Diffusion Tensor Peripheral Nerve Tractography ~ Histological Changes and Diffusion Anisotropy ~

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Background

The standard repertoire in the diagnosis of peripheral nervous system disorders includes clinical and electrophysiological examinations, supplemented by more invasive procedures, because peripheral nerves are difficult to delineate on conventional MRI due to their poor contrast with the surrounding tissues.

With the progress in MRI technology and techniques, several researchers have recently reported on successful diffusion tensor tractography (DTT) of the human peripheral nerves. Since the reliability of these DTT images has not yet been validated with detailed histological studies and quantitative analyses, it remains unclear whether DTT actually reflects anatomical degeneration / regeneration of the axonal fibers. We believe that under the present circumstances, when no proper tools are available yet for visualizing the peripheral nerves, it is important to evaluate the validity of assessment of peripheral nerve degeneration and regeneration using DTT.

Purpose

The objectives of this study are to determine

1. whether DTT can be used to delineate degeneration and regeneration of peripheral nerves after the injury.
2. whether recovery from the injury can be reliably evaluated by tracking the fibers distal to the lesion site, by examining the correlation with the histological and functional changes.

Materiarials & Methods

Animals and surgical procedures. Adult female Sprague-Dawley rats were used. All surgeries were performed under chloral hydrate anesthesia. The sciatic nerves were exposed and subjected to a crush injury using a brain aneurysm clip.

Magnetic resonance imaging. MRI was performed using a 7.0-Tesla MRI, PharmaScan 70/16 (Bruker BioSpin, Ettlingen, Germany) with a coil dedicated for small animals. To verify the feasibility of peripheral nerve DTT, we first performed DTT of the sciatic nerves of an *in vivo* model. For analyzing the degeneration and regeneration of nerves in detail, diffusion tensor MRI was conducted with phantoms consisting of excised sciatic nerve specimens (1 day, and 1, 3, 6, and 12 weeks after crush injury and intact nerves, n=6 each) embedded in 2% agarose gel with 5mM copper sulfate, using a spin-echo sequence based on the Stejskal-Tanner diffusion preparation.

Diffusion tensor analysis. DTT images were computed with the Volume One and dTV II SR software (Masutani Y et al. Eur J Radiol, 2003). We used one ROI technique, by choosing a seed ROI through which the fibers were tracked. To delineate the fibers, the ROI was placed 5mm proximal to the contusion. We measured the fractional anisotropy (FA) values in each sample at points 5 mm (proximal), 0 mm (epicenter) and -5 mm (distal) proximal to the contusion. The scanning parameters were as follows; TR = 4500 ms, TE = 40 ms, flip angle = 90 deg, FOV = 30 x 30 mm, slice thickness = 1.25 mm, reconstructed image resolution = 0.31 mm, matrix size = 96 x 96, b-value = 1000 sec/mm^2, MPG orientations = 12 axes.

Histological analysis. We performed electron-microscopic analysis of 6 nerve samples at the same time points as the diffusion tensor analysis. At a point 5 mm distal to the contusion, 80-nm-thick ultrathin sections were cut cross-sectionally and stained with uranyl acetate. The following parameters were calculated for each nerve: axon density, axon diameter, myelin sheath density,and myelin sheath thickness.

Functional analysis. The leg muscle contraction test, the Rota-rod test (to evaluate motor coordination), and the von Frey filament test (to evaluate mechanical sensitivity) were used to assess the recovery of function after the sciatic nerve injury. 6 rats were used for these functional evaluations. The tests were conducted at the same time points as the diffusion tensor analysis.

Statistical analysis. Pearson's correlation coefficients were calculated to determine correlations between these parameters and the FA.

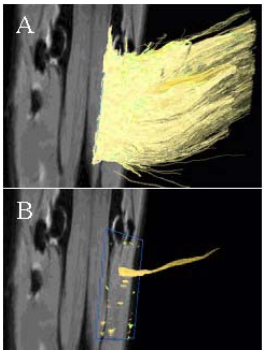

Fig. 1. *In vivo* sciatic nerve DTT. A:FA>0.15.B:FA>0.35. Using a more stringent threshold, DTT can clearly delineate nerves and exclude the surrounding tissues.

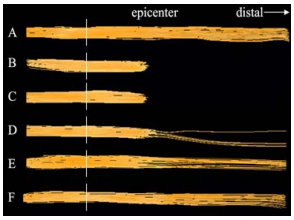

Fig. 2. Temporal analysis of injured sciatic nerves DTT. A:intact, B:1d, C:1w, D:3w, E:6w, F:12w after injury. The region of interest (ROI) was placed 5 mm proximal to the contusion (white line). It recovered gradually and reached the pre-injury level.

Results

Peripheral nerves could be reliably distinguished from the surrounding tissues by DTT in the *in vivo* study (Fig. 1A-B). Using a threshold FA value of 0.6, the recovery process of the contused peripheral nerves could be clearly visualized (Fig. 2A-F). The fibers could be tracked distally at 3 weeks after the injury, with the number of fibers increasing thereafter with time. For analyzing the histology in detail, we performed electron-microscope examination. Ultrathin sections from a site distal to the site of injury indicated that the axonal structures remained relatively stable until 1 day after the operation (Fig. 3B),with the beginning of disintegration (Fig. 3C). Regenerating nerve fibers, with myelinated fibers of a smaller caliber and thinner myelin sheath as compared with that of normal nerve fibers, were clearly detectable from 3 weeks after the injury (Fig. 3D), almost reaching the pre-injury level by 12 weeks after the injury(Fig. 3F). The FA value, a parameter for constructing the DTT, was correlated with the axon density and axon diameter ($p<0.05$) (Fig. 4A-D). In addition, the FA value at the epicenter was strongly correlated with each functional parameter ($p<0.01$) (Fig. 5A-C).

Discussion

Advantage is taken of the higher anisotropy values of peripheral nerves than the surrounding tissues in the detection and evaluation of peripheral nerves using DTT. FA values of the peripheral nerves were more strongly correlated with axon-related parameters, namely, axon density and axon diameter, than with myelin-related parameters. These findings support the theories that axonal membranes play a major role in anisotropic water diffusion in neural fibers and that myelination can modulate the degree of anisotropy(Beaulieu C,NMR in biomed, 2002). DTT of the peripheral nerves may become a novel tool for the evaluation of peripheral nerves if it is applied correctly and its properties and limitations are clearly understood.

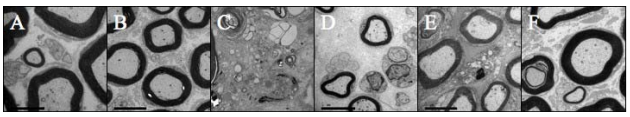

Fig. 3. Histological changes in distal sciatic nerves. A:intact, B:1d, C:1w, D:3w, E:6w, F:12w after injury. The series indicates disintegration starting within 1 week, progressive formation of myelinated axons from 3 weeks, with almost complete maturation of the axons by 12 weeks.

Conclusion

1. DTT can be used to reliably distinguish peripheral nerve from the surrounding tissues.
2. DTT is reliable for direct visualization of a contusion from immediately after the injury.
3. The FA values reliably reflect histological (esp. axonal) and functional changes, demonstrating the possible contribution of DTT to the evaluation of the some clinical events for peripheral nerve degeneration and regeneration.

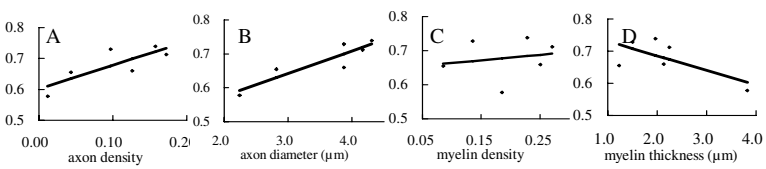

Fig. 4. Correlations between the FA value (distal) and (A) Axon density (r = 0.8034, P = 0.045). (B) Axon diameter (r = 0.9023, P = 0.014). (C) Myelin density (r = 0.1879, P = 0.7215). (D) Myelin thickness (r = -0.6817, P = 0.1359).

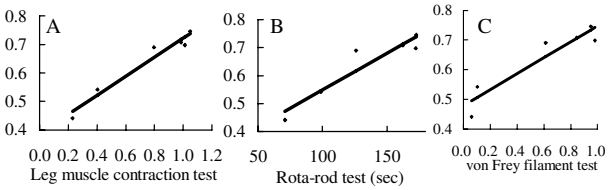

Fig. 5. Correlations between the FA value (epicenter) and (A) Leg muscle contraction test (r = 0.9773, P = 0.001). (B) Rota-rod test (r = 0.9415, P = 0.005). (C) von Frey filamet test (r = 0.9382, P = 0.005).