Evaluation of Acute Myocardial Ischemia Gene Therapy Efficacy Using Diffusion Tensor Imaging

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INTRODUCTION

Coronary heart disease is the main cause of ventricular systolic dysfunction and subsequent heart failure [1]. Evaluation of the efficacy of therapeutic interventions for restoring cardiac function via myocardial regeneration necessitates noninvasive techniques to characterize the cellular remodeling. Diffusion tensor imaging (DTI) [2] has been used to quantitatively examine the microstructure of the normal [3] and infracted myocardium [4], but not the post-infarction regenerating myocardium. The goal of the current study is to assess the utility of DTI for evaluating the cellular remodeling, hence the treatment efficacy, in the post-infarct regenerating myocardium.

MATERIALS AND METHODS

Animal Procedures: New Zealand white rabbits (2.5 – 3.5 kg), following ligation of the circumflex coronary artery, were either (N = 5 each) (a) given a novel ischemia-inducible plasmid construct expressing vascular endothelial growth factor in a water-soluble lipopolymeric carrier as described previously [5], or (b) left untreated to serve as control. Four weeks later, the animals were sacrificed, and the hearts were excised and perfusion fixed using 10% formalin. *Imaging:* DTI was conducted on a 7.0 T Bruker Biospec scanner using standard 3D spin echo sequence (500/22 ms TR/TE, 128 x 96 x 96 matrix size at 0.47 mm isotropic spatial resolution). Each dataset consisted of a non-weighted image and images with diffusion encoded (4 ms duration, 14 ms separation, nominal b-value of 1100 s/mm^2) along 12 optimized gradients directions [6]. *Post Processing:* Diffusion tensors were computed on a pixel-by-pixel basis via nonlinear least squares fitting. The fractional anisotropy (FA) index [7], mean diffusivity λ-mean, longitudinal diffusivity (i.e., largest eigenvalue) λ1, and transverse diffusivity λt (average of λ2 and λ3) were obtained from the diagonalized tensor eigenvalues. For consistency, ROI for the infarct region was defined in the apex of each heart using the papillary as landmark. To account for the variability among specimens, a second ROI of approximately same size was defined in the healthy left ventricular free wall. Each DTI parameter was averaged over the infarct-ROI and normalized to the healthy-ROI mean, and the parameters between the treated and control groups were compared via paired t test.

RESULTS AND DISCUSSION

Figure 1 shows a MRI image of a representative untreated heart with myocardial infarct and digitized H&E-stained histological section of the same heart. The infracted myocardium is characterized by lighter staining, reflecting the scarring and lower density of myocytes. The DTI-derived scalar diffusion parameters, ROI-averaged and normalized, for both the treated and control groups are tabulated in Table 1. The untreated myocardial infarct exhibits reduced diffusion anisotropy (specifically, a decrease of FA by 38%), which is consistent with a previous study of myocardial infarct in the rat heart [4]. Whereas the previous study correlated the reduction of diffusion anisotropy to increased disarray of the local myocardial fibers, the current work reveals that it is linked to an increase (25%) of the transverse diffusivity but unchanged longitudinal diffusivity. Compared to the control group, gene therapy of the myocardial infarct showed a significant increase of the FA, albeit its value remains reduced from the healthy myocardium. Examination of the individual diffusivities reveals that the FA increase is associated with an increased longitudinal diffusivity, while the transverse diffusivity remain elevated by the same degree compared to the healthy myocardium. Although the exact causes of the above observations need to be confirmed by histology, they likely reflect the cellular reorganization of the myocardium (e.g., increased myocyte density and more aligned myocardial fibers) induced by the gene therapy.

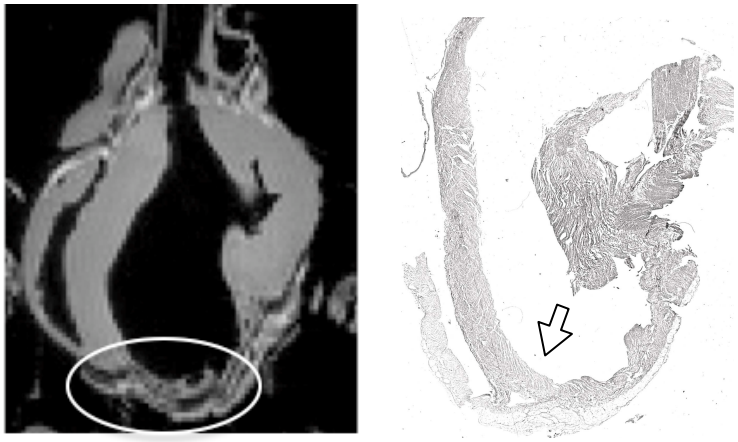

Figure 1. MR image (left) of an untreated heart with the infarct highlighted by the circle, and digitized histological section (right) revealing lighter H&E staining in the infarct (arrow).

Table 1. ROI-averaged and normalized DTI parameters obtained for the treated and control groups (mean ± SD, N = 5 each). Paired t tests reveal that the differences are significant between the group means in the FA ($p < 0.021$) and λ1 ($p < 0.008$).

Group	FA	λ-mean	λ1	λt
Control	0.62 ± 0.03	1.13 ± 0.03	0.99 ± 0.03	1.25 ± 0.05
Treated	0.80 ± 0.05	1.21 ± 0.03	1.13± 0.03	1.28 ± 0.03

CONCLUSIONS

DTI was used to characterize the cellular microstructure of untreated and gene therapy-treated myocardial infarct. Results show that myocardial infarct caused a reduction of the water diffusion anisotropy (i.e., FA) attributed to an increase of the transverse diffusivity. Moreover, gene therapy resulted in partial but significant recovery of the diffusion anisotropy caused in part by increased longitudinal diffusivity, both of which likely reflect cellular remodeling of the regenerating myocardium. These findings provide a basis and pave the way for *in vivo* DTI studies of the recovering heart to quantitatively evaluate the treatment efficacy of acutely myocardial ischemia.

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