diffusion coefficients (ADC) within injured cortical and striatal regions that reflected edema formation (Fig. 1). Coincident with these MR changes were significant increases in AQP4 expression on astrocytic end-feet in the border regions of injured tissues (Fig. 2). Striatal imaging findings were still present at 72h with a slow normalization of AQP4 expression in the peri-infarct regions. At 28 days AQP4 expression normalized in the border while striatal T2 and ADC values remained increased.

Conclusion

We show that induction of AQP4 is increased during the period of active edema formation in the peri-lesional area without regional correlation with edema. Finally, induction of AQP4 on astrocyte end-feet could participate in tissue preservation after ischemia in the immature rat brain.

1. de Castro Ribeiro M, et al (2006) Time course of aquaporin expression after transient focal cerebral ischemia in mice. J Neurosci Res 83:1231-1240; 2: Ashwal, S et al (2006) Serial magnetic resonance imaging in a rat pup filament stroke model. Exp Neurol Epub ahead of Print

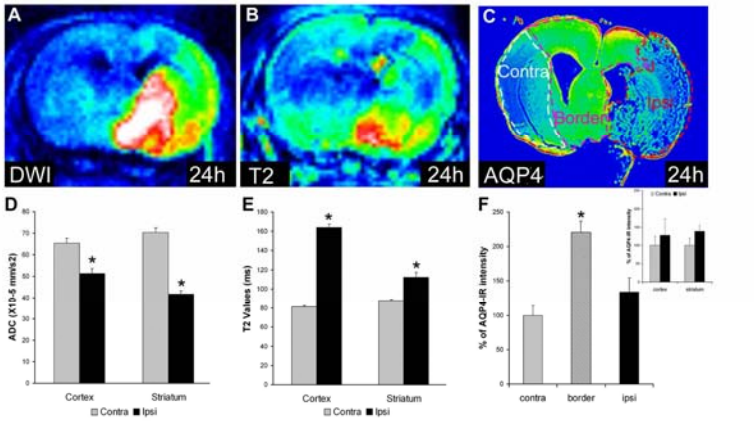

Fig 1. Macroscopic comparison of MRI data and AQP4 immunohistochemistry at 24 hrs after tfMCAO occlusion. A) Representative DW image showing decreased diffusion within the ischemic region (green to white). B) A T2WI from the same animal shows increased signal intensities which are suggestive of edema formation. C) False color illustration of the AQP4-IR intensity in the same rodent. D) Apparent diffusion coefficients (ADC) showed decreased values in the ipsilateral cortex and striatum, consistent with neuronal and glial swelling and thus reduced water diffusion. E) Concomitant increases in T2 values (increased water content) occurred at the same time in the ipsilateral ischemic tissue. F) Quantification of AQP4 in the cortex and striatum (insert) showed elevated AQP4-IR levels. AQP-IR of the border region was significantly increased when compared to the contralateral ($P<0.001$) and ipsilateral ($P<0.01$) tissues.

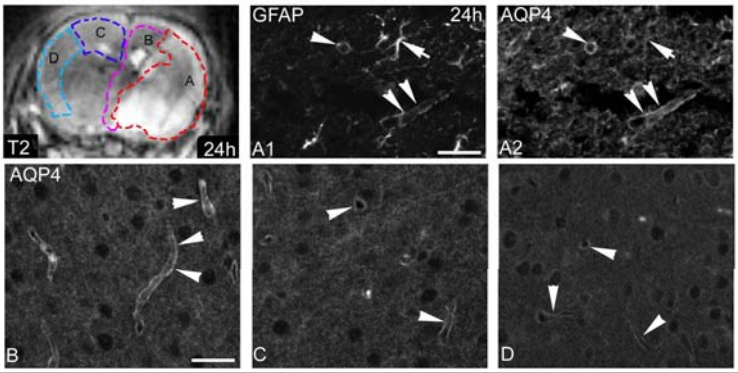

Fig 2. A) T2-images, at 24h after stroke onset, were used to localize the regions of interest. In ipsilateral cortex GFAP-IR (A1) was co-localized with AQP4-staining (A2) around blood vessels (arrow heads) and on processes (arrows). B) The intensity of the AQP4 staining was significantly increased around blood vessels (arrow heads) in cortex tissues in vicinity the regions where ADC was decreased and T2WI-values were increased. C) In frontal cortex of the contralateral hemisphere the intensity of AQP4-IR around blood vessels (arrow heads, D) was higher than in the contralateral parietal cortex (arrow heads, D). Bar : A-D=40□m