Bi-exponential T2 Decay in Hydrodynamic Vascular Gene Therapy

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Introduction

Intravascular delivery of naked plasmid DNA is a promising method for gene delivery for several diseases, including skeletal muscular disorders such as Duchenne's muscular dystrophy [1]. Monitoring gene expression can be difficult, and the mechanisms and effects of the procedure at the cellular level are under investigation in several potential target tissues including muscle and liver [2,3]. A less invasive method for measuring these effects might be beneficial. Here, the measurement of T2 signal decay is investigated.

Methods

Imaging was performed on a 1.5T MRI scanner (Signa HD, GE Healthcare, Milwaukee WI, USA). Five rhesus monkeys were imaged, with one forearm and the opposite foreleg injected in the first session, and the remaining two forelimbs injected in the second session, for a total of 20 pDNA injections. All experiments were approved by our institution's Animal Care and Use Committee.

The intravascular hydrodynamic pDNA delivery procedure (Pathway IV, Mirus Bio, Madison WI, USA) has been described in detail elsewhere [1,4]. In this study, an inflatable tourniquet proximal to the elbow (or knee) was inflated to 475 mm Hg for 2-5 min to temporarily suspend circulation in the forelimb. A solution of normal saline (volume adjusted by limb size) and 5-17 mg of pDNA encoding a luciferase reporter gene was injected into a forelimb vein at 1.50-2.0 ml/sec. The solution ideally extravasates into the myofibers, and swelling of the target muscles results. After the injection, the tourniquet was released. Transverse multislice 2D T2-weighted fat-suppressed FSE imaging was performed before and after the procedure to visualize edema in individual muscles (TR/TE/ETL/FOV/Matrix=3500/52/8/12cm/384x256). A CPMG T2-mapping sequence (T2map, GE Healthcare, Milwaukee WI) was used to acquire T2-decay curves in the same scan planes (2400/8.7/16/12/256x128). Because the sequence was limited to 16 echoes, it was acquired twice; once with minimum echo spacing (8.7-8.8 ms), and once with 24ms echo spacing, with the resulting T2-decay curves combined to measure both short and long T2 components. ROIs were drawn around each primary muscle group in each slice, and each muscle's ROIs were pooled to form a single volume of interest (VOI) for each muscle. Non-negative least square (NNLS) analysis [5,6] and multi-exponential curve fitting [6,7] were used to determine the number and relative magnitude of the T2 components.

Results

After injection using intravascular hydrodynamic delivery, the limb shows muscle swelling and edema; an example image from the T2-measurement sequence at TE=50ms is shown in Fig. 1 for one right leg (with an ROI shown for the lateral gastrocnemius muscle). NNLS fitting of the T2-decay curve for each muscle demonstrated two primary T2 components in post-treatment limbs (Fig. 2). For the same data, bi-exponential curve fitting also showed a significant long T2 component (Fig. 3), suggesting an increased extra-cellular water component. After measuring the change in muscle volume on the T2 weighted images from pre- to post-treatment, the change in intra-cellular (IC) volume was estimated. For the limbs analyzed here, the total muscle volume change was greater than zero for each muscle (●'s in Fig 4), but the mean estimated IC volume change (○ in Fig. 4) was seen to be approximately zero, and did not correlate with measured luciferase expression for individual muscles.

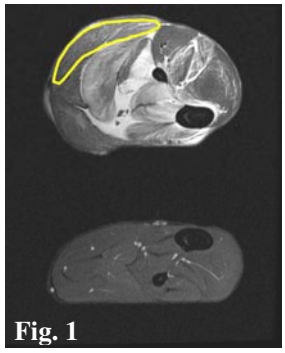

Fig. 1

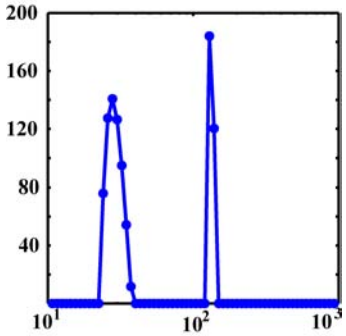

Fig. 2

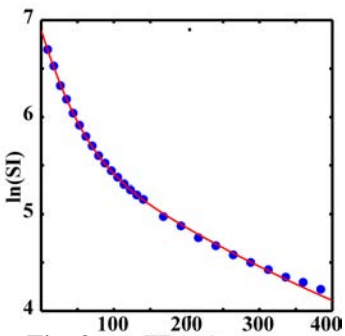

Fig. 3

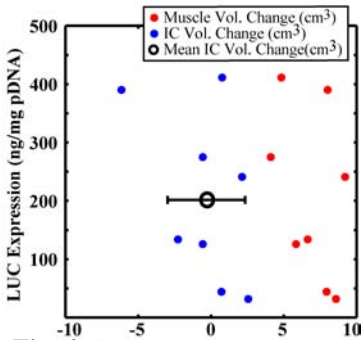

Fig. 4

Discussion

The forelimbs of non-human primates treated with intravascular hydrodynamic pDNA gene therapy demonstrate swelling and an increase in signal intensity on T2-weighted images. T2-decay measurements following treatment showed bi-exponential decay, and suggested an increase in the volume of the extra-cellular water compartment [6] but not the intra cellular component. This result is consistent with previous findings in a rat model, in which interstitial edema (i.e. extra-cellular water) was shown to be present by histology at one hour after treatment, and resolved by 24 hours post-treatment [2]. While the average intra-cellular volume change was zero, the large uncertainty might be related to difficulty in estimating muscle volume using the current measurement techniques, as well as grouping all voxels from a muscle into a single ROI.

References [1] Hagstrom JE, *et al., Mol Ther,* **10:** 386-98 (2004). [2] Toumi H, *et al. Mol Ther,* 13: 229-36 (2006). [3] Budker VG, *et al., J Gene Med*, 8: 874-88 (2006). [4] Vigen K, *et al.,Proc. 13th ISMRM,* 2151 (2005). [5] Whittall KP, MacKay AL, *J Magn Reson,* 134-52 (1989). [6] Gambarota G, *et al., Magn Reson Med*, 46: 592-99 (2001). [7] Ababneh Z, *et al., Magn Reson Med*, 54: 524-31 (2005).