Transplantation of Murine Embryonic Stem Cell-derived Cardiomyocytes Improves Cardiac Function in the Infarcted Heart

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Introduction

Embryonic stem cells (ESC) can be robustly induced to differentiate into cells of cardiac lineage making them a potential source of transplantable cells for myocardial regeneration. However conventional differentiation methods lead to low yields of murine ESC-derived cardiomyocytes (ESC-CM), making it difficult to perform in vivo studies. Furthermore, low enrichment of CM leads to concerns regarding teratoma formation. In our previous study, we have achieved highly enriched ESC-CM in high yields by inserting an cardiac specific antibiotic resistant gene in the ESC to allow selection for CMs in the presence of puromycin (1). The purpose of this study was to evaluate whether global and regional cardiac function can be restored by ESC-CM treatment using longitudinal cardiac MR (CMR) imaging on a athymic rat model of myocardial infarction (MI), which has been used extensively in examining in vivo efficacy of ESC-CM of human and murine origin [2]

Materials and Methods

Animal Preparation: Athymic (nu/nu) rats were subjected to a 60 minute reperfused MI. The initial infarct size was estimated at 24 hours after the surgery by delayed hyper-enhancement (DHE) MRI. The animals with infarction size in the range of 10-30% of the LV volume were assigned randomly to ESC-CM or vehicle group, whereas those outside this range were excluded. The vehicle (n=15) and ESC-CM (n=17) treated group received 50µl medium, which contains a cocktail of growth factors [2] or 5-10 Million of ESC-CM suspended in such media, respectively, on day 7 post-MI. Global LV functions were assessed with CMR at 1 day, 1- and 2-month post-MI, and also obtained from normal (non-infarcted) rats for comparison. Regional functions were assessed with cine displacement-encoded stimulated echo (DENSE) imaging [3] at 2-month post-MI. **MR Imaging:** All MR experiments were performed with a Unity INOVA console (Varian, Palo Alto, CA) interfaced to 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 combination of TEM transmit volume coil and surface receive coil (InsightMRI, Worcester, MA) was used. Animals were maintained at 37°C under gas anesthesia. ECG and respiration were monitored. Scanner was gated by ECG. After multi-slice cine imaging, cine DENSE data sets were acquired from three short axis (SA) slices of each heart from mid-ventricle to apex with 1.5-2 mm gap. The FOV is 4cm x 4cm, acquisition matrix is128x128 and tag spacing is 2mm. **Image Processing:** Global cardiac function parameters including end-diastolic volume (EDV), end-systolic volume (ESV), left ventricular ejection fraction (LVEF) were derived from cine MRI as previously described [4]. The k-space raw data from DENSE images was analyzed offline by home-made MATLAB programs (Mathworks, Natick, MA) to derive displacement map after phase unwrapping [5] and calculate orthogonal Lagrangian strains using finite element isoparametric triangle formula [6].

Results

Quantification of initial infarct size by DHE MRI led to relatively uniform infarct size in ESC-CM (20.4% ± 3.9%) and vehicle (19.4% ± 4.9%) group (P>0.5). At day 1 post MI, LVEF, ESV and EDV were not different between the ESC-CM and vehicle treated group; however, LVEF was significantly lower in infarcted rats compared to normal ones (Chart 1). At 1- and 2-month post-cell injection, LVEF is significantly greater in the ESC-CM treated than vehicle group (P<0.05 in both cases) while ESV is significantly smaller in ESC-CM group (P<0.05), suggesting the recovery of systolic function. DHE MRI at day 1 post MI suggests that infarct mostly locates in the anterior and lateral region at mid-ventricular level towards apex. Regional circumferential strains measured by DENSE suggest a significantly higher contractile function in the anterior and lateral regions at these levels in the ESC-CM treated hearts compared to the vehicle group (Chart 2). Ecc averaged over the entire wall on the slice also shows difference between the two groups. Our previous study showed that ESC-CMs have low proliferative capacity thus allows retaining of SPIO particles during serial MRI tracking and indeed SPIO-related hypointense regions were observed in ESC-CM treated hearts at 2-month post MI as shown in Fig 1b (yellow circle). Histological analysis suggests these regions consist of ESC-CMs and infiltrated macrophages, both containing SPIO (Prussian blue positive). Therefore although SPIO-related hypointense signals do not authentically represent grafted cells, they mark the location of graft formation and facilitate MRI and histology analysis. Finally, no evidence of teratoma formation was seen in any of the animals studied.

Conclusion: These data suggest that ESC-CM mediated a teratoma free myocardial repair with significant recovery of regional and global contractile function over the period of 2 months. We are further exploring the mechanism underlying the improvement of myocardial function.

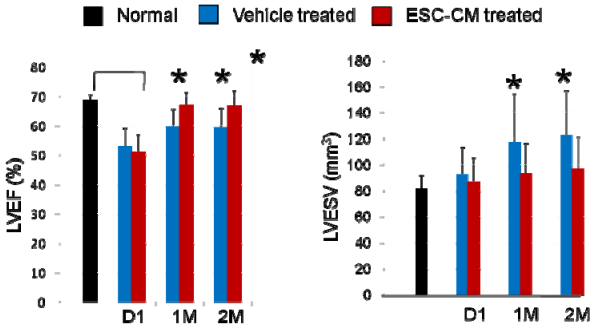

Chart 1 Long term effect on global cardiac function

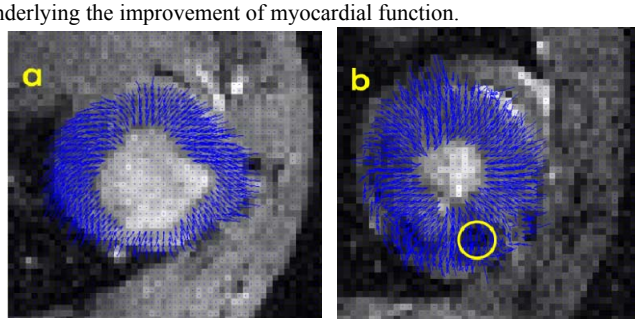

Figure 1 Displacement vector maps in ES phase overlaid with cine magnitude images. (a) and (b) are from a vehicle treated and ESC-CM treated animal, respectively. The yellow circle points to the graft zone grown from iron-labeled ESC-CM cells.

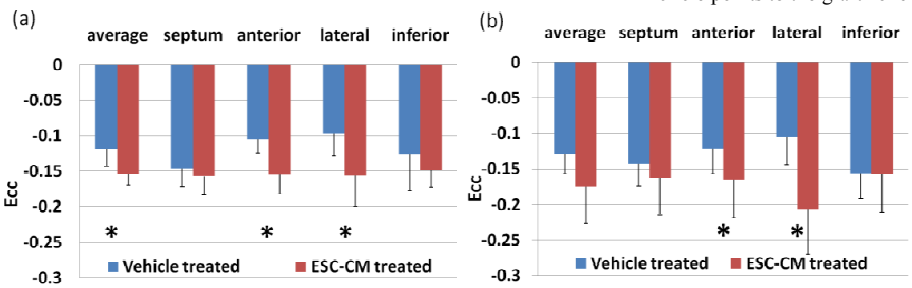

Chart 2 Circumferential strain from slices at mid-ventricle (a) and 1.5-2mm below towards apex (b). The circumferential strain of the ESC-CM treated subjects is higher than that of the vehicle treated subject in anterior and lateral wall (p=0.005 and 0.024 respectively at mid-ventricle slice and p=0.048 and 0.004 respectively at slice 1.5-2mm below).

References:

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