Detection of Myocardial Cell Hypertrophy in a Mouse Model of Hypertension: Transcytolemmal Water Exchange and its Sensitivity to Cell Size

Michael Jerosch-Herold[1], Otavio Rizzi Coelho-Filho[2], Richard Mitchell[3], Heitor Moreno Junior[4], and Raymond Y Kwong[5]

[1]Brigham & Women's Hospital, Boston, MA, United States, [2]Medicine, Brigham & Women's Hospital, [3]Harvard Medical School, [4]State University of Campinas (UNICAMP), [5]Brigham & Women's Hospital

BACKGROUND: The contrast-enhanced T1 relaxation rate in the cardiac muscle is frequently modeled as a linear response to the arterial concentration of an extracellular contrast agent, by assuming that the movement of water across the cytolemmal barrier is in the fast exchange (FX) limit. This assumption has been implicit in most of the recent studies of equilibrium myocardial contrast enhancement, its relation to extracellular matrix expansion and diffuse interstitial fibrosis [1, 2]. Moving away from the fast exchange limit, by increasing the extracellular concentration of a contrast reagent, introduces sensitivity of the myocardial T1 relaxation rate to the intra-cellular lifetime of water [3]. This provides a means of probing cell size. Currently, only endomyocardial biopsies allow detection of cell-hypertrophy, with the risks inherent to invasive catheter-based procedures. The goal of this study was the development of a technique suitable for detection of cardiac cell hypertrophy, a common physiological response in the heart muscle to cardiac disease and pathological stress. We tested the hypothesis that in the intra-cellular water lifetime (τ_{ic}) could be used to detect cell-size changes, and that it would correlate positively with histological measures of cell size in a mouse model of hypertensive heart disease.

METHODS: L-NAME (3mg/ml) or placebo were administered respectively to 17 (body-weight=37.2±2.3g) and 13 (body-weight=37.5±2.5g) male-wild-type mice. Mice were imaged at baseline and 7-weeks after treatment with a 4.7T small-animal MRI-system. T1 (> 5 T1 measurement / mouse study) was quantified with a modified Look-Locker gradient-echo cine technique, before and after fractionated Gadolinium-DPTA administration, using the following sequence parameters: TR/TE/flip=2.5 ms/1.8 ms/12°, in-plane resolution 190 µm, slice thickness 1 mm; repetition time per k-space segment: ~22 ms; number of averages: 6 (pre-contrast), or 4 (post-contrast). Signal intensity versus time curves for 6 myocardial segments and the blood pool were fit to an analytical expression for the inversion recovery, with correction for the radiofrequency pulse effects on the inversion recovery (Figure 1a). A parsimonious, 2-site H-exchange (2SX) model, with myocardial extracellular volume fraction (MECVF), and intra-cellular lifetime (τ_{ic}) as adjustable parameters, was fit by orthogonal least squares optimization to the measured myocardial T1 relaxation rates, with the T1 relaxation rates in the right ventricular blood pool as independent variable in the model. The blood hematocrit was determined at the end of each MRI study using a cartridge-based analyzer assay. Minor and major cell diameters were measured by FITC-labeled wheat germ agglutinin (Sigma) staining of cell membranes. Morphometric analysis was performed with a computer-based system (Image-Scope). The intra-cellular volume (V_{ic}) was calculated from the minor (D_{min}) and major (D_{maj}) cell-diameters using a cylindrical cell-shape approximation.

RESULTS: The heart of L-NAME-treated mice developed hypertrophy (weight-indexed LV mass 4.1±0.4 vs. 2.2±0.3 µg/g, p<0.001). The T1 relaxation rate exhibited a nonlinear dependence, which became apparent for T1 relaxation rates in blood greater than approximately 3 s^{-1} (Figure 1b), and more pronounced after development of cell hypertrophy. Minor cell diameter, major cell diameter, and V_{ic} was substantially higher in L-NAME-treated animals ($19.4 \cdot 10^3$ vs. $10.7 * 10^3$ µmm^3; p<0.0001), while D_{max}/D_{min} was smaller (4.2 vs. 3.4, p<1e-7), compared to controls. τ_{ic} was significantly higher in L-NAME-treated animals (0.453±0.10 vs. 0.234±0.06, p<0.0001). τ_{ic} increased significantly from baseline to 7-weeks in animals treated with L-NAME (p<0.0001), and strongly correlated with the minor cell diameter (r=0756, P<0.001), V_{ic} (r=0.875, r<0.001; Figure 1c), and more weakly with the major cell-diameter (r=0.478, p=0.02). τ_{ic} also correlated with weight-indexed LV mass (r=0.71, p<0.001).

CONCLUSIONS: Quantification of the intra-cellular lifetime of water (τ_{ic}) by MRI allows for a robust, non-invasive estimation of cell volume changes, validated here against direct morphological measurements of minor and major cell diameters. τ_{ic} correlated more strongly with D_{min} than D_{maj}, reflecting the fact that the dependence of τ_{ic} on D_{max} is weak for cylindrical shapes with $D_{max}/D_{min} \sim 4$. These results support the premise that under physiological conditions the temperature in the cell milieu and the water diffusion constant remain within a tight range, which means that the intra-cellular lifetime of water is predominantly sensitive to cell dimensions, and a parameter suitable for detection of cell hypertrophy.

Funding Sources and Cited References:

Supported by the American Heart Association (AHA 11POST5550053) and the National Institutes of Health/NHLBI (1R01HL090634)

[1] Flett AS, etl al, *Circulation.* 2010;122(2):138-144. [2] Jerosch-Herold M, et al. *American Journal of Physiology-Heart and Circulatory Physiology.* 2008;295(3):H1234-H1242; [3] Landis CS, et al, *Magn Reson Med* 2000;44:563-74.

Figure 1