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Ex vivo diffusion tensor MRI reflects microscopic structural remodeling associated with aging and disease progression in normal and cardiomyopathic Syrian hamsters

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Abstract

Dilated cardiomyopathy (DCM) is a major cause of mortality and morbidity in cardiac patients. Aging is often an ignored etiology of pathological conditions. Quantification of DCM and aging associated cardiac structural remodeling is important in guiding and evaluating therapeutic interventions. Diffusion tensor magnetic resonance imaging (DTMRI) has recently been used for nondestructive characterization of three-dimensional myofiber structure. In this study, we explored the potential of DTMRI in delineating microscopic structural remodeling in aging and DCM hearts. Six month ($n = 10$) and nine month old ($n = 11$) DCM (TO-2) hamsters and their age-matched controls (F1 β) were characterized. Both aging and DCM hearts showed increased diffusivity and decreased diffusion anisotropy. DTMRI images of DCM hearts also revealed a subgroup of imaging pixels characterized by decreased radial diffusivity and increased FA. The location of these pixels showed qualitative agreement with regions of calcium deposition determined by X-ray CT imaging. Histological analysis confirmed expanded extracellular space in aging and DCM hearts as well as substantial calcium deposition in DCM hearts. These results suggest that DTMRI may provide a noninvasive technique to delineate structural remodeling associated with aging and DCM progression at the tissue and cellular level without the use of an exogenous contrast agent.

Keywords

aging; calcium deposition; diffusion tensor MRI; dilated cardiomyopathy; fibrosis

INTRODUCTION

Dilated cardiomyopathy (DCM) is the most common cardiomyopathy, accounting for approximately 55% of all cases of cardiomyopathies. It is responsible for a high proportion of cases of heart failure and sudden cardiac death, as well as the need for transplantation (1). Anatomically, DCM is characterized by ventricular dilatation of the heart. At the cellular level, diffuse myocyte damage arises as a result of known (infections, drugs, etc.) or unknown causes. Muscle fibers are replaced by extracellular matrix proteins, resulting in a significant increase in interstitial collagen content (2). Myocardial biopsy is accurate and informative for

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delineating cardiac structures. However, such a method is destructive and may cause severe complications. Besides idiopathic DCM, aging alone can also lead to a dilated left ventricle (LV) and, more severely, DCM (3,4). Patients may often be misdiagnosed to classified diseases without considering aging as an etiology of pathological alterations. Therefore, it is clinically important to quantify the structural remodeling associated with DCM progression and aging for diagnosis, as well as for design and evaluation of therapeutic interventions.

Recently, diffusion tensor magnetic resonance imaging (DTMRI) has emerged as a nondestructive tool for 3D characterization of cardiac fiber structure (5–14). Several histological studies have validated the utility of DTMRI for the reconstruction of cardiac fiber and sheet structure (7,8,11,15–17). Furthermore, other diffusion parameters were found to be indicative of certain pathological states. In a previous study on infarct rats, we observed that the infarct region was associated with increased diffusivity and decreased diffusion anisotropy (13). Similar changes were also observed in patients after acute myocardial infarction (18) and a porcine model of myocardial infarction (17). More importantly, our study suggests that DTMRI is also sensitive to microscopic changes such as fiber disarray, which was recently confirmed by a similar study in post-infarct mouse hearts (19). These findings demonstrate the utility of DTMRI in delineating the diseased myocardium with large focal lesions. However, whether DTMRI is sensitive to more subtle changes in cardiac structure associated with aging and DCM progression has not been explored.

In the current study, we aimed at exploring the potential of DTMRI in elucidating the microscopic structural remodeling induced by aging and DCM in hamsters. The structural changes delineated by DTMRI were correlated with X-ray CT studies and histological analysis for validation and elucidation of possible mechanisms responsible for DTMRI-observed changes.

