In vivo MR Study of Muscle Damage Following an Electrotransfer Gene Delivery Protocol

I. J. Rowland[1], H. J. Simonsen[1]
[1]Danish Research Centre for Magnetic Resonance, Hvidovre, Denmark

Introduction:
In vivo gene delivery using electroporation-based methods is rapidly becoming an established technique. Gene transfer into muscle can, using electrotransfer techniques, be achieved with high efficiency (1). The application of short, high voltage electric pulses results in high electric currents passing through the tissue for short periods of time. Here we have used quantitative water diffusion measurements to investigate tissue damage that may occur as a result of the application of typical electrotransfer protocols, following animals for up to 14 days. To achieve maximum gene transfer into muscle, minimal irreversible muscle fibre damage is essential. The aim of this study was to investigate electric pulse induced tissue effects quantitatively using MR and assess the approach's suitability for optimising gene transfer.

Methods:
Male Sprague Dawley rats were anaesthetised using an IP administered Hypnorm/Dormicum/Atropine mixture. 10 pulses of 150 V/cm were applied for 20ms at 1Hz using a Cyto Pulse PA-4000 Electroporation device and a home-built needle system. The needle system consisted of an array of two rows of 4 needles inserted into hind limb muscle. Each needle was separated by 2 mm, the rows were separated by 9 mm. The needle arrays were positioned perpendicular to the long axis of the muscle fibres. Imaging was performed using a Varian SISCO 4.7 T system utilising a home built quadrature coil. The limb was positioned in the coil and aligned approximately parallel to the z-axis. T1W, T2W and quantitative diffusion images were acquired. Procedures were performed with permission of the Danish Animal Inspectorate.

Results:
A typical T2W image for an animal at 2 hours after electrical insult is shown in figure 1. Note the heterogeneity of the signal intensity with high intensity regions indicating the position of needle placement and maximum electrically induced oedema. To visualise whole muscle effects, quantitative diffusion data for ROIs covering the whole of an axial slice (excluding bone and sub-cutaneous lipid) through the limb were summed for 4 contiguous slices for each time point and is shown for all animals in figure 2.

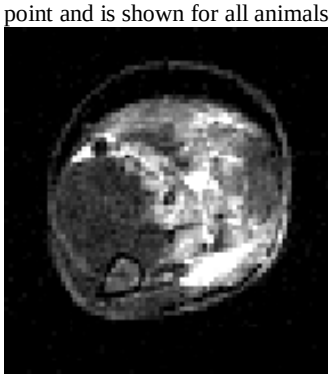

Figure 1: T2W image of a rat hind limb 2 hours after the application of 10 x 150 V/cm 20ms pulses applied at 1Hz. Note the heterogeneous high signal intensity attributable to tissue oedema and the apparently unaffected muscle group(s).

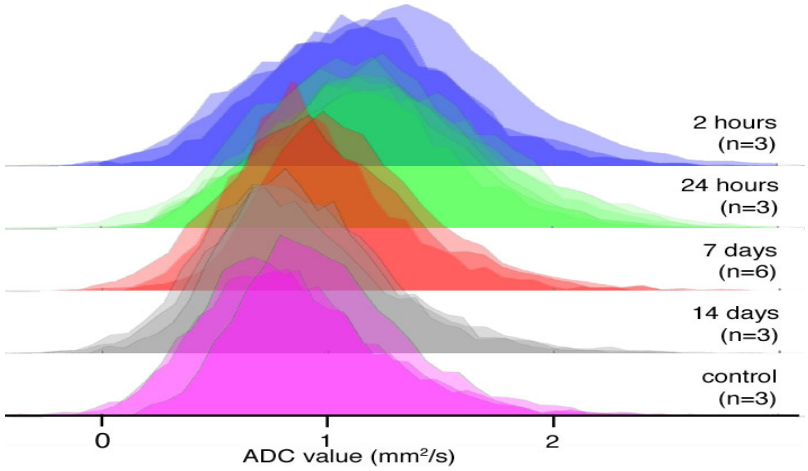

Figure 2: Normalised histogram representation of the summed muscle ADC values in 4 contiguous muscle slices following electrical insult for all animals. Note the broader distribution and higher ADC values for the 2 hour group and the 'sharpening' of the distribution with time due to muscle repair.

Discussion:
We have previously shown significant changes in T2W signal intensity and water diffusion attributable to muscle oedema following the application of high voltage electric pulses to rat hind limbs (2). The present study, however, has utilised pulses typically used for gene therapy applications and has demonstrated significant muscle effects using MR. Furthermore, this study shows that oedema reduces significantly over a 14 day period and that MR methods appear ideally suited to measure tissue effects due to electric pulses and may, therefore, be used to optimise gene transfer protocols.

Acknowledgements:
We would like to thank the Alfred Benzon Foundation for their financial support and Cyto Pulse Inc, USA for the loan of the electroporator.

References:
(1) Gollin H, McMahon J, Wells KE, Wells DJ. Gene Therapy 2003,10,504-512
(2) Rowland IJ, Vejby Søgaard L, Broberg N, Simonsen HJ, Stensgaard A. Proc 11th annual meeting ISMRM, 2003,1206