Endothelial Progenitor Cells Mediated Improvements in Post-Infarct Left Ventricular Myocardial Blood Flow Estimated by Spin Labeling CMR

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Introduction Endothelial progenitor cells (EPCs) have been shown to improve the cardiac function after myocardial infarction (MI), whether this improvement is associated with improved myocardial perfusion mediated by EPCs is not known. In this study, we first modified a spin labeling-based cardiac magnetic resonance (SL-CMR) method (1) and we validated it for quantification of regional myocardial blood flow (RMBF) in free breathing rats. We then demonstrated that EPCs mediated a significant improvement in post-MI myocardial perfusion.

Materials and Methods

Animal Preparation: Normal (not infarcted, n=5) hearts were used to validate the SL-CMR based RMBF quantification by standard fluorescent microsphere (FM) technique. To examine whether SL-CMR can detect EPC-mediated improvement of RMBF in post-MI hearts, human umbilical vascular endothelial cells (HUVECs) or Vehicle were injected in the infarct border zone of athymic rats during the MI surgery. Initial infarction size was estimated 1-day post-MI by CMRI. If the infarct size was within the range of [10%, 30%] of the LV mass, the rat would be included for further studies in the HUVEC or Vehicle group. The two groups were studied at 1-day and 2-weeks post-MI to measure RMBF.

Methods: All MR experiments were performed on a Varian DirectDrive™ console interfaced with a 4.7 T horizontal bore magnet and a 12 cm gradient insert capable of generating magnetic field gradients of up to 25 G/cm. A TEM transmit volume coil and a surface receiver coil (InsightMRI, Worcester, MA) were used in combination. For validation purposes, immediately before imaging the rat was cannulated in the carotid (for injection of microspheres into LV) and femoral artery (for withdrawing reference blood). During the SL-CMR, T_1 mapping under nonselective inversion was achieved by setting the inversion slice gradient close to 0, and was acquired interleavely with slice selective T_1 mapping for 2 averages. The T One by Multiple Read Out Pulses (TOMROP) sequence (2, 3) was gated on every other heart beat after a double gated hyperbolic secant inversion pulse (4). The parameters were: FOV = 35 × 35 mm^2, single short axis slice at mid-ventricular level with thickness = 3mm, acquisition matrix = 192 × 80, TE = 2.19 ms, bandwidth = 96 k, inversion pulses 11s apart, excitation flip angle = 12°. The respiratory waveform and k-space line acquisitions were simultaneously recorded (SA Instrument, NY) for retrospective gating to eliminate images acquired out of the quiescent phase of expiration. For each pixel, its T_1 value was derived by fitting signal intensities using a three-parameter fitting algorithm (2). In validation studies immediately after imaging, 0.2 Million FMs (Dye-Trak, Triton Technology, CA) in 200 µL saline was infused in 10 s followed by saline flush of 500 µL for 20 s. Meanwhile, arterial blood was withdrawn at 0.33mL/min via a syringe pump (Harvard Apparatus, MA) beginning 30s before FM injections and lasting for 3 min in total. Upon completion of the blood sampling, the heart was harvested while the rat was under anesthesia.

Results

Validations: The absolute RMBF values obtained from SL-CMR and FM methods were in excellent agreement(R = 0.79) (Fig. 2).

EPC transplantation: Initial infarct size measured at 1 day post MI suggested a relatively uniform distribution of infarct size in both experimental groups: 19 ± 3.9% in HUVEC (n=9) and 18 ± 4.9% in Vehicle group (n=8) without significant difference (P = 0.5). LV myocardium on the short axis slice was segmented into *I-B-R,* where the infarct (I) was defined on the delayed hyperenhanced (DHE) image acquired at the same location, border (B) zone was defined as 60° sectors neighboring the infarct segment, and the remote (R) zone encompassed the remaining myocardium.

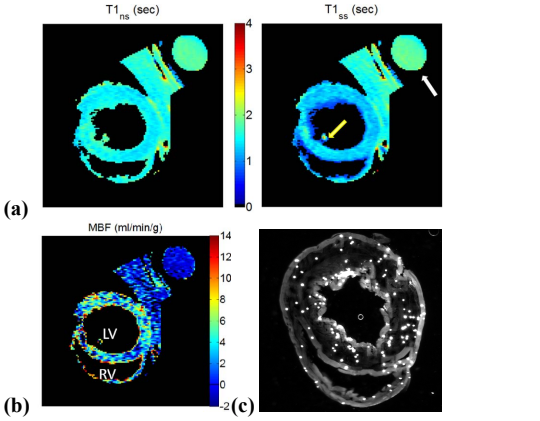

Figure 1. T_1 maps (a) and the MBF map (b) from Spin Labeling CMR and a corresponding section of FM images (c) from one representative heart. (a) The yellow arrow points to the cannulation tubing from the carotid artery; the white arrow points to the agarose gel phantom with calculated perfusion values close to 0 shown in (b).

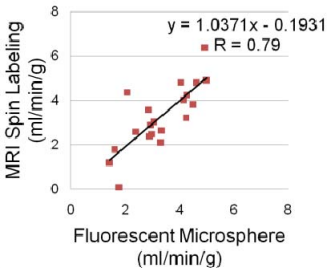

Figure 2. Correlation of perfusion values measured by SL-CMR and the FM method. Each subject presented four data points from whole slice average, septum, lateral, and mean of anterior and posterior segments (due to indistinct morphological features on FM images).

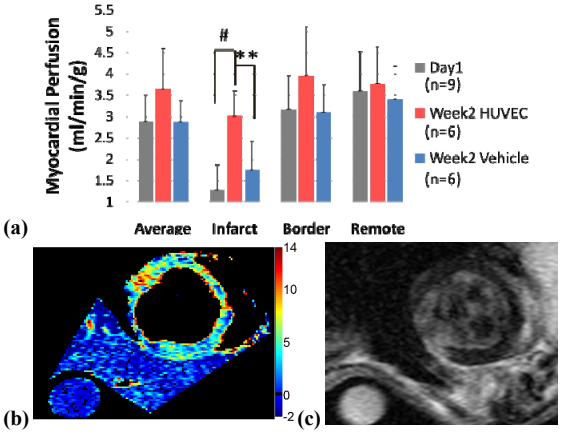

Figure 3. Myocardial perfusion at Day1 and Week2 post MI (a). A perfusion map in unit of ml/min/g (b) and the corresponding DHE image (c) from a HUVEC treated heart at 2-weeks post MI. # : p< 0.001, **: p<0.01

Conclusions SL-CMR allowed high resolution mapping of myocardial perfusion in rodent hearts. The absolute perfusion values by SL-CMR were in excellent agreement with those obtained by standard but invasive microsphere method. This noninvasive method enabled serial monitoring of myocardial perfusion improvement in response to stem cell engraftment.

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References 1. Kober et al, *MRM* 2004; 51:62-7. 2. Brix et al, *MRI* 1990; 8:351-6. 3. Pickup et al, *JMRI* 2004; 19:508-12. 4. Silver et al, *Nature* 1984; 310:681-3.