METHODS

Animal preparation

Six ($n = 10$) and nine ($n = 11$) month old TO-2 Syrian hamsters (Biobreeders Inc., Watertown, MA), an established animal model of DCM (20), were characterized. Normal six ($n = 8$) and nine ($n = 10$) month old F1 β hamsters were used as the controls. Hamsters of the same strain but at different ages were compared to assess the effect of aging. The hamster was heparinized and anesthetized with pentobarbital. The heart was excised and retrogradely perfused with modified Krebs–Henseleit buffer for 5 min to wash out the blood (13). The perfusate was then switched to 10% formalin to induce rapid fixation of the tissue. The heart was then removed from the perfusion column and suspended in 10% formalin for further fixation. The animal protocol was approved by the Institutional Animal Care and Use Committee of the Case Western Reserve University.

Diffusion tensor MRI

One day before imaging, hearts were rinsed and suspended in $1 \times$ PBS solution. Diffusion-weighted images were acquired on a 9.4 T vertical bore magnet (Bruker Biospin, Billerica, MA) at room temperature using a 20 mm birdcage RF coil. Standard Stejskal–Tanner spin-echo pulse sequence was used for diffusion encoding. Seven 1 mm thick short-axis slices were acquired to cover the whole LV. Imaging parameters were: TE, 18 ms; TR, 2.5 s; δ , 5 ms; Δ , 8.5 ms; independent diffusion directions, 6; FOV, 1.3×1.3 cm²; b, 800 s/mm²; number of averages, 6; matrix size, 128×128 ; resolution, 102×102 μ m². Total acquisition time was about 3.5 h for each heart.

Data processing

Diffusion weighted images were zero-filled to 256×256 matrix. Diffusion tensor matrix and the three corresponding eigenvalues were calculated from the diffusion-weighted image set using a MATLAB-based software developed in our laboratory (13,14). The primary eigenvalue, a measure of the water diffusivity along the myofiber, was referred to as the axial diffusivity ($\lambda_{\parallel}$). Water diffusivity perpendicular to the myofiber, calculated as the average of secondary and tertiary eigenvalues, was referred to as the radial diffusivity ($\lambda_{\perp}$) (21). All diffusivity parameters were normalized to those of the surrounding PBS solution to minimize the variation caused by temperature fluctuation. The fractional anisotropy (FA) map was generated to quantify the diffusion anisotropy. To quantify variations in diffusivity, dispersion values of diffusion parameters were calculated as the standard deviation of the diffusion parameter in each individual heart. DTMRI-determined myofiber helix angle (α_h) and myofiber transverse angle (α_t) were calculated to quantitatively describe the myofiber structure in the local wall-bound coordinates (14). Angular dispersion (AD) of the helix and transverse angles, defined as the standard deviation of the helix and transverse angles, respectively, was calculated to evaluate the coherence of fiber orientation at each transmural depth (13).

Epicardial and endocardial borders of the heart were manually traced. The LV cavity was fitted by a circle for the calculation of the LV diameter. LV wall thickness was calculated as the mean distance between epicardial and endocardial borders (13). The whole heart was transmurally divided into 10 layers from the endocardium to the epicardium. The middle four layers were defined as the mid-wall (MW) myocardium and the remaining layers that encompassed subendocardial and subepicardial regions were defined as border regions (BD).

X-ray CT imaging

X-ray CT images were acquired on a micro-CT/SPECT system (X-SPECT, Gamma Medica-Ideas, Northridge, CA). After the surrounding water was removed from the heart, the heart was mounted on a semi-cylindrical holder with the long-axis of the heart parallel to that of the holder. Imaging parameters were: tube voltage, 75 kV; tube current, 115 μ A; focal spot size, 20 μ m; slice thickness, 40 μ m; FOV, 2.3×2.3 cm²; matrix size, 768×768 ; resolution, 30×30 μ m². Total scan time was about 3.5 min for each heart.

Histology analysis

Following DTMRI and X-ray CT studies, 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 μ m. The tissue sections were stained with Masson's trichrome to identify myocardial lesions and von Kossa staining to assess calcium deposition (22).

Statistical analysis

All the results were expressed as mean $\pm$ SD. Unpaired Student's *t*-test was performed for intergroup comparison of DTMRI parameters between F1 β and TO-2 hamsters, as well as between 6 and 9 month old hamsters. A two-tailed value of $p < 0.05$ was considered as significant. One-way ANOVA was performed for comparison of helix and transverse angles at each transmural depth across the myocardium for all four groups.

