Multimodality validation and mechanistic explanation of functional restoration of murine model of myocardial infarction by mouse embryonic stem cells

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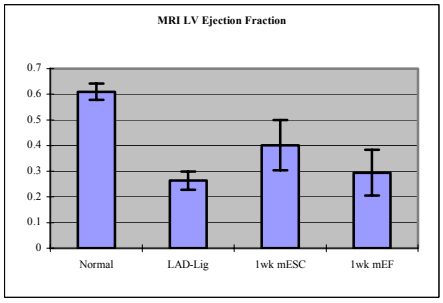
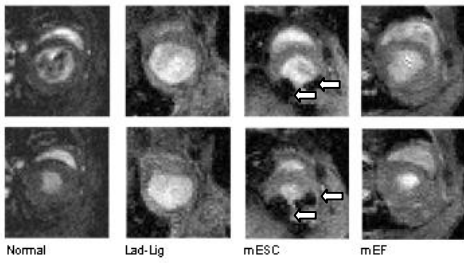
Background Much controversy exists regarding the exact mechanism of cell therapy in the restoration of acutely injured myocardium. Preclinical and clinical investigations suggesting angiogenesis, transdifferentiation, cell fusion, or paracrine effects have been reported.
Objective In this study, multi-modality validation of MR evaluation of myocardial restoration following transplantation of mouse embryonic stem cells (mESC) has been performed. Furthermore, the mechanisms underlying functional restoration of the injured myocardium in murine model of myocardial infarction have been investigated.
Method Sixty-five female 129Sv/J mice underwent LAD ligation to induce MI. The mice were randomized into the following groups (sample size): sham-operated control (n=10), LAD-ligated with normal saline (n=10), LAD-ligated with mESC (n=25), and LAD-ligated with mEF (n=20). The mice underwent *in vivo* MRI at 4.7T using a 15 cm horizontal bore magnet (Oxford Instruments, Oxford, UK) with GE Techron Gradients (12 G/cm) and a volume coil with inner diameter of 3.5cm (Varian, Palo Alto, CA). LV function was evaluated using ECG-triggered cine sequence (TE 2.8-ms, TR 160-ms, FA 60°, FOV 3.0cm2, matrix 128×128, slice gap 0-mm, slice thickness 1.0-mm, 8 NEX, and 12 cardiac phases). The data were analyzed using MR Vision software (Winchester, MA). LV ejection fraction (LVEF), end-diastolic (LVED), end-systolic (LVES) volumes and mass (LVM) were calculated by tracing the endocardial and epicardial borders in end-systole and end-diastole in all 12 cardiac phases. The *in vivo* MRI findings were confirmed by *ex vivo* pressure-volume (PV) loop analysis for systolic and diastolic functions. The right carotid was cannulated with the Millar catheter and advanced in a retrograde fashion through the aortic valve into the left ventricle. The PV relations were measured at baseline and during inferior vena caval occlusion. The measurements of segmental conductance were recorded and cardiac volumes were determined. When coupled with pressure, the generation of ventricular PV relationship allowed precise hemodynamic characterization of ventricular systolic and diastolic function and loading conditions. The explanted hearts underwent H&E and trichrome stains for myocardial infarction. Finally, RNA was extracted from the remaining explanted hearts for RT-PCR analysis of cytokines for mechanistic determination of myocardial restoration by mESC. RT-PCR was performed using SuperScript One-Step RT-PCR with Platinum *Taq* kits (Invitrogen, Carlsbad, CA). Mouse primers for the following cytokines were acquired: MMP-1, -2, -9, -14, TNF-α, VEGF- α, Collagen-2α, IGF1, TGF-β, ACE, IGF1, FGF1, Flk-1, Flt-1, and NFkB-1 (Applied Biosystems).
Results During the 1-week duration, the mESC-treated mice demonstrated significant improvement of the left ventricular ejection fraction (LVEF), LV mass, and LV volumes in comparison to NS- and mEF-treated mice ($p<0.05$) as shown in Figure 1. PV loop analysis confirmed these findings demonstrating an end-systolic elastance (Ees in mmHg/ul) in the mESC-treated group at week 1 of 8.80 ± 2.9 vs. NS-treated group of 4.58 ± 1.5. No significant improvement was observed in Ees in the mEF-treated group at week 1 of 6.38 ± 1.6 (n=4)] versus NS-treated group. Histology confirmed these findings as shown in Figure 2. RT-PCR demonstrated significant up-regulation of TNF-α and VEGF-α and down-regulation of MMP-1 and collagen-2α ($P<0.05$).
Conclusion This study demonstrates the therapeutic potential of mESC in comparison to mEF- or NS-treated murine model of acute myocardial infarction. Possible mechanistic explanation for the restorative effects of mESC includes combined anti-apoptotic, pro-angiogenic, and anti-remodeling effects, which together appear to play a significant role in restoring the injured myocardium.

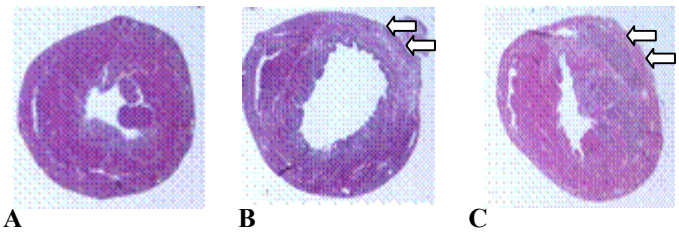


A



B

Figure 1. (A) LVEF at week 1 for normal sham-operated control, LAD-ligated (NS-treated), LAD-ligated (mESC-treated), and LAD-ligated (mEF-treated) mice. **(B)** Short-axis image at end-diastole and end-systole in normal sham-operated control, LAD-ligated (NS-treated), LAD-ligated (mESC-treated) with iron oxide labeled mESC (white arrows) at week 1 and LAD-ligated (mEF-treated) groups.



A B C

Figure 2. H&E stain of **(A)** normal sham operated myocardium, **(B)** LAD-ligated NS-treated demonstrates thinning of the myocardium in the anterolateral region, and **(C)** LAD-ligated mESC-treated demonstrates restoration of the myocardium in the same corresponding region.