DTI-based assessment of ischemia-reperfusion injury in mouse hind limb

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Introduction:

Skeletal muscle ischemia-reperfusion injury is commonly encountered in association with trauma, peripheral diseases, and compartment syndrome. Typically, prolonged skeletal muscle ischemia and reperfusion impose metabolic stresses that lead to energy depletion and cell swelling and eventually result in tissue damage and limb dysfunction [1]. T_2 is a widely accepted MRI measure for irreversible tissue damage. Recently, diffusion-weighted (DW) MR has been proposed as an earlier marker of tissue ischemia. For the brain, DW- MRI provides an early marker for detection of ischemia and a predictor for long-term injury [2]. The aim of this study was to investigate the diffusive properties of skeletal muscle during and after acute ischemia, to determine whether diffusion MRI is sensitive to muscle ischemia and to ischemia-induced muscle injury.

Materials and Methods:

Protocol: Two conditions of ischemia were studied: (a) by applying a tourniquet around the upper leg for 50 min; (b) by applying the tourniquet for 50 min and stimulating the dorsal flexors with 1 Hz twitches, which corresponds to 4-6 hr of ischemia [3]. A total of 11 DTI and T_2 scans were acquired: 2 before ischemia, 2 during ischemia (n=6 cuff and n=9 cuff and stimulation), 5 during reperfusion and 2 after 24 hr. Control measurements were obtained without any intervention (n= 3) and with only stimulation (n=4). Also force measurements and histology were performed.

Animal model: Female C57BL/6 mice were used. An electrode for electrical stimulation was implanted on the common peroneal nerve 2-3 weeks before scanning. Mice were anaesthetized with isoflurane (1.0-1.5% in air). The body temperature was maintained at 38°C and the animal's respiration was continuously monitored. After the MR experiments the mice were perfusion fixated with formaldehyde for histological analysis.

MRI: MR was performed with a horizontal 9.5 cm bore, 6.3 Tesla MRI scanner using a 1.5 cm solenoidal RF coil. A diffusion-weighted spin echo sequence with fat suppression was used with diffusion gradients applied along 6 non-collinear directions and one reference image was recorded without diffusion weighting. Scan parameters were: FOV=15x15 mm^2, matrix size=64x128, NSA=2, TE=30 ms, TR=1 s, Δ=13 ms, δ=8 ms and b-value=0 or 1142 s/mm^2. A multi-echo spin echo sequence with fat suppression was used to obtain T2 maps, with: six echoes, FOV=15x15 mm^2, matrix size=64x128, NSA=2, TE=13.3 – 78.9 ms, TR=4.

Data analysis: From the DTI datasets the diffusion eigenvalues, ADC (Trace(D)/3) and fractional anisotropy (FA) were calculated. Mean values were obtained from an ROI within the tibialis anterior (TA) and gastrocnemius (G). For each animal the values were normalized to the initial values before averaging all the datasets.

Results and Discussion:

For the TA the initial means (± SD) of the three eigenvalues λ_1, λ_2 and λ_3 were $(1.64 \pm 0.08)\times10^{-3}$, $(1.18 \pm 0.06)\times10^{-3}$ and $(0.87 \pm 0.04)\times10^{-3}$ $mm^2 \cdot s^{-1}$, respectively, while the mean ADC, FA and T_2 were found to be $(1.23 \pm 0.05)\times10^{-3}$ $mm^2 \cdot s^{-1}$, 0.31 ± 0.02 and 19.3 ± 1.5 ms, respectively. For the interventions reported below, the above MRI indices were normalized to the pre-ischemia, control situation.

The MRI indices remained constant in the control experiments without intervention (data not shown), indicating that there are no effects of anesthesia, etc on the parameters reported here.

In fig 1a the time course of ADC, FA and T2 for the TA with cuff only are plotted. The changes were small and the parameters largely normalized upon reperfusion. This was also the case for both interventions in the non-stimulated gastrocnemius (data not shown). Histology also showed no signs of damage. These results, along with previous studies [3] where the critical ischemic time was estimated to be 4 hr before severe changes in energy metabolism and muscle viability occur, indicates that there is no tissue damage in the case of only cuffing.

