

Multi-parameter MRI analysis of the time course of induced muscle damage and regeneration

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Introduction

MRI can detect pathological changes at the cellular and tissue level in skeletal muscle because the wide range of parameters are available in MRI to correlate with pathological and physiological states (1). Our work compared parametric MRI to test for the ability of different imaging modalities to discriminate the time course of damage and regeneration in a model of acute, toxin-induced muscle damage, and thus to temporally distinguish underlying tissue and cellular changes. We injected a myotoxin (BaCl₂) directly into mouse leg muscle to induce rapid and focal muscle damage with subsequent degeneration and regeneration. This myotoxin damages mature muscle cells but spares the satellite cells and muscle cell basement membranes (2). Full regeneration and restoration of function occurs in about three weeks. Our hypotheses are, first that MRI protocols are differentially sensitive to cellular changes during the phases of muscle damage, necrosis, inflammation and regeneration. We test this by using the several MRI methods mentioned above on sequential days during the course of damage and recovery. Secondly, we test whether MR modalities have different time courses corresponding to the temporal sequence of events at the cellular and tissue levels. We also made histological analyses of control and injected muscle to characterize cellular and tissue changes and to test whether they correspond to the time course of MR images.

Methods

We analyzed the changes in mouse lower limb musculature following localized injection of 50 μ L of BaCl₂ (0.125 M) by multi-modality MRI and post-mortem histological analysis of toxin-injected muscles compared to un-injected controls. MRI was conducted on a Bruker 4.7T horizontal bore magnet (Bruker Corp., MA) equipped with Varian INOVA spectrometer (Varian, Inc., Palo Alto, CA). A ¹H volume coil (25 mm inner diameter coil from Doty Scientific) was used to acquire MR images. Fourteen eight week old male C57Bl/6 mice were used for MR imaging on the 4.7T MR scanner with 4 pulse sequences: with and without magnetization transfer (MT) effect, single echo multi-slice sequence for T₂ measurements with three TE values (13, 30 and 60 ms), diffusion weighted imaging sequence to measure ADC values with three b values of 0, 573 and 1123 s/mm² and 3 dimensional (D) gradient echo sequence.

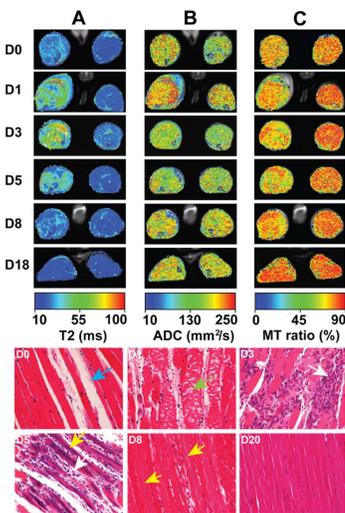


Figure 1. T₂, ADC and MT ratio maps for 0 - 18 days post injections of BaCl₂ and their corresponding histology (H&E) images.

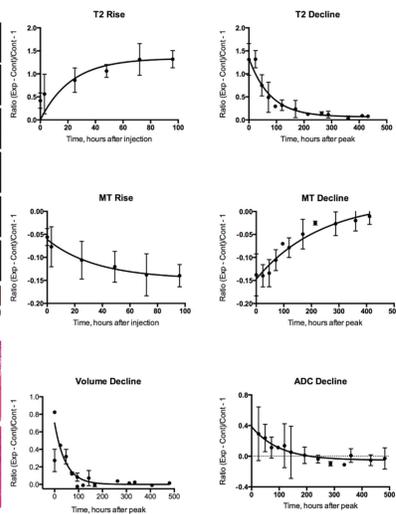


Figure 2. Nonlinear data fitting for rise or decline of MR parameters to determine their rate constants.

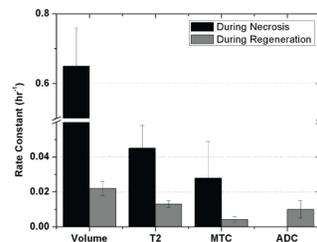


Figure 3. Rate constants for rise or decline of volume, T₂, MT ratio and ADC parameter.

Results and Discussion

The damages detected by several MRI modalities are transient and recover within 3 weeks. Diffusivity increases within the first hours after injection of myotoxin and subsequent edema changes nearly in parallel well before

cellular changes are noted by histology. The rate of rise in volume is statistically faster than the rates of rise of T₂ and MT ratio. Histological analyses showed changes in structural and cellular features of the leg that matched the progression of MR images. Figure 1 shows the temporal evolution of MR images (T₂, ADC and MT ratio) in one animal studied frequently from day of injection through day 18 of recovery and their relevant representative images of hematoxylin and eosin (H&E) staining muscle tissues from other mice at different time points after myotoxin injection. Muscle degeneration and regeneration processes are noticed with

edema (blue arrow), myofiber fragmentation (green arrow), macrophages (white arrow) and regenerating myofibers (yellow arrows). There is a clear separation between the evolution of MR image changes during damage and necrosis to about day 5 and regeneration thereafter, as expected because the underlying cellular processes are so different. The time course of changes in the microscopic structure of the leg musculature matched the time course of changes in the MR images, with maximal changes occurring at 3 to 5 days following injection of myotoxin. All data for volume, T₂, ADC and MT ratio were grouped into rising and falling phases so that the rates for each could be quantified as shown in Figure 2. Then time- and animal-averaged data were fitted into two sections to exponential curves by regression analyses. The data were fitted to the equation: relative intensity at time t = initial intensity + (plateau - initial intensity) * (1 - exp (K*t)), where K is the rate constant. The rate constants obtained are summarized in Figure 3.

Conclusions

A separation is possible in the kinetics of the MRI changes measured during development of necrosis and regeneration. Thus non-invasive multi-modality MRI has feasibility for identifying tissue and cellular necrosis and regeneration.

References

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