Functional Effects of Human Embryonic Stem Cell-Derived Cardiomyocyte Transplantation on Chronic Myocardial Infarction in Rats

A. Naumova[1], S. Fernandes[2,3], V. Yarnykh[1], V. Muskheli[2,3], C. Yuan[1], and C. E. Murry[2,3]

[1]Radiology, University of Washington, Seattle, WA, United States, [2]Pathology, University of Washington, Seattle, WA, United States, [3]Center for Cardiovascular Biology, Institute for Stem Cell and Regenerative Medicine, Seattle, WA, United States

Introduction

Cell transplantation using derivatives of adult or embryonic stem cells (ESCs) is a promising therapeutic approach for heart failure. Human ESC-derived cardiomyocytes (hESC-CM) can be efficiently generated by injecting differentiated cardiac-enriched hESCs progeny into the left ventricular wall of athymic rats [1]. It has been recently shown that hESC-CM transplanted into infarcted rat hearts in a pro-survival cocktail form human myocardial grafts which enhance regional contractility and prevent heart failure development [2]. These positive effects are cardiomyocytes-specific and were not demonstrated after the transplantation of non-cardiac hESC derivates. In that study, the hESC-CM were injected into the rat heart on the 4th day after infarction, when angiogenesis associated with wound repair is at its maximum and the survival rate of grafted rat cardiomyocytes is the highest [3]. However, in real clinical practice, the patients with severe heart failure are most in need of cell transplantation therapy. No studies were done to explore the restoration effects of hESC-CM transplantation on chronic myocardial infarction. The purpose of this study was to test the hypothesis that engrafting hESC-derived cardiomyocytes into the chronic infarction would improve heart function in rodents.

Methods

hESC preparation and engrafting: H7 human embryonic stem cell-derived cardiomyocytes were generated via directed differentiation with Activin-A and BMP4 [4]. Myocardial infarction in nude Sprague Dawley rats was induced by the ligation of the left main coronary artery (60 min) followed by reperfusion. One month after infarction-induced surgery rodent chest was reopened, and 10 million human cardiomyocytes were directly injected into the left ventricle in the border of infarct region (n=6). hESC-CM were injected into the rat heart in the multi-component pro-survival cocktail (PSC), targeting key points of potential death pathways [2]. Another group of rats had PSC injection into the infarcted area of the heart without hESC-CM (n=9). Control group of healthy nude rats (n=5) did not had any cardiac surgery.

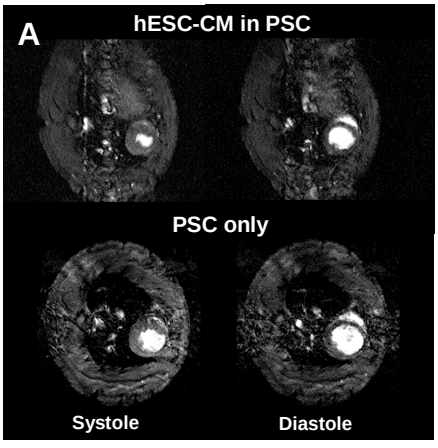
Echocardiography: All rats were studied by echocardiography 3 weeks post-infarction (before cell transplantation) to evaluate the extent of contractile dysfunction among all studied groups and to maintain the uniform functional baseline for all studied groups. Echocardiographic studies were repeated for the same animals one month after cell transplantation (8 weeks after myocardial infarction).

Magnetic resonance imaging (*MRI)*: Left ventricle geometry and function were studied by MRI 8 weeks post-infarction. Custom constructed 2-turn solenoid receive-only coil (4.5 cm diameter) connected to the interface box for 3T Achieva Philips human whole body scanner was used to image rat chest. Prospectively triggered, cartesian turbo-gradient echo cine (TFE CINE) sequence through the short-axis slices of the heart has been used with slice thickness 1.5 mm, TR/TE 8.3/3.8 ms, fov 70x49 mm, 2 signal averages, flip angle 30°, 2D acquisition matrix 232x163, phase interval 8.3 ms. For cardiac function analysis epicardial and endocardial borders of the left ventricle were manually traced using ImageJ 1.34s software (NIH, USA). Left ventricular chamber volumes at end systole and end diastole (ESV, EDV), stroke volume (SV), left ventricular mass (LVmass), cardiac output (CO) and ejection fraction (EF) were determined. Left ventricle wall thickening (LVTh) was evaluated at the infarcted and non-infarcted areas of the each slice.

Histology: After imaging, rat hearts were harvested, immersion-fixed in Methyl Carnoys and vibratome-sectioned. Uniform transverse sections were routinely processed and paraffin-embedded for evaluation of percentage infarct area (by picrosirius red) and quantitative measurements of total human graft (by counts of human nuclei labeled with a pan-centromeric in situ hybridization probe).

Results

At 2 months post-infarction all hearts showed a significant increase in LV chamber dimensions and LVmass. Particularly, ESV increased more than two-fold from 161±8mm^3 in non-infarcted animals (Mean±SE) to 357±68mm^3 in the hESC-CM group and 342±47mm^3 in the PSC group. EDV increased significantly also from 420±9mm^3 in control group to 708±65mm^3 and 624±51mm^3 (hESC-CM and PSC, respectively). EF was significantly reduced in all infarct-operated animals (Fig.1). Those changes indicate left ventricular remodeling of the infarcted rat hearts. There was no statistically significant difference in LV chamber volumes and ejection fraction between the hESC-CM and PSC groups by 2 month after infarction. Despite the EF reduction in hESC-CM group compare to healthy control (52±6% vs. 61±2%), the difference was not statistically significant (p=0.18). The stroke volume and coronal output were significantly higher in the cardiomyocyte-treated hearts vs. the PSC-only group, p<0.05 (SV: 351±16mm^3 vs. 281±14mm^3; CO: 133773±7532mm^3/min vs. 112146±6228mm^3/min). We also noticed trends in EF increase and better thickening of the infarcted wall in cardiomyocyte-treated animals vs. the PSC group (Fig.1). It was an excellent correlation between echocardiographic and MRI measurements of LV chamber dimentions and function (correlation coefficient between fractional shortening and ejection fraction was 0.81; end-systolic dimentions by MRI and echo 0.83; end-diastolic dimentions 0.75).



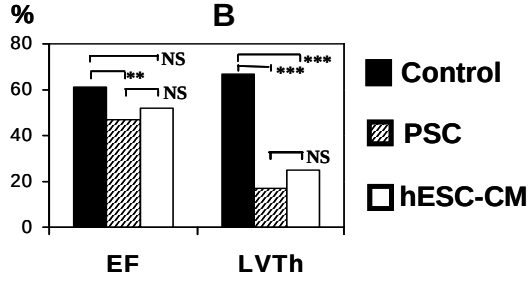


Figure 1. **MRI assessment of heart function in hESC-CM treated and untreated rat heart.**
A: Representative TFE CINE short-axis cardiac MRI of the hESC-CM treated and untreated animals.
B: Ejection fraction and left ventriclular wall thickening in infarcted area vs. healthy myocardium.
** p<0.01; *** p<0.001;
NS: difference is not statistically significant.

Discussion and Conclusion

This study showed that transplantation of hESC-CM to the border zone of mature infarction forms stable human grafts and moderately improves cardiac function in nude rats. Improvement of heart function was less pronounced in this study than if human cardiomyocytes were injected on 4th day after infarction [2]. We assume that effect of human cardiomyocyte transplantation on chronic myocardial infarction will become more apparent during a long term observation (3-4 months after cell injection), when graft size would reach its functional capability.

References:
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