Diffusion Tensor Magnetic Resonance Imaging of regeneration/degeneration after rat sciatic nerve injury

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Introduction

In the peripheral nervous system (PNS), accurate measurement of the changes in the nerve after injury may be highly beneficial clinically in saving the patient a needless operation or to avoid missing an opportunity for performing nerve reconstruction [1-3]. The non-invasive nature and high sensitivity to microscopic structural changes of diffusion tensor imaging (DTI) by measurement of water displacement has resulted in its wide application in developmental and pathological examinations in central nervous system (CNS) and PNS. Recently, an analytical approach examining DTI derived parameters has been proposed to assess the extent of axonal damage and demyelination [4,5]. In this experimental study, DTI was used to investigate spatiotemporally the processes of axonal degradation and demyelination, then remyelination, in cut or crush excised rat sciatic nerves using a 17.6 T magnet with a solenoid coil. In particular, orientation independent measures of water diffusion, fractional anisotropy (FA) of diffusion direction, and averaged rate of diffusion (<D>) were examined as DTI derived MR parameters for the quantification of the structural changes within the major peripheral nerve.

Materials and Method

Rat Sciatic Nerve Injury: Female Harlan Sprague-Dawley rats (180-200g) were anesthetized with isoflurane and the sciatic nerves exposed bilaterally at mid-thigh. Nerve crush was performed for 45 seconds with needle nose forceps. Nerve transections were made by cutting the nerve with surgical scissors. Proximal stump was bound and displaced from the distal stump to prevent axonal ingrowth. At 3, 7, 14 and 28 days post injury, nerves were removed, rinsed with saline and fixed in 4% paraformaldehyde.

Magnetic Resonance Imaging: Fixed rat sciatic nerves were placed in a 2mm diameter solenoid coil. Magnetic resonance imaging (MRI) was measured on a 17.6 T magnet (@ 750MHz), 89mm bore magnet with a Bruker Advance imaging and spectrometer system. Diffusion weighted images of normal nerve, as well as cut and crush nerves at 3, 7, 14 and 28 days post injury, were acquired by applying gradients to give diffusion sensitizing factor (b values)= 0 and 1250 s/mm2 in 21 directions with a spin-echo pulse sequence; imaging parameters were TR=2.7 s, TE=29.5 ms, 24 averages for a non-diffusion weighted image (b = 0) and 6 averages for diffusion weighted (b = 1250) images using Δ=17.5 ms, δ=1.5ms, 40 slices with thickness of 0.25mm, field of view (FOV) of $2\times2mm^2$, and data matrix of 58×58. Anatomy-based 15 ROIs were selected to get averages and standard deviations after the generation of the parameter images derived from the rank-2 tensor fit to the DWI data.

Results and discussion

Fractional anisotropy (FA) in normal nerve is high (0.85 ~ 0.90) and <D> is low (~ 2×10^{-4} cm^2sec^{-1}) because water diffuses preferentially in the longitudinal direction of well-structured nerve fibers which lead a restricted diffusion of water. In crush nerves, FA is the lowest (0.4~0.5) and <D> is the highest (~ 9×10^{-4} cm^2sec^{-1}) around 3 days post injury. As the post operative days increase, FA continues to increase and almost approaches to FA of normal nerve on 28 days post injury. In converse, <D> decreases to 4×10^{-4} cm^2sec^{-1}. It suggests that myelin and axon are degenerated after crush injury and their regeneration may start around 3 days post injury then continues toward normal status. However, cut nerves display a prolonged decrease in FA over the time after injury. FA keeps decreasing to 0.4 around 28 days post injury and <D> keeps increasing. This result suggests that. MR can image that the nerve becomes more demyelinated and less structured over time after cut injury. At 14 days post injury, FA of crush nerve (Fig.1) is high near the lesion then decreases linearly in a proximodistal gradient of 0.0285, which may indicate the myelin recovery rate in the crush nerve. At 8.5mm from the lesion, FA is down comparably to FA of cut nerve. <D> correlates with FA in these nerves in an inverse fashion; 4×10^{-4} cm^2 sec^{-1} at the lesion site then increases distally to 5×10^{-4} cm^2 sec^{-1}. Degenerated axons are usually recovered as fast as 1 - 4mm per day after crush injury [6]. Therefore, it suggests that FA or <D> appear to monitor the remyelinaton, starting at the lesion and propagating distally in the crush nerve. In contrast, FA of cut nerve (Fig.1) shows a prolonged decrease that correlates with an irreversibly damaged nerve.

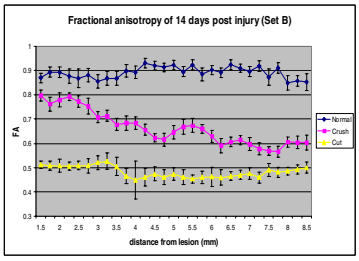

Fig.1 FA of crush and cut rat sciatic nerves 14 days post injury

At histopathology showing myelin by Sudan black stain (Fig.2), normal nerve (a) is characterized by closely packed, well-myelinated axons with relatively uniform and circular cross sections [1]. A number of differences are evident between the crush and cut nerve 14 days post injury. Well-myelinated axons with relatively thick and ring-shaped myelin sheaths are shown at 1 mm from the crush nerve 14 days post injury (b). The density of these axons decreases toward the distal end. Few myelinated axons are characterized at 8 mm from the lesion (e). The intermediate state appears at 3mm and 5mm(c,d). However, loss of structure without myelinated axon and myelin debris are observed in the cut nerve 14 days post injury. At 28 days post injury, a difference between crush and cut nerves becomes more conspicuous at 1mm distal to each lesion. Crush nerve is almost recovered to a level comparable to normal nerve with well-myelinated axons. Cut nerve has more loss of structure. These results show that FA or <D> in the crush/cut nerve matches with remyelination/demyelination process shown in the histopathology.

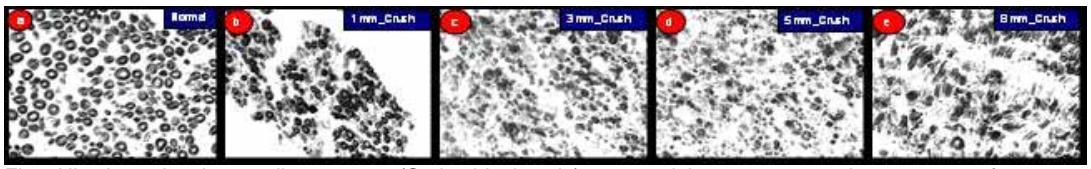

Fig.2 Histology showing myelin at 1600 × (Sudan black stain). a:normal; b:1mm, c:3mm, d:5mm, e:8mm from

Conclusion

DTI measured with the 17.6 T magnet demonstrates that MR can image regeneration of crush nerve starting proximal to lesion and proceeds distally as well as monitoring of the changes in the nerves with a time after the injury. Also MR can image the damage in cut nerve are irreversible. FA and <D> seem to be good indicators of (de)myelination and regeneration within rat sciatic nerve. Increasing FA and decreasing <D> may be used to follow the regenerating nerve after crush injury, and decreased FA and increased <D> may correlate with the irreversibly damaged to the nerve after cut injury. But FA appears to be more sensitive indicator because it reflects these changes more significantly.

References

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