Assignment of the NMR ^{2}H Double Quantum Filtered Signals in Nerves and Spinal Cords to their Anatomical Compartments

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Intoduction: The technique of ^{2}H double quantum filtered (DQF) NMR enables spectral separation of the different water compartments in sciatic and optic nerves on the basis of their different residual quadrupolat interaction (1). As a result one can measure the specific NMR parameters such as relaxation times and diffusion for each compartment (2). The spinal cord consists mostly of myelinated axons and a system of supporting membranes. Nerves of the peripheral systems consist in addition to the myelinated axons, of two matrixes of oriented collagen fibers, the outer compartment, the epineurium and the matrix surrounding the axons, the endoneurium. In the present work we report the assignment of the DQF signals to the anatomical compartments.

Theoretical Background: In the in-phase version of the DQF pulse sequence (3): $90^0 - \tau - 90^0 - t_{DQ} - 90^0 - \tau - 90^0 - t_{ZQ} - 90^0$ (Acq) the signal is proportional to $\sin^2(2\pi\nu_Q\tau)e^{-2\tau/T2}$. Thus selection of the value of τ, the creation time of the second rank tensors, can maximize the signal for each value of ν_Q, the quadrupolar splitting. Further enhancement of the resolution was obtained on the basis of the different T_1 of the compartments by inserting an inversion pulse before the DQF pulse sequence.

Results: ^{2}H DQF spectra of two rat sciatic nerves are given in Fig. 1. Three pairs of quadrupolar split satellites are observed: signals A, B and C with a quadrupolar splittings of about. 1500, 500, and 200 Hz respectively. A narrow signal (D) is also observed at longer creation times. Using the T_1 weighted version, signals B and C are resolved with inversion times of 135 and 65 ms respectively, **We have assigned signal A to the water in the epineurium, signal B to the intramyelin water, signal C to the water in the endoneurium and signal D to the intraaxonal compartment.** The assignment was based on a series of ^{2}H DQF measurements and was corroborated by histology.

The following four experiments indicate that the compartments corresponding to signals A and C contain collagen and that they can be assigned to the **epineurium and endoneurium respectively:**

1. Incubation of rat sciatic nerves in collagenase resulted in a very rapid disappearance of signal A and a slower decline of signal C.

2. Signals A and C but not B and D are affected by shift reagents.

3. The ratio of signals C and B is smaller in nerve I than in nerve II in Fig 1. For the same nerves transmission electron microscopy (TEM) showed smaller amount of collagen fibers in the endoneurium for nerve I. The intensity of signal A is similar for the two nerves as is the amount of collagen in the epineurium detected by TEM (not shown).

4. Mechanical stretching of rat sciatic nerve by 20% caused approximately 50% increase in the quadrupolar splitting of signals A and C while having no effect on signal B. This result is typical to stretching of collagen fibers.

The following experiments suggest that signals B and D can be assigned to **myelin and intraaxonal water** respectively **:**

1. Signal B, with a splitting of 500 – 600 Hz is the only quadrupolar satellite detected in porcine spinal cord (Fig. 2) which lacks epineurium and endoneurium.

2. Relative to porcine sciatic nerve, in porcine optic nerve which contains a larger fraction of myelin, signal B is more prominent. It is smaller for the vagus nerve, which is partially un-myelinated. In all cases signal B was not affected by collagenase and in rat sciatic nerve it was also unaffected by the stretching of the nerve.

3. Wallerian degeneration, a spontaneous degenerative process of the distal portions of nerve, is known to affect the nerve axons and myelin sheaths. Indeed signals of compartments B and D are greatly reduced in the injured nerve.

4. It was previously found by ^{2}H DQF diffusion measurements in rat sciatic nerve, that the perpendicular diffusion of signal D was highly restricted indicating diffusion in a vessel of approximately 7μm diameter, which is the known average diameter of the axon (3). However, some contribution from cellular water to signal D cannot be ruled out.

Conclusion: The complete assignment of the different ^{2}H DQF signals opens opportunities to study the properties of the different anatomical compartments of the nerves and the water exchange among them.

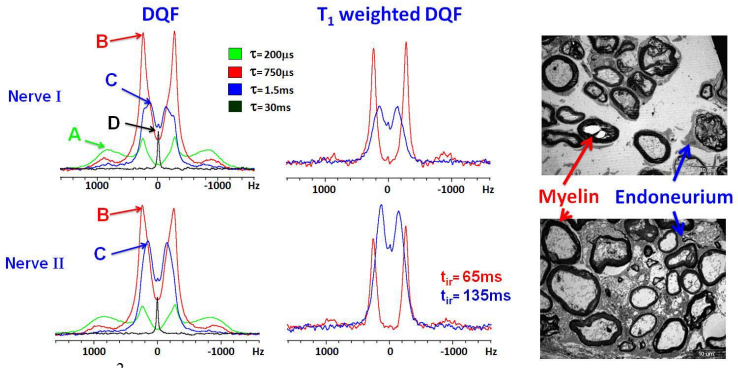

Fig 1. ^{2}H DQF spectra and TEM images of two rat sciatic nerves.

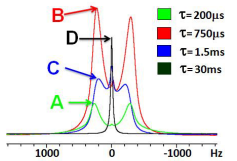

Fig 2. ^{2}H DQF spectra of porcine spinal cords.

References: 1. H. Shinar et al., JMR,**129** (1997),98.
2.Y. Seo et al., MRM, 42 (1999), 461.
3.U. Eliav et al. JMR, **137** (1999),295.