A High-Throughput MRI Method for Individualized Assessment of Left Ventricular Hypertrophy in Mice

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Introduction

Mouse models of myocardial hypertrophy are being increasingly used to characterize molecular genetics of this common cardiovascular complication and the associated heart failure [1]. Cardiac MRI has been established as a gold standard for quantifying left ventricular (LV) mass in mice *in vivo* [2-3]. The purpose of this study is to further improve the speed of this method, with no sacrifice in accuracy, for the high-throughput assessment of LV hypertrophy in mice, and to maximize the test sensitivity by applying an individualized baseline control.

Methods

Animal preparation: Thirty 14 week-old male C57/Bl6 mice were administered isoproterenol (ISO, 30mg/kg/day via a mini-osmotic pump at a rate of 1 µl/hr) for 7 days (from day 1 to day 7) to induce myocardial hypertrophy. Seven days prior to ISO administration, half of the animals (group A, n=15) started to receive drinking water containing 0.7 g/L of Enalapril (an ACE inhibitor) to attenuate the ISO stress, while the other half (group B, n=15) received normal drinking water constantly.

***Cardiac MRI*:** All animals were subjected to cardiac MRI twice, on days 0 and 7 respectively, with the same protocol. Mice were anesthetized with 1.5 ~ 2.5 vol. % isoflurane, and their body temperature was maintained with warm air. The imaging was performed on a 7 T Bruker Pharmascan system with a standard 38 mm resonator. A modified fast low angle shot (FLASH) gradient echo sequence gated to both the ECG and the respiratory movement was used to acquire 10 phases of heart images per cardiac cycle (NEX=2). Three contiguous 1-mm-thick short-axis slices, with an in-plane resolution of 187×187 μm^2, were collected at the mid-ventricular region of the left heart with the 2nd slice overlapping the greatest LV diameter. Areas of LV walls on these 3 slices at the end-diastolic phases were manually delineated, and an LV mass index was calculated (by multiplying the wall volume by the specific gravity of myocardium (1.05 g/cm^3) and dividing by the animal weight).

Heart weight measurement: Animals were euthanized after the 2nd imaging, and hearts were excised for their wet weight and the heart weight index (normalized by the body weight).

Results

Our modified cardiac MRI protocol, which saved a substantial amount of time by acquiring 3 mid-ventricular slices instead of the whole LV, provided LV mass data that correlated closely with the post-mortem heart weight index (r=0.84, n=30, day 7 data), indicating a sufficient level of accuracy. Total imaging time (including anesthesia, pilot scans, and the final short-axis acquisitions, or the so-called "back to cage" time) was 18 min/animal on average, which was ~ 10 min faster than a whole LV scan in our lab. In previous publications a similar study required 45 min to 1 hr. This improved speed would allow a single imaging system to provide sufficient throughput for a large scale *in vivo* LV mass study and/or repeated measurements for a longitudinal study.

This method was validated in a mouse model of myocardial hypertrophy (Fig.1). The development of LV hypertrophy was assessed by cardiac MRI at the baseline (day 0) and day 7 post-ISO treatment, respectively. All individuals showed a notably increased LV mass as compared to their own baseline (Fig. 1). The mean percentage increase of LV mass index was 31 (0.016±0.001 at day 0 vs. 0.021±0.002 at day 7, group B, n=15). In order to establish a benchmark for attenuated LV hypertrophy, another group of animals (group A) were given Enalapril in addition to ISO. As shown in Fig. 1, the thickening of LV wall at days 7 was minimal as compared with that at day 0. The mean percentage increase of LV mass was 21 (0.014±0.002 at day 0 vs. 0.017±0.002 at day 7, n=15). A discernible attenuation of LV hypertrophy was demonstrated by the MRI method ($p<0.01$). This difference was also detected using the post-mortem measurement of heart weight index at day 7 (0.0060±0.0005 of group B, n=15, vs. 0.0053±0.0006 of group A, n=15, $p < 0.01$), however, with no reference to the "starting" LV mass at day 0 (in fact group A had a slightly lower mean, see Fig. 1).

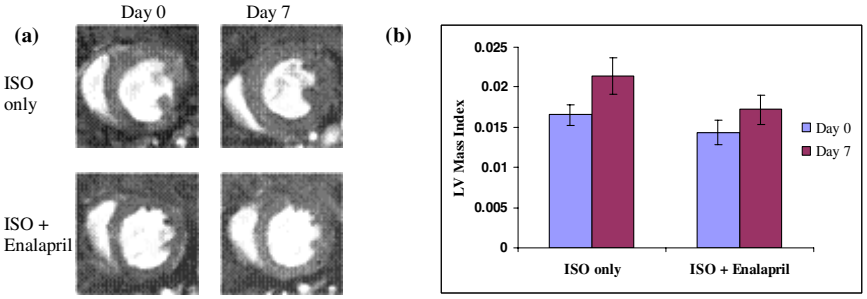

Fig 1: Cardiac MRI assessment of ISO-induced myocardial hypertrophy in mice. (a) Representative short-axis images from a mouse in the "ISO only" group (top panel), and another one from the "ISO+Enalapril" group (bottom panel). (b) Graphic presentation of the mean±SD of LV mass index from ISO-induced hypertrophic hearts and those co-treated with Enalapril.

Conclusion

This 3-slice-cardiac MRI method has proven to be fast (18 min/animal) and accurate for LV mass assessment in mice. Taking advantage of its baseline-control capability, this method should provide higher statistical power than the single-shot autopsy method, require a smaller sample size, and more importantly, be less dependent on the starting body weight or heart weight of the animals. It can be applied to the high-throughput screening for novel genes which contribute to the development or attenuation of myocardial hypertrophy and the associated heart failure in knockout mice, as well as pre-clinical testing of candidate drugs for related heart diseases.

References

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