MR Properties of Neural Tissue Following Experimentally Induced Inflammation

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Introduction

Some components of inflammation commonly occur in a wide spectrum of nervous system diseases, including multiple sclerosis, stroke, dementia and traumatic brain injury. Distinguishing the processes of inflammation from neural tissue degeneration, such as axonal loss and demyelination, is challenging because of similar qualitative changes in the MR signal intensities in the T_1, T_2 or Magnetization Transfer (MT) weighted images. The purpose of this study was to measure how the process of inflammation alone affects quantitative MR measurements. To achieve this goal, we have used an experimental animal model of neural tissue inflammation [1] by injecting tumour necrosis factor alpha (TNF-α) into rat sciatic nerves.

Experimental Methods

We measured MR properties of rat sciatic nerve *in vitro* two days after TNF-α (2 µl of 8 000 U/ µl) injection in 16 samples. As controls, four untreated and four nerve samples with PBS injections (with no TNF-α) were also measured. Histopathological samples were obtained for the histomorphometric assessment of the myelin content, axonal integrity and extracellular volume fraction (toluidine blue stain) and for the immunohistologic evaluation of the inflammatory process (using antibodies for ED1, a macrophage and monocyte cytoplasmic antigen) .For quantitative evaluation of neural tissue integrity, a computer-assisted image analysis (CAIA) was performed on the toluidine blue-stained samples using image analysis software (Image-Pro Plus 4.5, Media Cybernetics, Silver Spring, MD). The myelin content and extracellular matrix volume (EM) fraction were calculated as percentages of total sampled area.

All MR measurements were performed at 20°C and 1.5 T on a 20 cm bore superconducting magnet (Nalorac Cryogenics Corp, Martinez, CA) controlled by a SMIS spectroscopy console (SMIS, Surrey, England). The MR measurements consisted of the following:

- T_1 relaxation (35 TI values logarithmically spaced from 1 to 32,000 ms);
- multicomponent T_2 relaxation (CPMG, TE/TR=1/10,000 ms, 2000 even echoes sampled and 100 averages)
- quantitative Magnetization transfer (MT) was measured using a continuous wave (cw) saturation pulse of 7 s duration. For the standard MTR evaluation, the RF saturation pulse amplitude, $\omega_1/2\pi$ was 670 Hz and the offset frequency of the saturation, Δ was 5 kHz. To quantitatively evaluate MT data [2] seven RF saturation amplitudes ($\omega_1/2\pi$ = 85, 170, 330, 670, 1330, 2670 and 5340 Hz) and 26 off-resonance frequencies Δ (ranging from 0.014 to 250 kHz) were applied. The repetition time TR was 10 s, the number of averages was eight.

T_1 data were analyzed assuming mono-exponential behavior. All T_2 decay data were fitted to a multicomponent T_2 model in which the relaxation of each T_2 component has a Gaussian distribution on a logarithmic time scale [3]. Moreover, the single measure of the T_2 relaxation, $\langle T_2 \rangle$ was evaluated. $\langle T_2 \rangle$ represents an average of the T_2 relaxation spectrum and is equivalent to the mono-exponential estimate of T_2 decay that is usually assessed in clinical MR. Quantitative MT data were fitted to a "two-pool" model [2] quantifying the exchange between an unrestricted liquid pool and a semisolid macromolecular pool of restricted mobility. The model estimates: R, the rate of exchange of longitudinal magnetization between liquid and semisolid pools as well as M_{0B}, the fraction of magnetization that resides in semisolid pool and undergoes MT exchange.

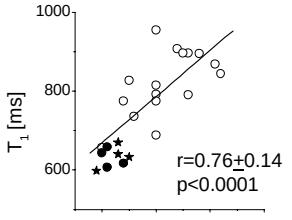
Results

Two days following TNF-α injection, a large number of inflammatory cells, and red blood corpuscles were present. Most axons were well myelinated and appeared normal, except for the increased distance between them (increased extracellular volume fraction). The immunohistologic staining revealed the presence of considerable inflammatory cells (ED1+ macrophages and monocytes). Most of the measured MR parameters changed with TNF-α treatment (Table 1).

	EM [%]	T1 [ms]	<T2> [ms]	Intermediate. T2 [ms]	M_{0B} [%]	R [s^{-1}]	MTR [%]
Control	42±2	631±17	78±3	42±3	10±2	47±2	70±1
TNF-a	52±2	819±20	96±5	78±4	5±1	47±2	65±2

The MR parameters were then compared to the quantitative morphometric measure extracellular volume fraction, EM of nerve samples. The average relaxation times, T_1 and $\langle T_2 \rangle$ and the value of the intermediate T_2 component increased with EM. The MT semisolid fraction M_{0B} and the MTR decreased, whereas the MT exchange rate constant, R was independent of EM.

Most of the MR parameters are strongly correlated with histopathology ($p<0.005$). For example, Fig1 shows T_1 as a function of extracellular volume fraction (EM) for all measured samples (normal controls solid circles, PBS controls – stars, TNF-α treated – open circles).



Discussion

TNF-α injections into nerve provide an excellent means to induce inflammation with minimal damage to axons or myelin. The quantitative comparison between measured MR parameters and histopathology showed that most of the MR parameters were sensitive to inflammation and may be used as a semi-quantitative evaluation of the degree of inflammation. On average the T_1 and $\langle T_2 \rangle$ relaxation times increased by approximately 28 and 25% respectively, whereas MTR decrease was small (approximately 5%). The changes in more quantitative MR measures, such as the value of intermediate T_2 component (approximately 83% increase) and MT macromolecular fraction M_{0B} (approximately 50% decrease) were more pronounced. Other MR parameters, such as relative curve areas of the T_2 component spectra changed only slightly with inflammation (in contrast to the processes of demyelination [3]). Interestingly, the MT exchange rate, R seems to be independent of inflammation. The moderate change in MTR due to inflammation may be explained by conflicting contributions of the MT and direct effects to the MTR [4]. In the case of inflamed nerves, the decrease in MTR caused by decreased M_{0B} is counteracted by decreased longitudinal relaxation rate, R_A.

The multi-component T2 relaxation seems to be the best technique in distinguishing between the processes of inflammation and myelin loss. The quantitative measure of MT is less successful due to competing contributions of demyelination and pH-sensitive changes in the MT effect.

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3. Webb, S et al Magn Reson Med, 2003. **49**: p. 638-645. 4. Henkelman RM et al NMR in Biomed, 2001. **14**: p. 57-64.