MRI-Guided Intravascular Plasmid DNA Delivery

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Introduction

Intravascular delivery of naked plasmid DNA (pDNA) is being developed as a means of gene delivery in a variety of diseases [1-3], including skeletal muscular disorders such as Duchenne's muscular dystrophy. One method of delivering pDNA vectors suspends blood flow into the region of interest (for example, forelimb muscles) and delivers at high pressure a solution of saline and the pDNA which extravasates into the muscle fibers. Monitoring of gene expression is difficult, involving invasive and potentially imprecise post-treatment biopsies of the targeted tissue in humans, or post-mortem examination in animal subjects. The objective of this work is to develop and evaluate techniques for monitoring intravascular pDNA delivery using 3D T1-weighted volume imaging and fluid-sensitive sequences for determining the distribution of the injected solution.

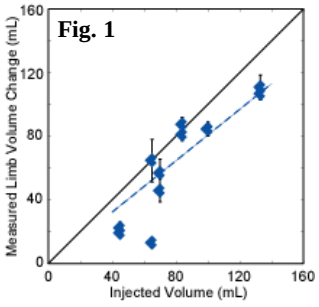
Methods

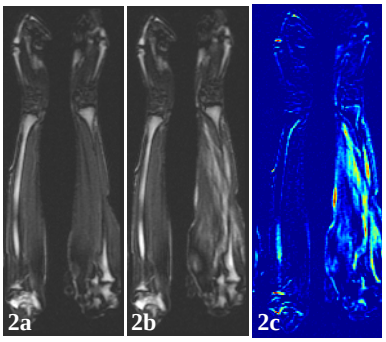
All imaging was performed on a 1.5T MRI scanner (Signa Excite 1.5T, GE Healthcare, Milwaukee WI). Three rhesus monkeys were each injected and imaged in two scan sessions. One forearm and the opposite foreleg were injected in the first session, and the remaining two forelimbs were injected in the second session, for a total of 12 pDNA injections. All experiments were approved by our institution's Animal Care and Use Committee. T1-weighted 3D SPGR images (64 slices, slice thickness = 1.1-1.4 mm, matrix = (1.1-1.4mm)x(1.1-1.4mm)) covering one forelimb were acquired to determine the total arm volume. The volume for each arm was determined after threshholding, filtering, and dilation and erosion operations on the image volume. High-resolution T2-weighted fat-suppressed FSE images transverse to the limb were also acquired in a baseline measurement (FOV = 12x12, Matrix = 384x256, 16 slices, 3 NEX, T_{scan} = 5:40).

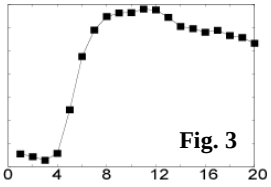
An inflatable cuff placed immediately proximal to the elbow (or knee) was inflated to 475 mm Hg to temporarily suspend circulation in the forelimb. A solution of normal saline and 5 mg of pDNA encoding a luciferase reporter gene was injected into a forelimb vein under high pressure using an MR-compatible injector (Spectris, Medrad, Indianola, PA). In this situation, the solution ideally extravasates from the postcapillary venules into the myofibers [3], resulting in swelling of the target muscle. A multiphase 3D SSFP sequence was used to monitor the injection, with the injected saline expected to have high signal. After the injection, the cuff was released, and the 3D SPGR acquisition was repeated to confirm the resulting increase in limb volume and delivery of the solution distal to the pressure cuff. Transverse T2-weighted fat-suppressed FSE scans were repeated, and showed increased levels of fluid in the forelimb muscles.

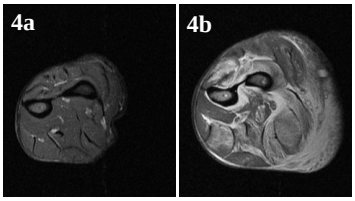
Results

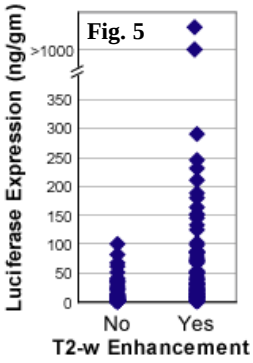
The volume of each injected forelimb was measured from the 3D SPGR images, and the pre- and post-injection difference was compared to the known injectate volume (Fig. 1). With the exception of one injection, in which the cuff was noted to be not properly inflated, all or nearly all of the solution was delivered to each forelimb (b=0.81+/-0.04, R^2=0.75). The multi-phase 3D SSFP acquisitions demonstrated swelling of the forelimbs, and the arrival of fluid into the muscles. Fig. 2a is one slice of a pre-injection phase; Fig. 2b shows the same slice 35s after the injection began; and Fig. 2c gives the difference image. The time course of signal enhancement in one muscle group is shown in Fig. 3. The pre-injection T2-weighted FSE images (one slice shown in Fig. 4a) were compared to post-injection images (Fig. 5b); individual muscles were identified, and the presence of fluid causing signal enhancement within the muscles was noted. Regions of signal enhancement were compared to luciferase expression for six injections that used the pDNA (pCI-Luc-K) vector [3]; the results are shown in Fig. 5. While the mean enhancement level was higher for enhanced muscles, some enhanced muscles did not show significant expression.











Discussion

We have demonstrated the use of MRI for monitoring and verifying the delivery of a naked pDNA and normal saline solution to the forelimbs in non-human primates. Pre- and post-injection volume measurements using a 3D SPGR acquisition may be used to verify the delivery of the solution volume. Multiphase 3D SSFP acquisitions allowed monitoring during the injection. High-resolution T2-weighted FSE imaging allowed the identification of individual muscles, and indicated the distribution of the injected solution in the muscle tissue. The results suggest that signal increase in the T2-weighted images is a necessary factor, but alone may not be sufficient for determining gene expression.

References

[1] Zhang G, *et al.*, *Hum Gene Ther*, **12:** 427-38 (2001). [2] Herweijer H, *et al.*, *Gene Ther*, **10:** 453-58 (2003). [3] Hagstrom JE, *et al.*, *Mol Ther*, **10:** 386-98 (2004).