Cardiac Diffusion MR Microscopy of Rabbit Heart

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Introduction

The homogeneous cardiac function is a result of harmonious interplay of numerous muscle cells specialized depending on functions that they perform, contraction (normal contractile myocyte) and conduction (the Purkinje fiber) [1]. The specialized muscle cells have various sizes and densities depending on where they are located in the heart. And considerable structural complexity exists in the extracellular space that contains blood vessels, collagen, and connective tissues, etc. [2, 3]. Diffusion tensor magnetic resonance imaging (DTMRI) has emerged to characterize the structure of the cardiac tissue for a decade, with popular spatial resolutions of tens of cellular level. In this experimental study using a 17.6 T magnet, we explored the potential of microscopic high angular resolution diffusion imaging (MHARDI) achieving a cellular level spatial resolution as a non-invasive tool that is sensitive to subtle changes in the heterogeneous microstructure and arrangement of the cardiac tissues. Diffusion tensor images acquired with two b-values (1000 s/mm^2 and 2000 s/mm^2) were compared to investigate that the tensor invariants may alter depending on the diffusion weighting factor, determining relative contribution of the fast and slow components to the diffusion signal.

Materials and Methods

Isolated heart preparation: Isolated perfused hearts (n = 3) of New Zealand White male rabbits (3 ~ 5 kg) were prepared according to the animal protocol approved by the UF Institutional Animal Care and Use Committee. The isolated hearts were fixed in situ during an intravascular formalin-perfusion fixation procedure that was carefully standardized. And the fixed hearts were kept in a refrigerator until MR imaging experiments.

HARDI: MR experiments of the isolated hearts were performed on a 17.6 T / 89 mm vertical wide-bore magnet (Bruker Instrument spectrometer and console, Billerica, MA). The RF coil used for the in vitro imaging was an Alderman-Grant birdcage coil, diameter = 25 mm, length = 35 cm. The temperature in the magnet was maintained at 19 - 20°C. HARDI of 21 directions was performed using a standard PGSE pulse sequence, achieving an isotropic in-plane resolution of 50 µm with a transverse slice of 500 µm. Diffusion sensitizing factors (b-values) were 1000 s/mm^2 and 2000 s/mm^2 using Δ = 13.4 ms and δ = 1.8 ms. Imaging parameters were TR = 3000 ms, TE = 25.1 ms, 1 average. The pilot images with three orthogonal planes were collected to check whether or not the isolated heart imbedded in the dense FC-43 solution moved during a long scan (~ 7 hours).

Data analysis: The tensor processing of HARDI data sets was performed using fanDTasia™ (©2008, Barmpoutis, http://www.cise.ufl.edu/~abarmpou/). Pixel-based analysis of selected ROIs from a variety of regions (free wall in the LV, interventricular septum, and papillary muscles in the left ventricular cavity) was conducted. Trace, axial diffusivity ($\lambda_{//}$), radial diffusivity ($\lambda_{\perp}$), and fractional anisotropy were calculated using MATLAB (MathSoft, Cambridge, MA).

Results and Discussion

Figure 1 demonstrates that MHARDI has the potential to follow elaborately the subtle change in the structure of an isolated rabbit heart. Transmural and regional heterogeneity of the tensor invariants (Fig.1 and Table 1) might represent the structural diversity in the cardiac tissue. Table 1 shows the change of the fractional anisotropy depending on two diffusion weightings. Since the diffusion MR signal measured using a low b value of 1000 s/mm^2 is likely biased to the fast component (Fig. 2), decrease of the fractional anisotropy and the primary eigen value in a high b-value of 2000 s/mm^2 might result from increase in contribution of the slow component to the diffusion signal. Considering that each pixel includes only one or two myocyte(s) (length of 120 ~ 130 µm and width of 25 ~ 30 µm), this result may reflect direct influence of the intra/extracellular compartments on the molecular diffusion of water. Understanding of the source of diffusion signal is currently on the study using the diffusion MR microscopy of single myocyte. Interestingly, structure in stripe shape is found transmurally in the upper half of the ventricular interseptum (Fig. 1, yellow box). The diffusion anisotropy and eigen vector maps imply that the finger-like structure may represent another structure that is not associated with myocardial fibers and sheets. Tendinus cords in the ventricular cavities are also visible in transverse images of MHARDI (Fig. 3). To my knowledge, since the cords truly contain the free-running Purkinje fibers and connective tissue [4], this finding may lead subsequent progress for the Purkinje fiber diffusion imaging. The structural change of the purkinje fibers in the aging or pathological heart may be monitored using this innovative Purkinje fiber diffusion imaging.

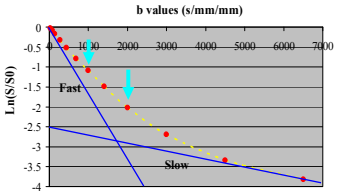

Fig. 2 Plot of the logarithm of signal intensity as a function of b-values for an isolated rabbit heart. Arrows indicate b-values employed for HARDI. Dotted line represents the best-fit biexponential function.

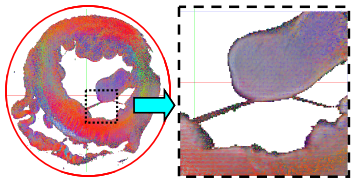

Fig. 3 A transverse primary eigen vector map demonstrating the free-running Purkinje fibers.

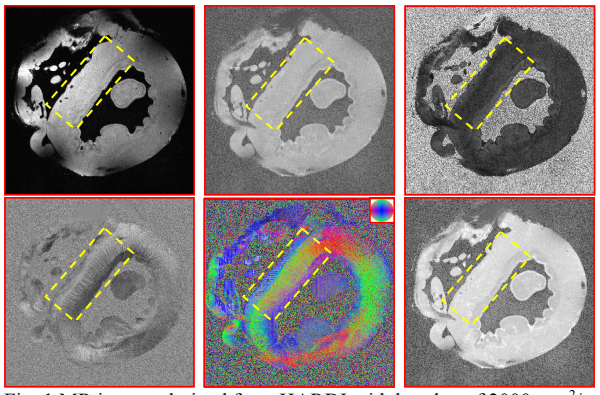

Fig. 1 MR images derived from HARDI with b-value of 2000 mm^2/s. Images are arranged from the left in the top, images of S0, primary eigen value, and FA, respectively. And from the left in the bottom, images of a tensor component (Dxz), the primary eigen vector, and the trace, respectively.

Parameters	b	FW			IS		PM
		endo	myo	epi	left	right	
ADC (X 10^{-3})	1000	1.16	1.14	1.17	0.94	1.22	0.93
	2000	1.12	1.1	1.15	0.91	1.12	0.95
$\lambda_{//}$ (X 10^{-3})	1000	1.5	1.59	1.72	1.54	1.77	1.37
	2000	1.45	1.48	1.49	1.41	1.64	1.38
$\lambda_{\perp}$ (X 10^{-3})	1000	0.99	0.92	0.89	0.63	0.94	0.71
	2000	0.96	0.91	0.90	0.66	0.95	0.74
FA	1000	0.28	0.34	0.39	0.51	0.39	0.39
	2000	0.27	0.30	0.32	0.45	0.33	0.37

Table 1 Diffusion parameters depending on b-values at various regions in the LV. Each value corresponds to the mean of selected ROIs. Standard deviation of parameters except the FA were less than 5% of the corresponding mean. FW: free wall, IS: interseptum, PM: papillary muscle. b is b-value (s/mm^2).

Conclusion

High angular resolution diffusion imaging achieving an in-plane resolution of 50 µm could reveal the tissue structures such as the free-running Purkinje fibers and stripes in the ventricular interseptum as well as the heterogeneous microstructure in the myocardium. The microscopic HARDI with an optimized b-value may be a powerful tool for non-invasive monitoring of electro-mechanical property and its well-coordinated function. Histology of the tissue part that displays the stripe structures and MHARDI of the procured free-running Purkinje fibers are currently underway

References: 1. P. Kohl Circ Res 2003; 93: 381-3. 2. Aliev et al. Cardiovas Res 2002; 53: 48-58.
3. J. Frank and G. Langer J Cell Biol 1974; 60: 586-601. 4. A. Ansari et al. Anat Rec 1999; 254: 92-7.

Acknowledgement: This work was funded by seed grants (JRF) from the McKnight Brain Institute and the Department of Radiology, UF.