

1H MRI assessment of cardiac function following transplantation of human embryonic stem cell-derived cardiomyocytes for cardiac repair

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Introduction

Rehabilitation of cardiac function and replacement of cardiomyocytes lost after infarction are major goals in experimental cardiology. Current insights into stem cell plasticity and recent discoveries in this field have opened up new perspectives for regenerating the infarcted heart. Human embryonic stem cells (hESCs) are attractive candidates for heart regeneration, because these cells have an unquestioned ability to differentiate into functional cardiomyocytes, have tremendous proliferative capacity prior to differentiation [2] and continue to proliferate after differentiation into cardiomyocytes (even in vivo [3]), and, the most importantly, possess the cellular elements required for electromechanical coupling with the host myocardium. For these reasons, grafts of hESC-derived cardiomyocytes may contribute to systolic force generation in the injured heart [1, 2]. The possibility to generate human myocardium by injecting differentiated cardiac-enriched hESCs progeny into the left ventricular wall of athymic rats has been shown recently [3]. This novel approach is permitting studies of human myocardial development and physiology and supporting the feasibility of their use in myocardial repair. The purpose of this study was to test the hypothesis that engrafting hESC-derived cardiomyocytes into infarct zone would prevent left ventricular remodeling and increase regional wall motion after reperfused myocardial infarction in rodents. The physiological consequences of creating human myocardium in the rat heart were examined non-invasively using MRI.

Methods

hESC preparation and engrafting: H7 human embryonic stem cell-derived cardiomyocytes (hESCCs) were generated via directed differentiation with Activin-A and BMP4. Myocardial infarction (MI) in 300-g nude Sprague Dawley rats was induced by the ligation of the left main coronary artery (60 min) followed by reperfusion. At day 4 the chest was reopened, and 10 million human cardiomyocytes were directly injected into the previously injured left ventricular wall. hESCCs were injected into the rat heart (n=24) in Matrigel along with the multi-component pro-survival cocktail (PSC), targeting key points of potential death pathways [4]. Another group of rats had PSC injection into the infarcted area of the heart without hESCCs (n=14). A third group had serum-free medium (SFM) injection only (without hESCCs and PSC, n=5), while a fourth group did not have surgery or any other intervention and served a functional control (n=9).

Echocardiography: All rats were studied by echocardiography 2 days post-infarction (before cell transplantation) to evaluate the extent of contractile dysfunction among all studied groups. Animals with fractional shortening more than 40% were excluded to maintain the uniform functional baseline for all studied groups.

Magnetic resonance imaging (MRI): 4 weeks after infarction all animals were studied by MRI using a 4.7T Varian scanner. High-resolution ECG-gated spin-echo multi-slice short-axis ¹H MR images for end systole and another for end diastole were obtained to quantify left ventricular function (FOV of 50 mm²; 2D matrix of 256x128; slice thickness 1.5 mm without gap between slices, TR=400ms; TE=13ms; flip angle=90°). Epicardial and endocardial borders were manually traced for determination of left ventricular volumes at end systole and end diastole (ESV, EDV), left ventricular mass (LVmass), and left ventricular ejection fraction (LV EF). Left ventricle wall thickening (LVTh) was evaluated at the infarcted and non-infarcted (opposite and mid-septim) areas of the each slice.

Histology: After imaging, rat hearts were fixed and vibratome-sectioned at 500-μm thickness to ensure equivalent sampling. These uniform transverse sections were routinely processed and paraffin-embedded for evaluation of percentage infarct area (by picosirius red) and quantitative measurements of total human graft (by counts of human nuclei labeled with a pan-centromeric in situ hybridization probe). Implanted cells were phenotyped with the histo- and immunohistochemical markers.

Results

At 4 weeks post-MI all hearts showed significant increase in LV chamber dimensions and LVmass. Particularly, ESV increased from 139±9 mm³ in control group (Mean±SE) to 301±25 mm³ in hESCCs group, 339±14 mm³ in PSC and 414±28 mm³ in SFM group. EDV increased from 357±26mm³ in control group to 595±30 mm³ in hESCCs group, 634±13 mm³ in PSC, and 710±27 mm³ in SFM. LVmass increased from 599±38mg (healthy rat heart) up to 759±34mg in SFM group (p<0.001). LV ejection fraction was significantly reduced in all animals following infarction, p<0.001 (Fig. 1). Those changes indicate left ventricular remodeling of rat heart four weeks after MI. However, the degree of the remodeling and severity of LV dysfunction were significantly less in hESCCs-treated group compare to vehicle-injected hearts (PSC and SFM groups). Importantly, cell-treated hearts had higher EF compared to PSC or SFM groups (p<0.01), demonstrating improved contractile function (Fig 1). Human cardiomyocyte grafting also attenuated the increase in LV chamber volume compared to the SFM group (p<0.01). Moreover, the infarcted anterior wall of hESCCs-treated rat hearts showed 2.5-fold greater systolic thickening compared to untreated infarcted animals, p<0.001 (Fig. 1, 2). Histologically, hearts receiving hESC-derived cardiomyocytes had the biggest human myocardial grafts we have seen to date, reaching up to 10% of the infarct area.

Conclusion

This study shows that transplantation of hESC-derived cardiomyocytes improves left ventricular function after infarction, yielding a 20% relative increase in LV ejection fraction and a 2.5-fold increase in regional wall motion. The strong positive effect of human cardiomyocyte therapy offers a new approach to limit the adverse consequences of myocardial infarction and encourages further study in models that closely reflect human myocardial infarction.

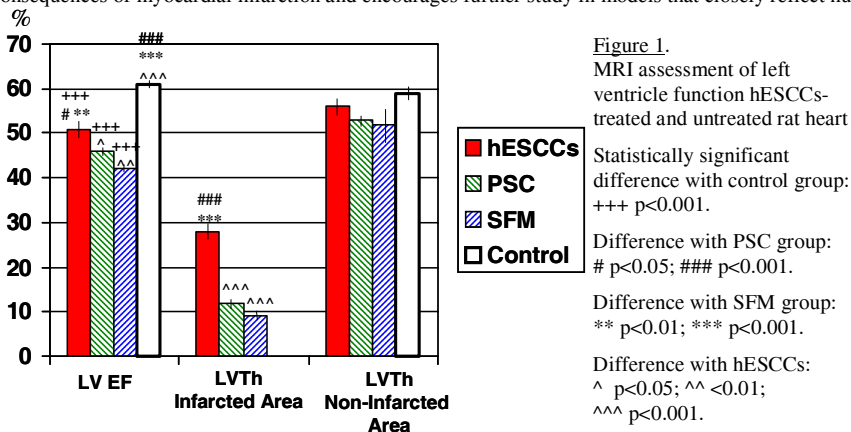


Figure 1. MRI assessment of left ventricle function hESCCs-treated and untreated rat heart. Statistically significant difference with control group: +++ p<0.001. Difference with PSC group: # p<0.05; ### p<0.001. Difference with SFM group: ** p<0.01; *** p<0.001. Difference with hESCCs: ^ p<0.05; ^^ <0.01; ^^> p<0.001.

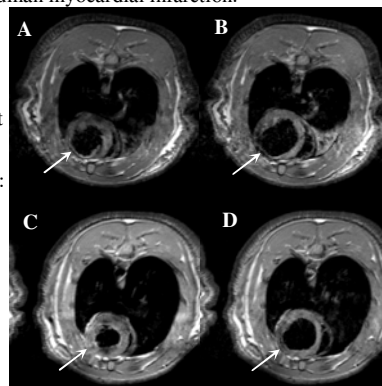


Figure 2. Typical short-axis ¹H MRI of rat thorax at the level of the heart at end-systole (left) and end-diastole (right): PSC (top) and hESCCs + PSC (bottom). Note the improved LV wall thickening of infarcted area (arrow) in hESCCs-treated hearts (C, D) compare to untreated animals (A, B).

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