RESULTS

Morphological changes

Animal characteristics are shown in Table 1. Compared to age-matched controls, LV dilated significantly by 19 and 13% in 6 and 9 month old TO-2 hamsters, respectively, accompanied by 16 and 23% wall thinning, respectively ($p < 0.05$). Aging showed no significant impact on

LV diameter or wall thickness in either of the strains. Heart weight, body weight, and heart-to-body weight ratio were similar among all groups.

Myofiber orientation

Figure 1 shows the helix and transverse angles calculated from the primary eigenvector. All four groups showed a continuous shift of the helix angles from about $+50^\circ$ at subendocardium to -45° at subepicardium (Fig. 1a, c). Transverse angles were within $\pm 10^\circ$, indicating that myocardial fibers were circumferentially orientated in the short-axis plane (Fig. 1b, d). ANOVA analysis showed no significant difference in the helix and transverse angles among all groups.

To quantify the coherence of fiber orientation, the dispersion in helix and transverse angles was calculated at each transmural depth (Fig. 1e, f). The helix angle measured from 9 month old TO-2 hamsters exhibited a greater dispersion that reached statistical significance from the endocardial region to the midwall. Increased helix angle dispersion manifested in 6 month TO-2 hamsters also, but the dispersion was less pronounced. There was no statistical difference in the transverse angle dispersion between TO-2 and F1 β hamsters in either age group.

Water diffusivity and fractional anisotropy

Increased diffusivity was observed in aging and DCM hearts (Fig. 2a). Specifically, the axial, radial, and average diffusivities were greater in 6 month TO-2 hearts (0.62 ± 0.02 , 0.38 ± 0.01 , and 0.46 ± 0.01 , respectively) than the age-matched F1 β hearts (0.56 ± 0.03 , 0.32 ± 0.02 , and 0.40 ± 0.02 , respectively) ($p < 0.05$). Nine month old groups also exhibited the same trend. Axial, radial, and average diffusivities increased significantly from 0.61 ± 0.03 , 0.38 ± 0.04 , and 0.46 ± 0.04 to 0.64 ± 0.04 , 0.43 ± 0.04 , and 0.50 ± 0.04 , respectively ($p < 0.05$). Compared to 6 month groups, both F1 β and TO-2 hamsters showed increased water diffusivity in 9 month groups ($p < 0.05$).

In addition to increase in water diffusivity, aging and DCM hearts also showed decreased diffusion anisotropy. In F1 β groups, 9 month old hamsters showed decreased FA (0.33 ± 0.04) compared to 6 month old controls (0.38 ± 0.04 , $p < 0.05$). In TO-2 groups, FA was 0.33 ± 0.02 for 6 month hamsters and 0.28 ± 0.03 for 9 month hearts ($p < 0.05$). The comparison between TO-2 and F1 β hamsters in their corresponding age groups was also statistically significant.

Variations in diffusion parameters

Dispersion of diffusion parameters is shown in Fig. 2b. Compared to age-matched controls, TO-2 hamsters showed increased dispersion in axial, radial, and average water diffusion ($p < 0.05$), suggesting a wider range of variation in diffusion parameters in DCM hearts. The dispersion of average diffusivity in TO-2 hamsters increased by 20.2 and 26.3% in 6 and 9 month old groups, respectively ($p < 0.05$). Aging also resulted in a significant increase in the dispersion of average diffusivity by 15.2 and 21.1% in F1 β and TO-2 groups, respectively ($p < 0.05$).

To further evaluate the regional heterogeneity of diffusion parameters, we compared the diffusivity and FA values at mid-wall and border regions (Table 2). Mid-wall myocardium exhibited lower diffusivity and higher FA than those at border regions in all groups ($p < 0.05$). This difference was most pronounced for $\lambda_\perp$ in 9 month old TO-2 hamsters, with an average 36% increase in $\lambda_\perp$ in midwall than the mean $\lambda_\perp$ of the whole heart.

Representative distributions of FA and radial diffusivity ($\lambda_\perp$) from each group are shown in Fig. 3a–d. All four groups showed a subpopulation of imaging pixels characterized by low FA but high $\lambda_\perp$ values, with TO-2 hearts showing an increased number of pixels that belonged to

this subpopulation. These pixels were mainly located near the epicardial and endocardial border regions in both TO-2 (Fig. 3e) and F1 β hearts (data not shown). In addition, TO-2 hearts also showed an increased number of pixels with low $\lambda_{\perp}$ and high FA values (Fig. 3c, d). These pixels were mostly located at the mid-wall region (Fig. 3f). The 9 month old TO-2 group had the most manifested two-end shift (Fig. 3d). Unlike TO-2 hearts, there was no distinct subpopulation of pixels with high FA but low $\lambda_{\perp}$ values in F1 β hearts (Fig. 3a, b). Furthermore, pixels of high FA but low $\lambda_{\perp}$ values were randomly distributed in F1 β hearts (data not shown).

Comparison of DTMRI data with X-ray CT and histology

Figure 4 shows the radial diffusivity map (Fig. 4a), FA map (Fig. 4b), and X-ray CT image (Fig. 4d) from a representative TO-2 hamster. Pixels with low $\lambda_{\perp}$ (1 SD below the mean value) were encoded by red color in Fig. 4a. Pixels with high FA (1 SD above the mean value) were encoded by green color in Fig. 4b. Co-registration of low $\lambda_{\perp}$ and high FA pixels revealed that most of the low $\lambda_{\perp}$ pixels were associated with high FA values (Fig. 4c). The geometrical location of these pixels qualitatively agreed with those high-intensity pixels in X-ray CT images (Fig. 4d), indicative of calcium deposition in these regions.

Figure 5 shows the characteristic histological sections of both TO-2 and F1 β hearts. Although expanded extracellular space was present in the whole myocardium in TO-2 hearts, fibrosis developed mainly at mid-wall regions (Fig. 5a), accompanied by calcium deposition (Fig. 5b). Compared to 6 month hearts (Fig. 5c), 9 month hearts exhibited increased extracellular space in both TO-2 and F1 β hamsters (Fig. 5d).

DISCUSSION

To the best of our knowledge, this study was the first DTMRI study to delineate structural remodeling associated with aging and/or DCM progression. Major findings were that aging and DCM hearts were associated with increased water diffusivity and decreased diffusion anisotropy. However, a small fraction of myocardium in DCM hearts was associated with increased diffusion anisotropy and decreased radial diffusivity. Comparison with X-ray CT imaging and histology analysis suggested that there was significant calcium deposition in these regions.

Cell loss and structure degeneration are typical changes in aging hearts (23,24). Our histological examination also revealed expansion of extracellular space in 9 month old groups (Fig. 5). These cellular changes may serve as the underlying mechanisms for changes in diffusion characteristics observed in the current study. The expanded extracellular space imposed less restriction on water diffusion, leading to an increase in water diffusivity. Furthermore, the expansion of the extracellular space and the degeneration of an organized myofiber structure also lead to a more isotropic diffusion environment, causing reduced diffusion anisotropy. Such changes were also observed in DCM hearts both in histological examination and DTMRI data. These observations are consistent with previous reports that tissue necrosis develops with DCM progression (20,25).

The pathological remodeling process in DCM also induced a change in diffusion parameters that was characterized by low radial diffusivity and high diffusion anisotropy. Areas that are associated with low radial diffusivity and high diffusion anisotropy were largely located in the mid-wall region with substantial tissue fibrosis and calcification, which was confirmed by histology and X-ray CT. It is possible that excessive deposition of densely packed calcium salts in interstitial space further hindered water diffusion in the transverse direction. With diffusion along the fiber direction unaffected, such reduction in radial diffusivity led to an increase in diffusion anisotropy.

The development of cardiac lesion in DCM hearts is frequently accompanied by both calcium deposition and expansion of extracellular space. These two pathological processes may complicate the interpretation of DTMRI data, as they led to changes in diffusion parameters in opposite directions, which gave rise to observed increases in diffusivity dispersion (Fig. 2b). This problem may be alleviated by acquiring images at higher spatial resolution. More importantly, multi-parametric analysis with more sophisticated imaging classification methods may enable DTMRI to uniquely differentiate these two pathological processes.

Despite changes in diffusion parameters in both DCM and aging hearts, transmural courses of the helix and transverse angles were similar to those of the normal heart in both TO-2 and aging hearts, albeit with increased AD (Fig. 1). These observations suggest that myocardial fiber orientation was preserved despite DCM progression and aging. Such findings are consistent with the study on post-infarct myocardium in both humans (18) and animals (13). One possible explanation for the preservation of myocardial fiber orientation is that chronic development of DCM and aging-associated remodeling did not induce a dramatic change in the extracellular collagen matrix, which served as a scaffold for cardiac myocytes. However, tissue fibrosis can lead to less coherent organization of myocardial fibers at the microscopic level, which was reflected in the observed increase in AD in TO-2 hearts.

The current study was performed on formalin-fixed hearts. Compared to viable myocardium, diffusion coefficients of water may have different values in fixed hearts. However, the trend of DCM and aging associated alterations in diffusion parameters remains the same for *ex vivo* and *in vivo* models, as the physical processes governing such changes are the same. Techniques for *in vivo* DTMRI have been developed in the past two decades (6,26,27) and have been applied to the characterization of the cardiac fiber structure in post-infarct myocardium (18) as well as in patients with hypertrophic cardiomyopathy (28). With the advancement of more sensitive and robust *in vivo* DTMRI techniques, DTMRI may provide a useful tool in delineating aging and DCM induced myocardial remodeling in clinical settings.

In summary, we investigated the potential of DTMRI for sensitive delineation of microscopic structural remodeling associated with DCM progression and/or aging in the current study. Our results show that diffusion parameters are sensitive to pathological changes in myocardial structure that occur at the microscopic level. Although *in vivo* cardiac DTMRI with high spatial resolution remains a challenge at this point, our results show that DTMRI has the potential to contribute significantly to the characterization of myocardial remodeling as a consequence of various pathologies.

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Abbreviations used

AD	angular dispersion
BD	border regions
DCM	dilated cardiomyopathy

DTMRI	diffusion tensor magnetic resonance imaging
F1 β hamster	normal hamster
FA	fractional anisotropy
LV	left ventricle
MW	mid-wall
TD	transmural depth
TO-2 hamster	cardiomyopathic hamster

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Figure 1.

Myocardial fiber orientation. (a) A representative map of helix angle; (b) a representative map of the transverse angle; (c) transmural course in helix angle; (d) transmural course of transverse angle; (e) angular dispersion (AD) of helix angle; (f) AD of transverse angle. TD: transmural depth. * $p < 0.05$ between 9 month TO-2 and F1 β hearts. † $p < 0.05$ between 6 month TO-2 and F1 β hearts.

Figure 2. Diffusivity parameters. (a) Normalized axial diffusivity ($\lambda_{||}$), radial diffusivity ($\lambda_{\perp}$), and average diffusivity (λ_{ave}); (b) dispersion values of $\lambda_{||}$, $\lambda_{\perp}$, and λ_{ave} over the whole heart region. * $p < 0.05$ compared to age-matched controls. † $p < 0.05$ compared to strain-matched 9 month groups.

Figure 3.

Distribution of radial diffusivity ($\lambda_{\perp}$) and fractional anisotropy (FA) values. (a–d) Representative plots of $\lambda_{\perp}$ versus FA from 6 month F1 β (a), 9 month F1 β (b), 6 month TO-2 (c), and 9 month TO-2 hamsters (d), respectively. (e) Diffusion-weighted image of a 9 month TO-2 heart superimposed with highlighted pixels of high $\lambda_{\perp}$ values (1 SD above mean) and low FA values (1 SD below mean). (f) Diffusion-weighted image of a 9 month TO-2 heart superimposed with highlighted pixels of low $\lambda_{\perp}$ values (1 SD below mean) and high FA values (1 SD above mean).

