Dynamic Alterations in Myocardial Fiber and Laminar Sheet Structure of Rat Hearts in Diastole and Systole Quantified by Diffusion Tensor MRI

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Introduction

Changes in myocardial fiber orientation and laminar sheet organization of the heart are postulated to be a key determinant of its contractile performance. Streeter et al. reported increased transmural shift of myofiber helix angle during contraction by histologic examination of hearts fixed at systolic and diastolic states respectively [1]. However, diastolic and systolic data were from two separate groups of animals so that no direct association of changes in fiber orientation can be reached. With respect to cardiac sheet structure, LeGrice et al. observed organized laminar sheet structure with significant regional variations at rest [2]. However, it is not clear how myofiber contraction affects the laminar sheet structure in a dynamic manner. Furthermore, the mechanical significance of such sheet organization needs to be determined. Recently, diffusion tensor imaging (DTI) has been validated as a non-destructive approach for rapid characterization of myofiber and laminar sheet structure [3]. In this study, alterations in myofiber and laminar sheet structure of the heart in both diastole and systole were evaluated in Langendorff perfused rat hearts using DTI after arresting the hearts first in diastole with KCl and then in systole with barium chloride.

Methods

Isolated heart perfusion. Hearts from 2-4 month old Sprague-Dawley rats (N=11) were retrogradely perfused with oxygenated Krebs buffer (37°C). A fluid-filled latex balloon was inserted into left ventricle (LV) through mitral valve. The balloon was connected to a pressure transducer to record left ventricular pressure and heart rate. Cardiac work was evaluated by rate-pressure-product (RPP).

DTI of hearts arrested in diastole. Cardiac arrest was induced by perfusing the heart with oxygenated cardioplegic (high [KCl]) solution (20°C). DTI of arrested hearts at diastolic state was performed on a Varian 4.7T scanner using a 2 cm solenoid coil. A multi-slice spin-echo sequence with diffusion sensitizing bipolar gradient was used to acquire short-axis diffusion-weighed images. Imaging parameters were: TE, 36 ms; TR, 1.3 s; Δ, 20 ms; δ, 6 ms; b-value, 948 s/mm^2; slice thickness, 1 mm; inter-slice distance, 0.5 mm; number of slices, 7; number of averages, 4; data matrix, 128×128. Image acquisition required about one hour.

DTI of hearts arrested in systole. Upon the completion of image acquisition, the arrested heart was perfused with normal Krebs buffer to resume contraction. Once cardiac work was stabilized, the balloon was deflated and taken out of the ventricle. A PE-50 tubing was inserted into LV to drain the remaining fluid. Sustained systolic arrest was achieved by Ba^{2+} induced contraction [4]. Specifically, heart was perfused with modified Tyrode solution with 0.078 mM Ca^{2+} for 1.5 min, followed by Tyrode solution with 2.5 mM $BaCl_2$ for 5 min. Adenosine was added to both solutions at 1 mg/min to maximally dilate the coronary vessels. At the end, heart was rapidly fixed in systole by perfusing with 5% formalin. Immediately after systolic fixation, heart was suspended in 5% formalin and imaged in the same coil. DTI of 11 continuous short-axis images were acquired using the same parameters as the diastolic images except that TR was 1.7 s. Slice thickness was adjusted according to ventricular shortening in longitudinal direction.

Calculation of myofiber and laminar sheet orientations. Primary, secondary and tertiary eigenvalues and eigenvectors of diffusion tensor were calculated. The primary, secondary and tertiary eigenvectors was found to coincide with myofiber, sheet and sheet normal directions respectively [3]. All eigenvectors were transformed from magnet Cartesian coordinates to the wall-bound myocardial coordinates. A prolate spheroid was fit to the epicardial surface to define the local wall-bound myocardial coordinates. Orientation angles of myofiber were specified by helix and transverse angles [5]. Orientation of sheet was specified by sheet angle, defined as the angle between sheet direction and radial axis. Transmural myofiber and laminar sheet structure were characterized in a 20° wide area in lateral region in basal, mid-ventricular and apical slices. Transmural shift of helix angle was calculated as the helix angle at endocardium minus that at epicardium.

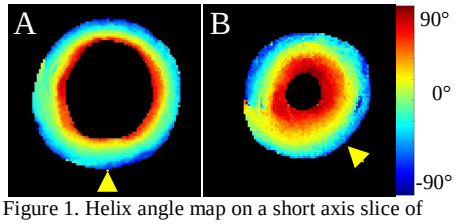

Figure 1. Helix angle map on a short axis slice of heart in (A) diastole; (B) systole. Arrows point to lateral region.

Statistical analysis. All results were expressed as mean±SD. Paired student's t-tests were used for comparison of the parametric variables. A 2-tailed value of $p<0.05$ was considered as significant.

Results

The ratio of RPP before and after DTI of diastolic arrested hearts was 0.94±0.08, indicating preserved myocardial viability during the experiment. Left ventricular wall thickness in lateral region at base, midventricle and apex was 2.5±0.4 mm, 2.6±0.4 mm and 2.4±0.3 mm respectively in diastole, and increased to 3.6±0.4 mm, 3.9±0.3 mm and 3.6±0.4 mm respectively in systole ($p<0.001$ for each pair of data).

Hearts exhibited considerable shift in fiber orientation during contraction (Figure 1). The helix angle in systole was significantly increased at endocardium (5% to 65% transmural depth), but was significantly decreased at epicardium (85% to 95% transmural depth, Figure 2A). At endocardium, mean increase in helix angle was 14°±3° at base, significantly higher than the 6°±3° increase at apex ($p<0.001$). Transmural shift of myofiber helix angle at base, midventricle and apex was 103°±14°, 97°±12° and 100°±13° respectively in diastole, and increased to 132°±9°, 134°±9° and 133°±11° respectively in systole ($p<0.001$ for each pair of data). No significant changes in transmural distribution of myofiber transverse angle were observed.

Sheet structure in systole also changed significantly from that in diastole (Figure 2B). Transmural shift of sheet angle was observed in both systolic and diastolic heart. These observations of sheet structure in diastolic arrest were consistent with findings by LeGrice et al made in histologically sectioned tissues. The dynamic responses of cardiac sheet architecture to systolic contraction comprised changes in the mean absolute sheet angle, which is an indicator of sheet slope in the transmural direction. In systole, the mean absolute sheet angle decreased by 19°±5°, 15°±4° and 16°±3° at base, midventricle and apex respectively, indicating flattening of the laminar sheet due to contraction.

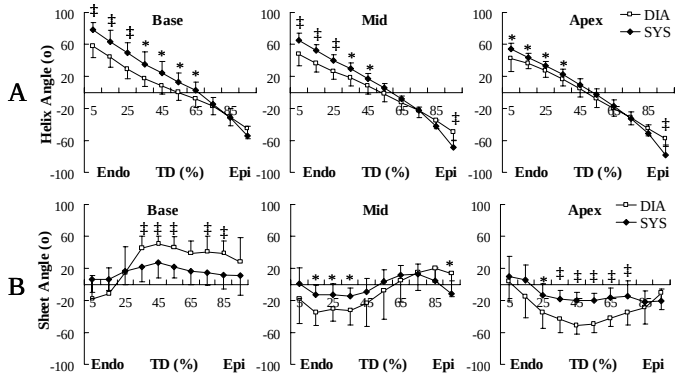

Figure 2. (A) Myofiber helix angle; (B) Laminar sheet angle from 5% to 95% transmural depth (TD) at base, midventricle (Mid) and apex in diastole (DIA) and systole (SYS). * $p<0.05$, ‡ $p< 0.01$.

Discussion and Conclusion

The current study presents the first observations that directly quantify dynamic structural alterations of myofiber and laminar sheet from diastole to systole. The transmural shift of myofiber helix angle increased about 30° in systole. No significant changes were observed for transverse angle. In diastolic arrested heart, transmural distribution of sheet angle delineated by DTI agreed with that from histologic analysis [2]. In systolic arrested heart, decreased absolute value of sheet angle indicated marked sheet flattening, which may also contribute to wall thickening in addition to myocyte shortening.

Reference

1. Streeter DD, et al., Circ. Res., 1969.24(3), p.339-47.
2. LeGrice IJ, et al., Am. J. Physiol., 1995.269(2 Pt 2), p.H571-82.
3. Tseng WY, et al., J. Magn. Reson. Imag., 2003.17(1), p.31-42.
4. Munch DF, et al., Am. J. Physiol., 1980.239(3), p.H438-42.
5. Chen J, et al., Am. J. Physiol., 2003.285(3), p.H946-54.