Diffusion Tensor MRI Reflects Age-Associated Changes in Normal and Cardiomyopathic Syrian Hamsters

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Introduction

The T02 Syrian hamster is a widely used animal model of dilated cardiomyopathy (DCM). Myocardial remodeling in T02 hamsters involves the development of tissue fibrosis, frequently accompanied by the calcification of the scar tissues. Previously, Gd-enhanced MRI has been used to delineate the scar tissues in T02 Syrian hamsters [1]. However, such study cannot characterize the detailed structure of myocardial fibers and scar tissues. Recently, diffusion tensor MRI (DTMRI) has been used as a non-destructive tool to elucidate myocardial remodeling in post-infarct hearts [2]. In the current study, we aimed to explore the potential of DTMRI in staging disease progression in cardiomyopathic Syrian hamsters.

Methods

Subjects Six (n=4) and nine (n=4) month old T02 hamsters were characterized in this study. Normal age-matched F1B hamsters were used as the controls (n=4 for each age group). Excised hearts were retrogradely perfused with Krebs buffer to wash out the blood and perfusion-fixed with 10% formalin for DTMRI studies.

Diffusion Tensor MRI One day before imaging, hearts were rinsed and suspended in 1X PBS. Diffusion weighted images were acquired on 9.4T Bruker scanners under room temperature using spin-echo sequence with bipolar diffusion gradient. Seven 1 mm thick short-axis slices were acquired to cover the whole left ventricle (LV). Imaging parameters were: TE, 20 msec; TR, 2.5 sec; δ, 5 msec; Δ, 8.52 msec; Diffusion direction, 6; FOV 1.3x1.3 cm^2; b, 1000 s/mm^2; Number of averages, 4; Matrix size, 128x128; Resolution, 102x102μm^2.

Data Processing Diffusion tensor matrix and the three corresponding eigenvalues were calculated from diffusion-weighted image set using a MATLAB-based software developed in our lab. Averaged diffusivity map, defined as the average of the three eigenvalues, was normalized to that of the surrounding PBS solution to minimize the variation in diffusivity caused by temperature fluctuation. Fractional anisotropy (FA) map was generated to quantify diffusion anisotropy.

Histology Following DTMRI study, hearts were sliced at 1 mm thickness from base to apex along the LV long-axis for histological analysis. Each slice was embedded in paraffin and sectioned at 4 μm. The tissue sections were stained with Masson's trichrome to identify myocardial lesions and with von Kossa to assess calcium deposition. Scar locations from histology were compared with DTMRI images.

Statistical Analysis All results were expressed as mean ± SD. Unpaired student's *t*-test was used for intergroup comparison of the parametric variables. A 2-tailed value of P<0.05 was considered significant. Two-way ANOVA was performed for comparison of helix and transverse angle from the four groups.

Results

Compared to the controls, T02 hamsters exhibited significant ventricular dilation in both 6- and 9-month groups. Significant wall thinning was also observed in T02 hamsters. In addition, 9-month old F1B hamsters also exhibited enlarged left ventricle compared with 6-month old F1B hamsters (P<0.05). (Table 1).

Table 1. LV diameter and wall thickness (WT) of Syrian hamsters. n=4 for each group.

	LV Diameter (mm)	WT (mm)
9 month F1B	5.48±0.09 * †	1.36±0.06 †
6 month F1B	3.96±0.59 †	1.50±0.37
9 month T02	5.72±0.30	1.05±0.05
6 month T02	5.74±0.64	1.08±0.21

*P<0.05, 6 mo vs 9 mo; †P<0.05, F1B vs T02.

A significant increase in all three diffusion eigenvalues was observed in 9 month old groups of both T02 and F1B hamsters (Fig. 1). As a result, normalized average diffusivity increased with age from 0.46±0.01 to 0.51±0.04 for T02 hamsters and from 0.40±0.03 to 0.45±0.03 for F1B hamsters (P<0.05). 9-month old T02 hamsters also showed a significantly decreased FA by 19% compared with 6-month old T02 hamsters.

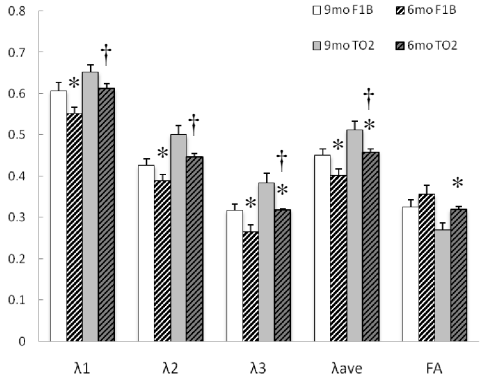

Fig. 1. Primary, secondary and tertiary eigenvalues, averaged diffusivity and FA values in six and nine month old hamsters. All values are normalized to the corresponding values of surrounding PBS solutions. *P < 0.05, 6 mo vs 9 mo; †P<0.05, F1B vs T02.

In addition to age-associated changes in diffusion parameters, 6-month old T02 hamsters exhibited increased diffusivity compared to their age-matched controls (P<0.05). 9-month old T02 hamsters also showed a trend of increased diffusivity. However, no statistical significance was detected. Besides the changes in diffusivity, T02 hamsters also showed a trend of decrease in FA in both age groups (Fig. 1). For those regions with average diffusivity 1 SD above the mean values, a 35% reduction in FA was observed.

Histology revealed that fibrotic lesions expanded with age in T02 hamsters (Fig 2a&b). Both T02 and F1B hamsters showed enlarged extracellular space in 9 month old groups (Fig.2c&d).

Helix angles changed continuously from +60 degree at endocardium to -40 degree at epicardium in all groups. Transverse angles were within ±10 degrees. Two-way ANOVA revealed that age or CM had no significant effects on helix or transverse angles.

Conclusion

An increase in diffusivity and a decrease in diffusion anisotropy associated with aging and pathological remodeling were observed in both T02 and F1B hamsters. Histology examination revealed that fibrotic lesions expanded with age, leading to increased extracellular space. This increase in extracellular space may elicit less restriction on water diffusion, resulting in increased diffusivity and decreased diffusion anisotropy. The preserved transmural helix and transverse angles suggest that the global fiber structure for both collagen scaffold and myocytes was well preserved in CM and aged hearts. This study shows that DTMRI has the potential to quantitatively stage microscopic tissue remodeling without the need for exogenous contrast agent.

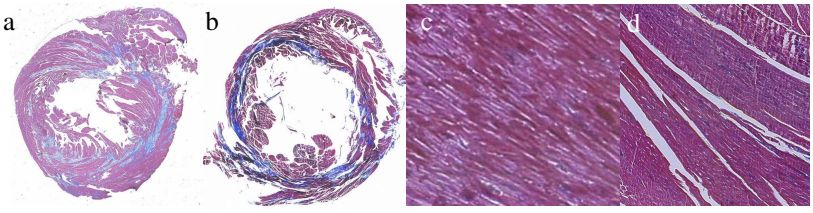

Fig. 2. Mid-LV slices stained with Masson's trichrome. Scar tissue has no normal myocyte staining. (a) 6mo T02 hamster; (b) 9mo T02 hamster; (c) High power view of a 6 mo F1B hamster; (d) High power view of a 9 mo F1B hamster. Increased extracellular space was reflected by the wider white stripes.

Reference

[1] Nanjo S, et.al, Int Heart J. 2006; 47:607-16.

[2] Chen J, et.al, Am J Physiol Heart Circ Physiol. 2003; 285:H946-54.