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Original article

Cell therapy enhances function of remote non-infarcted myocardium

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ABSTRACT

Cell transplantation improves cardiac function after myocardial infarction; however, the underlying mechanisms are not well-understood. Therefore, the goals of this study were to determine if neonatal rat cardiomyocytes transplanted into adult rat hearts 1 week after infarction would, after 8–10 weeks: 1) improve global myocardial function, 2) contract in a Ca^{2+} dependent manner, 3) influence mechanical properties of remote uninjured myocardium and 4) alter passive mechanical properties of infarct regions. The cardiomyocytes formed small grafts of ultrastructurally maturing myocardium that enhanced fractional shortening compared to non-treated infarcted hearts. Chemically demembranated tissue strips of cardiomyocyte grafts produced force when activated by Ca^{2+}, whereas scar tissue did not. Furthermore, the Ca^{2+} sensitivity of force was greater in cardiomyocyte grafts compared to control myocardium. Surprisingly, cardiomyocytes grafts isolated in the infarct zone increased Ca^{2+} sensitivity of remote uninjured myocardium to levels greater than either remote myocardium from non-treated infarcted hearts or sham-operated controls. Enhanced calcium sensitivity was associated with decreased phosphorylation of cTnT, tropomyosin and MLC2, but not changes in myosin or troponin isoforms. Passive compliance of grafts resembled normal myocardium, while infarct tissue distant from grafts had compliance typical of scar. Thus, cardiomyocyte grafts are contractile, improve local tissue compliance and enhance calcium sensitivity of remote myocardium. Because the volume of remote myocardium greatly exceeds that of the grafts, this enhanced calcium sensitivity may be a major contributor to global improvements in ventricular function after cell transplantation.

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1. Introduction

Cell-based therapies for cardiac repair hold great promise for the treatment of myocardial infarction. It has been demonstrated that transplantation of many cell types (including cardiomyocytes) into experimentally induced myocardial infarctions improves myocardial performance as assessed by decreased cavity dilation, increased ejection fraction, decreased infarct expansion, and increased developed systolic pressure [1–6]. These encouraging results have led to the initiation of several clinical trials (reviewed in reference [7]). Very little is known, however, about the mechanism by which cell transplantation improves heart function. In fact, at least seven general mechanisms have been hypothesized in the literature: re-muscularization, attenuated post-infarct ventricular remodeling, paracrine effects leading to increased angiogenesis or to increased survival of cardiomyocytes near the infarct border zone, immunomodulation of the infarct environment, improvements in the extracellular matrix, and recruitment of resident cardiac progenitors [7]. Thus, the underlying mechanisms by which cell-based therapies can contribute to the improvement of myocardial performance clearly warrant investigation.

The overall design of this study was to investigate the active and passive mechanical properties of transplanted cells and host myocardium as a means to determine the cellular-tissue basis of improved whole-heart function following cell transplantation. Neonatal rat cardiomyocytes (NRCs) were grafted into the infarct region of adult rat hearts 1 week following permanent occlusion of the left anterior descending coronary artery to address four main questions: (1) