Early Detection of the Pathologies in Experimental Allergic Neuritis Using High b Value q-Space Diffusion MRS

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Introduction

Experimental allergic neuritis (EAN) is an animal model of immune-mediated inflammatory demyelination in the peripheral nervous system (1). This model is similar to the Guillain-Barre Syndrome in humans (1). The EAN model is characterized by two major pathologies: inflammation and demyelination with axonal loss. These pathologies are clinically expressed by severe paralysis of the hind limbs and tail at the peak of the disease (16-17 days post immunization). The first clinical signs are apparent only 10-13 days after immunization (1).

The water signal decay in diffusion experiments at high b values was shown to be multi-exponential in sciatic nerve (2) as well as in other neuronal tissues such as rat (3) and human brains (4) and bovine optic nerve (5,6). One way to analyze NMR diffusion experiment in restricted geometries is by using the q-space approach that gives the displacement distribution profile for the water molecules in the tissue. In brain tissue and optic nerve, the slow diffusing component was shown to have narrow displacement profile that hardly changes with the diffusion time (3,5). This slow diffusing component was tentatively assigned to restricted diffusion of water in axons.

In this work we used a combination of high b values q-space ^{1}H and ^{2}H DQF diffusion MRS for characterization of the EAN model in rat sciatic nerve. The q-space diffusion approach provided early detection of the EAN pathology, enabled to probe the demyelination that occurs during the progression of the disease and detected the partial remyelination that occured at the chronic stage of the disease.

Methods

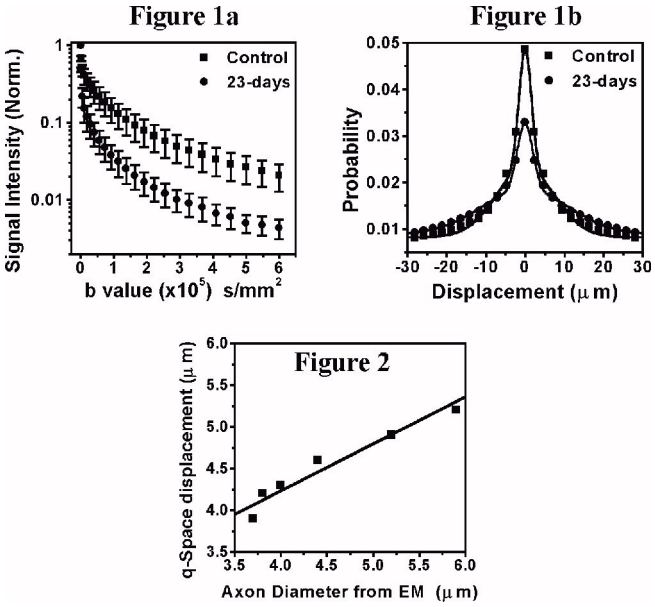
EAN was induced in 17 Female Lewis rats that were immunized by an injection of 200 µl of inoculums containing 10mg of bovine peripheral myelin (BPM) and 4mg of mycobacterium tuberculosis emulsified in complete Freund´s adjuvant (CFA). Six rats served as controls and were immunized without BPM. The sciatic nerves were excised for the NMR experiments at days 5, 9, 16, 23 days post immunization and at the chronic stage of the disease (day 90-150).

NMR experiments were performed on an 8.4T spectrometer equipped with a Micro5 gradient probe. Diffusion experiments were performed using the stimulated echo diffusion pulse sequence with the following parameters: TR/TE=3000/30ms, δ=4.5ms, G_{max}=160 gauss cm^{-1} giving maximal q value of 3065cm^{-1}. Three diffusion times were examined: 23, 63 and 163 ms. In these experiments the diffusion gradients were applied perpendicular to the long axis of the nerve. q-Space analysis of these experiments was performed as described before (5) by Fourier transformation of the diffusion data to obtain displacement distribution profiles. These profiles were fitted to a bi-Gaussian function. The diffusion anisotropy was measured perpendicular and parallel to the long axis of the sciatic nerve with b value of 2000s/mm^2 at a diffusion time of 63ms. T_2 was measured by the CPMG sequence. ^{2}H DQF diffusion experiments were performed as described before (7) with the following parameters: TR=1500ms, creation time (τ) of 28ms (to detect only the axonal component), Δ/δ=59/9 ms and t_{DQ} of 78 ms resulting in maximal q value of 1886 cm^{-1} (taking into account the coherence order).

Results and Discussion

Figure 1a shows the water signal decay of the control and the 23 days post immunization (peak of demyelination) groups at diffusion time of 163ms. Figure 1b shows the respective displacement distribution profiles showing broadening of both the broad and narrow displacement components. q-Space analysis showed that as the disease progresses the displacement of the slow diffusing component increases from 3.9±0.4 to 5.2±0.4µm for control and day 23 post immunization groups ($p<0.002$), respectively. The increase in the displacement of the slow diffusing component was apparent already at day 9 post immunization (4.6±0.1µm, $p<0.04$) although no clinical signs of the disease were apparent at this stage. At the chronic stage of the disease remyelination occurs and the displacement of the slow diffusing component decreased (4.3±0.8µm) approaching the control value. Very good correlation was found between the mean axon diameters as deduced from electron microscopy and the q-space ^{1}H diffusion MRS experiments (Fig. 2)

T_2 curves did not show significant changes in any of the stages of the disease. Diffusion anisotropy showed a significant decrease only 16 days post immunization (at the peak of clinical signs). The diffusion anisotropy factor decreased from 0.64±0.12 in the control group to 0.44±0.02 ($p<0.03$) for the day 16 post immunization group. q-Space profiles obtained from the ^{2}H-DQF diffusion experiments performed on control sciatic nerves at long creation time (30ms), to detect mainly the axonal water signal, were found to be very similar to the q-space profiles of the slow diffusing component observed in the ^{1}H diffusion experiments (displacements of 3.1 and 3.3µm, respectively). The comparison between the ^{1}H and ^{2}H-DQF q-space profiles for the control and 23 days post immunization rats showed that the isotropic water fraction (which is not detected by the ^{2}H-DQF technique) increased significantly at the peak of the disease suggesting axonal loss and formation of edema.



Conclusions

Diffusion at high b values enables early detection of the pathologies associated with the EAN model already at day 9 post immunization when clinical signs are not yet observed. q-Space analysis of diffusion experiments enables extraction of structural information that correlates well with electron microscopy. ^{2}H-DQF diffusion experiments on the axonal water produce displacement profiles which were very similar to that of the ^{1}H slow diffusing component corroborating the assignment of this component to intra-axonal water.

References

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