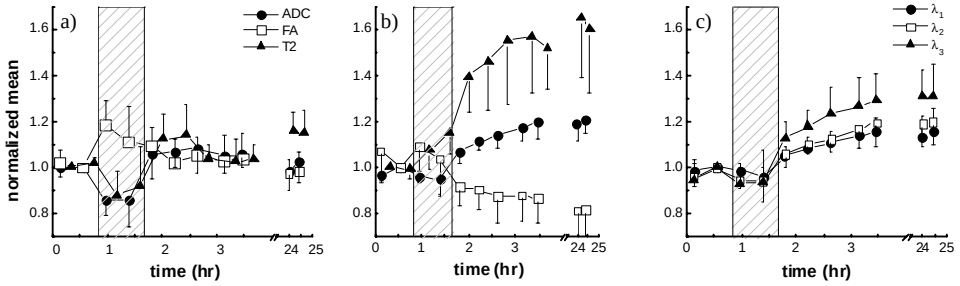

Fig 1. *Relative changes in MRI parameters for the TA. **(a)** ADC, FA and T_2 for occlusion only, **(b)** ADC, FA and T2 for occlusion and stimulation of dorsal flexors, **(c)** DTI eigenvalues for occlusion and stimulation. Bar represents ischemic period.*

Fig. 2 shows typical examples of parametric images collected during the occlusion and stimulation protocol. During the intervention parameter values remained essentially constant (Fig 1b), except for a slight T2 prolongation in the TA region that was electrically stimulated. Large reductions in high-energy phosphates have been reported under these conditions [3]. This suggests that energy depletion alone is not accompanied by water diffusion changes in skeletal muscle.

Examples of parametric maps of ADC, T2 and FA collected in the reperfusion phase following an episode of cuffing/stimulation are depicted in Fig. 2 c and d. Fig 1b shows a steady increase of ADC and T2 and decline of the FA in TA. These indices remained essentially unchanged in the other muscles. The change in FA in the TA region was mainly due to an increase in λ3 compared to λ1 and λ2 (Fig 1c). The latter change in diffusion indices is most probably caused by cell swelling and disintegration. The latter is corroborated by histology, which showed loss of striation and monocyte infiltration in the TA (not shown). Welsh et al. [3] reported that reperfusion following this type of intervention results only in a partial recovery of energy metabolism. For these reasons, it is likely that the observed changes in diffusive properties report on irreversible tissue damage. It is important to note that fig 1b shows similar time courses for changes in DTI indices and T2 and that the relative change in T2 is larger than that in ADC and FA.

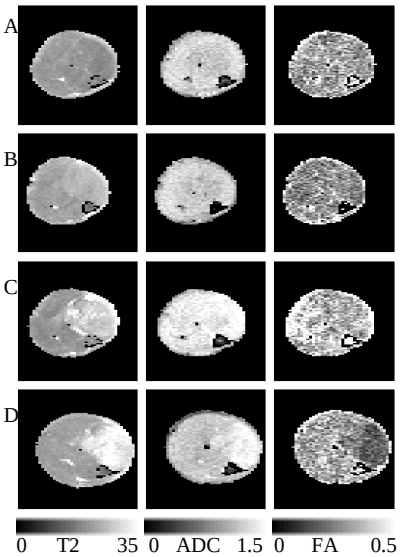

Fig. 2. *Parametric images at different stages of occlusion and stimulation of dorsal flexors. **(a)** before intervention, **(b)** during cuff and stimulation, **(c)** 1.5 hr after start reperfusion, **(d)** after 24 hr. T_2 (ms), ADC ($x10^{-3}$ $mm^2 \cdot s^{-1}$).*

Conclusion:

DTI indices remained unchanged during severe muscle ischemia and dynamically changed following reperfusion. These findings suggest that DTI can be used to measure the development of muscle injury induced by ischemia-reperfusion.

References:

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