Time course of water diffusivity in the rat brain after global ischemia

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Introduction

Global cerebral ischemia, usually caused by a heart attack, is characterized by delayed neuronal loss and reactive gliosis, especially in the CA1 region of the hippocampus. The aim of this study was to evaluate the time course of changes in the apparent diffusion coefficient of water (ADCw) in the CA1 region after ischemia/reperfusion injury in vivo. The diffusion properties of the tissue change during many pathologies as well as under physiogical conditions and influence signaling in the brain, including synaptic and extrasynaptic transmission [1]. Diffusivity in the brain reflects cell morphology, extracellular space volume changes [2] and/or extracellular matrix composition [3]. Therefore, we correlated the ADCw results with immunohistochemical analysis of the damaged tissue, focusing on neuronal loss, reactive gliosis and the expression of apoptotic markers.

Subjects and methods

Experiments were performed *in vivo* on 2-month-old Wistar male rats. Animals were anesthetized with sodium pentobarbital (60 mg/kg i.p.). To induce global cerebral ischemia, a bilateral 15 min occlusion of the common carotides combined with hypoxic conditions (6%O2 / 94%N_2) was used. We employed diffusion-weighted (DW) MRI to determine ADCw in control rats and after 1 hour, 6 hours, 1 day, 3 days, 7 days and 5 weeks of reperfusion following global ischemia. Sham operated animals were measured 3 days after surgery. DW images were obtained using a stimulated echo sequence at six different diffusion weightings (75 - 1732 s/mm^2), and the diffusion gradient direction pointed along the rostrocaudal direction. ADC_W maps were evaluated in the CA1 region of the hippocampus (Fig. 2). Following MR measurement, the animals were perfused transcardially with 4% paraformaldehyde under deep anesthesia. Coronal brain slices (40µm thick) were used for immunohistochemical analysis. Astrocytes were identified using antibodies against GFAP and nestin (reactive astrocytes); neurons were identified using an antibody against NeuN; antibodies against cleaved caspase-3 and cleaved poly-ADP-ribose polymerase-1 (PARP-1) were used to identify cells undergoing programmed cell death.

Results

In the CA1 region of hippocampus global cerebral ischemia leads to an increase in ADCw (762 ± 5 µm^{2}s^{-1}, n = 6) during the first two hours of reperfusion. No significant differences from control values (714 ± 7 µm^{2}s^{-1}, n = 7) were found between 6 and 24 hours after ischemia or in sham operated animals. Subsequently, ADCw significantly increased to 756 ± 9 µm^{2}s^{-1} and 859 ± 12 µm^{2}s^{-1} (n = 6) after three days and after five weeks of reperfusion, respectively. The time course of ADCw changes is shown in the plot below (Fig. 1). Immunostaining with an antibody against GFAP (red) and NeuN or nestin (green) revealed neuronal loss and reactive gliosis in the CA1 region of the hippocampus (Fig. 3a). Both casp-3 and PARP-1 were mostly activated in neurons in the CA1 region 6 hours after ischemia, and later during reperfusion (about 5 weeks) they became detectable in astrocytes (Fig. 3b).

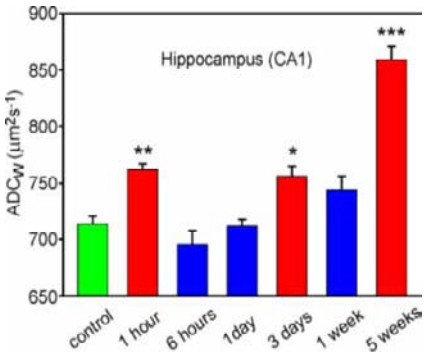

Figure 1: Plot shows time course of ADC_W values after reperfusion. Significant differences (One-way ANOVA) compared to controls are marked by asterisks (* p<0.05, ** p<0.01, *** p<0.001).

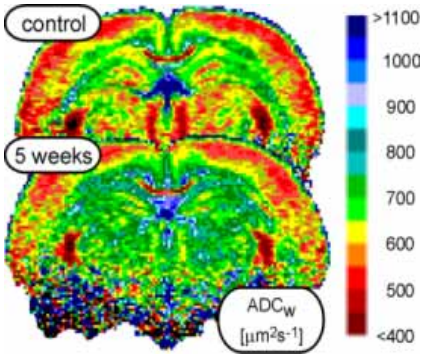

Figure 2: ADCw map acquired in the brain of a rat 5 weeks after ischemia shows an increase in water diffusibility in the hippocampus as compared to a control animal.

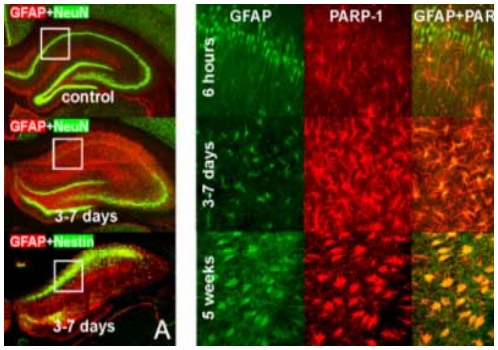

Figure 3: (A) Note the decreased NeuN and increased GFAP/nestin immunoreactivity (reactive gliosis) in the CA1 region. (B) PARP-1 is mostly activated in neurons at 6 hours after ischemia; after 5 weeks of reperfusion it becomes detectable in astrocytes.

Discussion and Conclusion

The increase of ADCw at the begining of reperfusion is probably caused by an increase in extracelular space (ECS) volume due to a regulatory volume decrease after ischemia [4]. The second peak in ADCw, three days after ischemia, coincides with neuronal death in the CA1 region, which can lead to the enlargement of the ECS. As we showed previously [3], changes in extracellular space volume affect ADC_W; an increase in ADC_W reflects an increase in ECS volume and vice versa. The marked rise in ADCw five week after global ischemia might reflect changes in astrocyte morphology and membrane properties. Caspase-3 and PARP-1 immunoreactivity in this period implies either ischemic preconditioning in astrocytes or ongoing programmed cell death in astrocytes (apoptosis).

References and Acknowledgement

1. Syková E. (2004) Extrasynaptic volume transmission and diffusion parameters of the extracellular space. *Neuroscience* 129:861–876.
2. van Der Toorn A., et al. (1996) Dynamic changes in water ADC, energy metabolism, extracellular space volume, and tortuosity in neonatal rat brain during global ischemia. *Magn. Res. Med.*, 36:52-60.
3. Syková E. et al. (2005) Reduced extracellular space in the brain of TN-R and HNK-1-sulphotransferase deficient mice. *Eur. J. Neurosci.*, 22:1873.
4. Syková E. et al. (1999) Glial swelling and astrogliosis produce diffusion barriers in the rat spinal cord. *Glia* 25:56-70.

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