Diffusion Anisotropy Correlates Compound Action Potential in the Optic Nerve from Mice of Retina Ischemia

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Introduction:

Injury to optic nerve after retinal ischemia has previously been examined to show a progressive pattern of neuropathology with axonal injury followed by demyelination. However, the functional assessment using diffusion tensor imaging (DTI) has not been evaluated to determine which diffusion parameter reflects the physiological state of the injured nerve. In vivo electrophysiology, such as the visual evoked potential (VEP), has been used to evaluate optic nerve function, but is challenging in mice in vivo. In contrast, the compound action potential (CAP) amplitude measured by in vitro recordings is more robust. The CAP amplitude is considered a quantitative index of the white matter tracts reflecting the coherent transduction of neuronal impulses along white matter tracts. The attenuation of CAP amplitude could reflect a decrease in the number of normally functioning axons or an increase in the number of impaired axons due to axonal and/or myelin damage. Thus, CAP amplitude is an effective structural and functional measurement of white matter integrity. Herein, in vitro recordings were performed to correlate electrophysiological and DTI measures on optic nerves from control and mice at 3 and 7 days after retinal ischemia. The axonal and myelin integrity of optic nerve from control and retinal ischemic mice were evaluated to establish a correlation between DTI and CAP measurements and to determine which DTI parameter is best suited to reflect optic nerve function after retinal ischemia.

Method:

Animal Model: Seven to eight weeks old C57/BL6 mice (n = 25) were employed including 9 control and 16 retinal ischemia animals for the study. Retinal ischemia was induced in mice after intraperitoneal injection of a cocktail of ketamine and xylazine. Briefly, the anterior chamber of the right eye of the mouse was canulated with a 32-gauge needle attached to a saline-filled reservoir raised above the animal to increase intraocular pressure (IOP) to 110 mmHg for 60 min. At the end of the ischemic period, the needle was removed from the anterior chamber allowing the reperfusion to the retina.

Electrophysiology: Electrophysiology was assessed on the right optic nerve from control and retinal ischemia mice at 3 and 7 days after reperfusion. The right optical nerve was removed and allowed to recover. The in vitro nerve was held by two suction pipettes bathed with normal artificial cerebrospinal fluid. The recording temperature was maintained at 25°C. Normal (anodal) stimulation was used allowing the action potential to be triggered at (or in the proximity) of the orifice of the suction electrode. The half maximal amplitude of CAP was measured in all nerves. After electrophysiological assessments, nerves were immersion fixed in 4% buffered PFA for subsequent diffusion tensor MRI study.

MRI: MRI of the optical nerves was performed using a Varian 4.7T UNITY-INOVA scanner employing a custom-built solenoid coil for transmission and receiving. A Stejskal-Tanner spin-echo diffusion-weighted sequence was used to acquire diffusion weighted images with the following parameters: TR 1s, TE 55ms, Δ 45 ms, δ 4 ms, slice thickness 0.5 mm, field-of-view 0.5×0.5 cm^2, (Gx,Gy,Gz) = (1,1,0), (1,0,1), (0,1,1), (-1,1,0), (0,-1,1), and (1,0,-1), and b = 0 and 1805 s/mm^2 . In plane resolution was 52×52 μm^2

Statistics: ANOVA was performed to compare the relative anisotropy and half maximum amplitudes of optic nerves from mice at control, 3 and 7 days after retina ischemia. Statistical significance was accepted at $p < 0.05$.

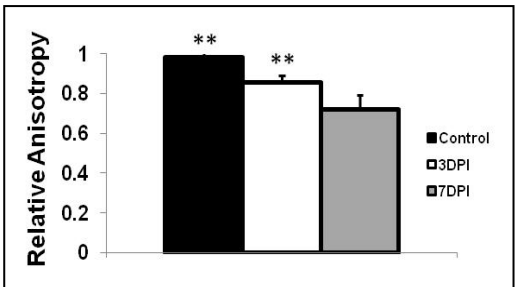

Figure1. The relative anisotropy of the optic nerves from control mice, and retina ischemia (RI) mice at 3, and 7days after reperfusion. **. p<0.005

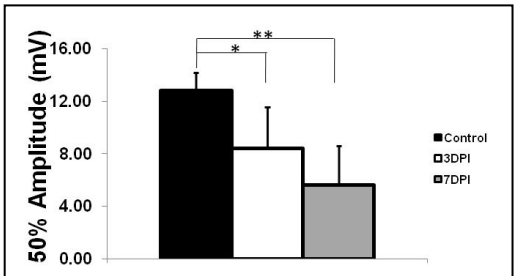

Figure2. The half of maximum amplitude of optic nerve action potentials from the control mice, and retina ischemia (RI) mice at 3, and 7days after reperfusion.*, p<0.05, **, p<0.005

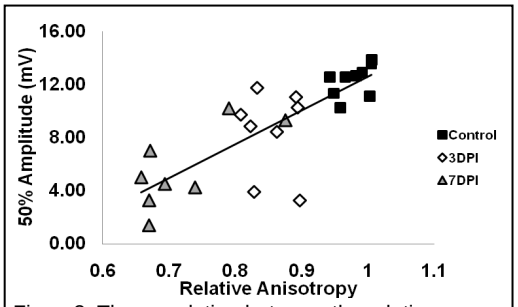

Figure3. The correlation between the relative anisotropy and half maximal amplitude of the compound action potential (Amp-50%), r =0.80.

Results and Discussion:

In our DTI measurements, we found a 25% decrease in axial diffusivity with normal radial diffusivity 3 days after retinal ischemia, suggesting axonal injury without demyelination. At 7 days, there was no additional changes in axial diffusivity compared to 3 days, while radial diffusivity significantly increased by 50% consistent with significant demyelination due to sustained axonal injury. The trend of decreased relative anisotropy (Fig. 1) was progressive over 3 and 7 days after retinal ischemia compared to controls. The half maximal amplitude (Amp-50%) also decreased at 3 and 7 days after retinal ischemia (Fig. 2), with a similar trend as seen in RA. The Amp-50% correlated with the relative anisotropy (r = 0.80; Fig. 3), suggesting that Amp-50% is affected by both axon and myelin integrity.

The evolution of functional electrophysiological assessment after retinal ischemia with progressively worsening half maximal amplitude was consistent with a progressive degeneration of optic nerves after retinal ischemia. These preliminary results suggest that the optic nerve function is likely to be affected by both axon and myelin injury with a more severe impact when both axon and myelin damage is present. The decreased diffusion anisotropy may optimally reflect optic nerve function after retinal ischemia. In conclusion, despite the presence of axonal injury may be responsible for the long term disability of visual function, the overall functional assessment would be best to be evaluated by diffusion anisotropy than axial or radial diffusivity alone.

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