Analysis of water diffusion and magnetization transfer ratio in optic nerves after crush injury

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Introduction

The purpose of this study was to assess the potential of apparent diffusion coefficients (ADC) and magnetization transfer (MT) measurements to elucidate the processes of axonal degeneration in the optic nerve after traumatic injury. It has been shown that magnetic resonance determination of ADC can indicate the orientation of axons and characterize injury of white matter in species with myelinated systems such as rats, cats, and humans [1]. The magnetization transfer ratio (MTR) has been shown to be an indicator of tissue injury (e.g., myelination) [2].

Materials and Methods

Eight Sprague-Dawley rats were subjected to left optic nerve crush using standard techniques. Briefly, anesthetized adult female rats were fixed in a stereotaxic frame and an incision was made behind the left eye. The intraorbital position of the left optic nerve was exposed and then crushed 2 mm behind the globe for 10 seconds with No. 5 jeweler's forceps. Nerve injury was verified by the appearance of clearing at the crushed site. Under anesthesia with 2% isoflurane, all animals were subjected to MRI in a 4.7 T MRI system (Biospec 47/40, Bruker BioSpin, Karlsruhe, Germany). Transverse sections in which both right and left optic nerves were delineated were carefully selected and imaged at 1week or 1month after the surgery. For ADC measurement, b values ranging from 10 - 1000 sec/mm^2 were employed parallel and perpendicular to the optic nerve orientation. The other imaging parameters were: TR/TE = 2000/30 msec, FOV = 3 x 3 cm, matrix size = 128x128, diffusion time = 17 msec, diffusion pulse duration = 5 msec, and NEX = 2. On those images, transverse (t-) and longitudinal (l-) ADCs (mm^2/sec) were calculated in the crushed optic nerve and the contralateral side as a control. Anisotropy was simply defined as l-ADC/t-ADC. MT saturation was applied for SE sequences (offset = 4500Hz) and the MTR was calculated in each location. After imaging, the rats were sacrificed by an overdose of anesthesia, and the optic nerve was immediately isolated and fixed in paraformaldehyde. The nerves were stained immunohistochemically for myelin, paranodes, and sodium and potassium channels.

Results and Discussion

The t-ADC, l-ADC and diffusion anisotropy of the intact optic nerve in the adult rats were 0.46 ± 0.15, 1.41 ± 0.19 and 3.38 ± 1.18 (n=7), respectively, in our previous study [3]. The t-ADC and l-ADC changed to 0.98 ± 0.19 ($p<0.01$) 0.93 ± 0.30, $p<0.05$) in the crushed optic nerve 1 week after injury, resulting complete loss of diffusional anisotropy (0.98 ± 0.42, $p<0.05$). The t-ADC returned toward normal levels 1mo after injury. The l-ADC tended to recover but it remained lower than that in the control, leading to less diffusional anisotropy. In the contralateral, the t-ADC did not change (0.50 ± 0.04) but the l-ADC reduced (1.04 ± 0.10, $p<0.05$) 1week after injury while the t-ADC increased (0.75 ± 0.15) and the l-ADC recovered (1.56 ± 0.17) to the normal level 1mo after injury. At both time points, the diffusional anisotropy was reduced (2.09 ± 0.33 and 2.17 ± 0.61). The MTR in the crushed optic nerve was lower than that in the contralateral at both time points (30.8 ± 12.9 vs. 37.9 ± 4.4, $p<0.05$ for 1w, 30.0 ± 10.1 vs. 35.3 ± 5.7, $p<0.05$ for 1mo). The results were related to myelin basic protein staining.

The results in the quantitative analysis revealed that the t-ADC increased while l-ADC decreased in the crushed optic nerve 1 week after injury, resulting complete loss of diffusional anisotropy. We postulated that the increased t-ADC was due to low density of the axons presumably due to edema, axonal loss, or both. The t-ADC retuned toward normal levels 1 month after injury. The l-ADC tended to recover but it remained lower than that in the control, leading to less diffusional anisotropy as well. In the contralateral optic nerve, the t-ADC did not change but the l-ADC reduced 1 week after injury while the t-ADC increase and the l-ADC recovered to the normal level 1 month after injury. At both time points, the diffusional anisotropy was reduced. The MTR in the crushed optic nerve was lower than that in the contralateral optic nerve.

In conclusion, optic nerve crush results in reproducible loss of anisotropy due to increase in t-ADC and increase of MTR. Post traumatic degeneration of the nerve could be monitored with MR. This method is appropriate for evaluating response to therapy intended to be protective for posttraumatic degeneration.

Table 1. Changes in apparent diffusion coefficients (ADCs; x10^{-3} mm^2/sec) and magnetization transfer contrast ratio (MTCR) in the optic nerves in crushed and contralateral sides 1 week and 1 month after surgery (N=4)

	Control*	Contralateral side		Crushed side	
	(intact)	1 week	1 month	1 week	1 month
t-ADC (x10^{-3} mm^2/sec)	0.46 ± 0.15	0.50 ± 0.04	0.75 ± 0.15	0.98 ± 0.19	0.87 ± 0.47
l-ADC (x10^{-3} mm^2/sec)	1.41 ± 0.19	1.04 ± 0.10	1.56 ± 0.17	0.93 ± 0.30	1.23 ± 0.46
Diffusional anisotropy	3.38 ± 1.18	2.09 ± 0.33	2.17 ± 0.61	0.98 ± 0.42	1.87 ± 1.28
MTR (%)	--	37.9 ± 4.4	35.3 ± 5.7	30.8 ± 12.9	30.0 ± 10.1

t-ADC: transverse ADC (perpendicular); l-ADC: longitudinal ADC (parallel); * from the previous study [3]

References: 1. Le Bihan Nature Rev NeuroSci 2003;4:469. 2. Paus et al. Brain Res Bull 2001;54:255. 3. Takahashi et al. Radiology 2000; 216:881.