Biphasic expression of aquaporin 4 during the course of brain inflammation

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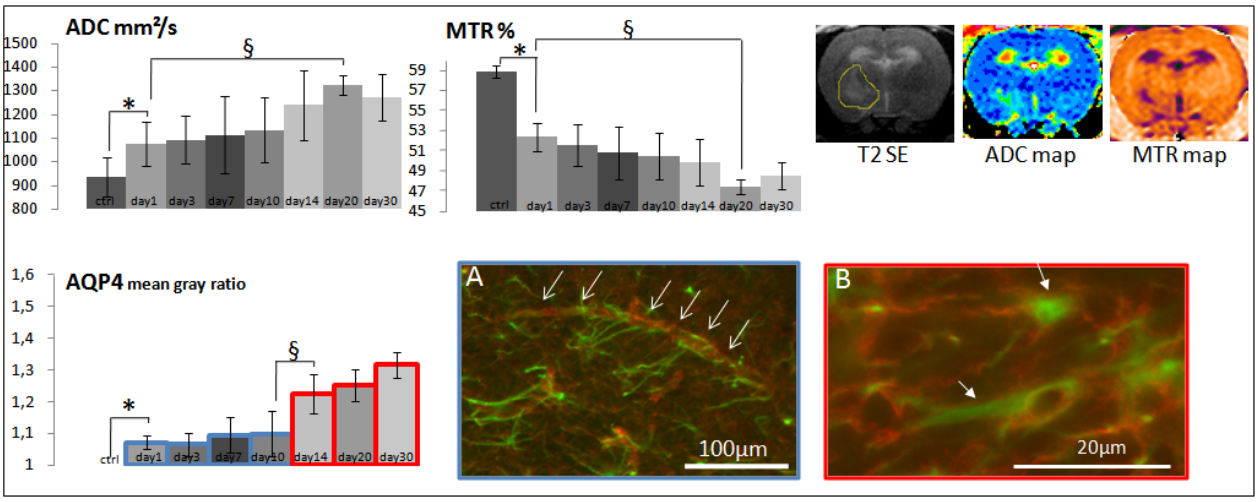
Objective: Aquaporin 4 (AQP4), the major selective water channel throughout the central nervous system, was found to facilitate water removal in case of interstitial edema[1] and was also involved in astrocyte migration during glial scar formation[2]. Because brain inflammation involves both, vasogenic edema and gliosis, we hypothesized a major role of AQP4 in this process. Edema and gliosis being two temporally different stages of inflammation, we further hypothesized that AQP4 regulation could vary during the course of inflammatory process according to the prevailing edematous or cicatricial stage.

Methods: A toxic model of brain inflammation was induced by a stereotaxic injection of L-α-lysophosphatidylcholine in 53 rats. Apparent diffusion coefficient (ADC) derived from MRI was used as a marker of interstitial edema and magnetization transfer ratio (MTR) as a marker of myelin content. Animals were sacrificed at day 1, 3, 7, 10, 14, 20 (n=8 per group) and 30 (n=5) post injection and immunostaining was performed using AQP4, ED1 and GFAP for water channel, macrophages and astrocytes, respectively. A second group of animal (n=15) was used to confirm histological observations with RNA quantification (RT-PCR).

Results and Interpretation: We found a significant correlation between AQP4 expression and markers of interstitial edema (ADC, r=0.6) or disease progression (MTR, r=0.66) in line with our previous data[3]. More detailed analysis revealed two temporally different steps of AQP4 up-regulation. First, AQP4 up-regulation was moderate during early stages of inflammation and mainly located at blood brain barrier (BBB) interface. This coincided with the active phase of inflammation (peak of ED1+ macrophages and pro-inflammatory cytokines) and with a significant vasogenic edema increase (high ADC values). At this stage AQP4 could be interpreted as insufficient to remove interstitial water excess that continued to increase. Second, a highly significant AQP4 up regulation was observed but delayed (day 14) and with a different location, not only at BBB interface but on the whole membrane of astrocytes. This could again be interpreted as insufficient to remove all interstitial water excess (high ADC values) but probably involved in the glial scar as depicted with GFAP.



Time course of ADC and MTR measured within the inflammatory lesion (yellow contour on T2 weighted image) and semi-quantitative AQP4 expression assessed histologically within the same area. ADC and MTR exhibited modifications as soon as day 1 (*:p<0.05, Wilcoxon test) compared to the contralateral hemisphere that progressively amplified until day 20 post injection (§: p<0.05, one way ANOVA with post hoc test of Bonferroni). Corresponding AQP4 expression, first, increased moderately (blue, example A) with a location at BBB interface and, next, demonstrated a delayed amplification (red, example B) with a pan-astrocytic expression. AQP4 in red and GFAP in green; arrows indicate AQP4 staining along vessels (A) or astrocyte whole membranes (B).

1. Papadopoulos MC, Manley GT, Krishna S, Verkman AS. Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. Faseb J 2004;18:1291-1293.
2. Saadoun S, Papadopoulos MC, Watanabe H, Yan D, Manley GT, Verkman AS. Involvement of aquaporin-4 in astroglial cell migration and glial scar formation. J Cell Sci 2005;118:5691-5698.
3. Tourdias T, Dragonu I, Fushimi Y, et al. Aquaporin 4 correlates with apparent diffusion coefficient and hydrocephalus severity in the rat brain: a combined MRI-histological study. Neuroimage 2009;47:659-666.