Figure 4.

Geometric distribution of imaging pixels with low radial diffusivity ($\lambda_{\perp}$), high diffusion anisotropy (FA), or both in a representative 9 month TO-2 heart. (a) Radial diffusivity map with pixels of low $\lambda_{\perp}$ values (1 SD below mean) highlighted in red; (b) FA map with pixels of high FA values (1 SD above mean) highlighted in green; (c) coregistration of pixels with both low $\lambda_{\perp}$ and high FA values highlighted in yellow; (d) X-ray CT image of the same heart.

Figure 5. Histological analysis. (a) Masson's trichrome stained slice from a representative TO-2 heart. Normal myocyte was stained red and fibrotic tissue was stained blue. (b) von Kossa stained slice of the same heart for identification of calcium deposition. Normal myocyte was stained pink and calcium salts were stained dark brown. (c, d) High power view (200 $\times$) of Masson's trichrome staining of 6 month (c) and 9 month (d) old F1 β hamsters. Nine month old normal F1 β hamsters manifested expanded extracellular space (d). Expansion of extracellular space was also observed in TO-2 hamsters. This figure is available in color online at www.interscience.wiley.com/journal/nbm

Table 1

Animal characteristics

		LV Diameter (mm)	Wall Thickness (mm)	Body Weight (g)	Wet Heart Weight (g)	HW/BW (%)
6 month	F1β (n = 8)	4.79 ± 1.20	1.39 ± 0.27	114 ± 8	0.80 ± 0.06	0.70 ± 0.05
	TO-2 (n = 10)	5.70 ± 0.57*	1.17 ± 0.17*	106 ± 10	0.79 ± 0.10	0.70 ± 0.08
9 month	F1β (n = 10)	5.17 ± 0.94	1.47 ± 0.26	126 ± 14	0.89 ± 0.15	0.70 ± 0.01
	TO-2 (n = 11)	5.82 ± 0.46*	1.12 ± 0.11*	116 ± 10	0.90 ± 0.13	0.70 ± 0.01

* *p* < 0.05 compared with age-matched controls.

Table 2

Axial ($\lambda_{||}$), radial ($\lambda_{\perp}$), and average (λ_{ave}) diffusivities, and FA in midwall (MW) and border regions (BD)

		6 mo F1 β	6 mo TO-2	9 mo F1 β	9 mo TO-2
$\lambda_{ }$	MW	0.54 $\pm$ 0.03 [†]	0.59 $\pm$ 0.02 [*]	0.58 $\pm$ 0.03	0.60 $\pm$ 0.04
	BD	0.58 $\pm$ 0.02 [†]	0.63 $\pm$ 0.02 ^{*†}	0.62 $\pm$ 0.03	0.67 $\pm$ 0.04 [*]
$\lambda_{\perp}$	MW	0.30 $\pm$ 0.03 [†]	0.35 $\pm$ 0.01 ^{*†}	0.34 $\pm$ 0.04	0.37 $\pm$ 0.04
	BD	0.37 $\pm$ 0.03 [†]	0.45 $\pm$ 0.02 ^{*†}	0.43 $\pm$ 0.04	0.53 $\pm$ 0.04 [*]
λ_{ave}	MW	0.38 $\pm$ 0.03 [†]	0.43 $\pm$ 0.01 [*]	0.42 $\pm$ 0.04	0.43 $\pm$ 0.04
	BD	0.44 $\pm$ 0.03 [†]	0.51 $\pm$ 0.02 ^{*†}	0.50 $\pm$ 0.04	0.56 $\pm$ 0.04 [*]
FA	MW	0.40 $\pm$ 0.05 [†]	0.36 $\pm$ 0.02 ^{*†}	0.35 $\pm$ 0.05	0.31 $\pm$ 0.04 [*]
	BD	0.37 $\pm$ 0.04 [†]	0.32 $\pm$ 0.01 ^{*†}	0.32 $\pm$ 0.04	0.27 $\pm$ 0.03 [*]

* $p < 0.05$ compared to age-matched controls.

[†] $p < 0.05$ compared to strain-matched 9 month old hamsters.