Delineation of Microscopic Cardiac Tissue Remodeling in Cardiomyopathic Syrian Hamster with Diffusion Tensor MRI

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Abstract
The purpose of this study is to explore the potential of diffusion tensor MRI (DTMRI) for sensitive detection of alterations in cardiac structure in cardiomyopathic (CM) Syrian hamster hearts. DTMRI studies were performed on formalin fixed hearts of both T02 (CM) and F1B (control) hamsters. T02 hamsters exhibited increased trace values in scattered regions that were 40% higher than the trace in normal myocardium. Histology revealed that increased trace regions were in accordance with fibrotic scar locations. This study suggested that DTMRI may be used to assess the structural integrity of the myocardium.

Introduction
The CM Syrian hamster is a well-characterized animal model for cardiomyopathy progressing to heart failure. Myocardial fibrosis develops in distinct stages throughout the myocardium. A unique feature of these scar tissues is that both replacement fibrosis with collagen and scar calcification increase dramatically. We have observed previously that the average collagen fiber orientation is well aligned with the nearby muscle fiber orientation [1]. Recent studies of rat hearts with myocardial infarction suggested that DTMRI may provide a rapid and nondestructive technique for localizing discrete infarct zones and characterizing changes in the fibrous tissue after myocardial infarction [2]. However, whether this technique is sensitive to changes associated with CM comprising diffuse fibrosis and calcification is unknown. This study was aimed to explore the potential of DTMRI for quantifying the location and extent of diffuse scar tissue in remodeling CM Syrian Hamster hearts.

Methods
Subjects
Hearts from 5 CM Syrian Hamsters (Bio T0-2 strain) and 5 normal Syrian Hamsters (Bio F1-B strain) were studied. After excision, hearts were retrogradely perfused with Krebs buffer to washout the blood and fixed with 10% formalin. On the day before imaging, hearts were rinsed and suspended in 1x PBS.

Diffusion Tensor Imaging
DTMRI of excised hearts was performed on a Varian 4.7T scanner using a birdcage RF coil. A multi-slice spin-echo sequence with diffusion sensitizing bipolar gradient was used to acquire short-axis diffusion-weighted images. Imaging parameters were: TE, 45 msec; TR, 2.6 sec; Δ, 20 msec; δ, 10 msec; FOV, 1.5x1.5 cm²; slice thickness, 1.0 mm; number of averages, 8; diffusion gradient, G¼2 G/cm and G½8 G/cm. Images were acquired with 128x128 data matrix and zero-filled to 256x256. These parameters yielded an in-plane resolution of 59x59 µm² after zero-filling. A total of 11 short-axis images covering the whole left ventricle were acquired.

Data Processing
Diffusion tensor matrix and three corresponding eigenvalues were calculated from the 12 diffusion-weighted images. A trace map, i.e., sum of the three eigenvalues, was generated. The trace map was normalized to that of the surrounding buffer salt solution to minimize the effect of diffusivity variations due to temperature fluctuation. The myocardial fibrous scar tissue were defined as the zones with highest trace values, or > 1 SD above the mean trace for the whole slice. RA and helix angle of the primary eigenvector was calculated to compute fiber orientation.

Histology
Following DTMRI study, hearts were sliced at 1 mm thickness from base to apex along the LV long axis to enable direct correlation of slice locations between MRI and histological analysis. Each slice was embedded in paraffin and sectioned at 4 µm. The tissue sections were stained with Masson’s trichrome and picrosirius red for the identification of myocardial lesions. Scar location determined from histology was compared with the corresponding DTMRI images.

Statistical Analysis
All results were expressed as mean±SD. An 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.

Results
All three eigenvalues increased in the scar tissue of CM heart, indicating increased magnitude of water diffusion. As a result, the scar tissue appeared brighter on the trace map (Fig 1.a). Normalized mean trace value was 1.58±0.09 in normal myocardium, 1.66±0.12 in overall CM myocardium, and 2.02±0.08 in scar tissue (p<0.001 compared to normal). The primary, secondary, and tertiary eigenvalues, normalized by the mean eigenvalue of the surrounding water were 0.67±0.03, 0.50±0.03, and 0.41±0.05 respectively in normal myocardium; 0.70±0.04, 0.54±0.05, and 0.41±0.05 in CM myocardium; and 0.81±0.02, 0.66±0.03, and 0.55±0.03 in scar tissue (p<0.001 compared to normal). The location, size and shape of these high trace value regions were closely correlated to those of scar tissue as defined by Masson’s trichrome staining (Figure 1.b). High power images revealed increased extracellular space in the scar due to myocyte death and subsequent scar formation (Figure 1.c).

The transmural variations of helix angle of both CM and normal heart demonstrated continuous transition from +60° at endocardium to –40° at epicardium. Further analysis revealed no significant difference of angular dispersion and RA in CM and normal myocardium. Histological study revealed that mean orientation of deposited collagen fibers concided with that of nearby myocytes. (Figure 1.d).

Conclusion
The magnitude of water diffusion increased in scar tissue of CM Syrian Hamster’s heart, which is similar to findings reported previously in rat hearts with chronic infarction [2]. Histological examination revealed increased extracellular space in scar tissue. This increased extracellular space together with an intrinsically more porous collagen matrix may elicit less restriction on water diffusion along deposited collagen fiber, resulting in increased water diffusivity. The preserved RA and transmural helix angle variation indicated that the global fiber structure for both collagen scars and myocytes was well preserved in CM heart. This study suggests that DTMRI might offer a non-invasive method to detect and quantify small and diffuse deposition of scar tissue in CM heart in the myocardial remodeling process without the need for exogenous contrast agents to delineate late hyper enhancement.

Reference: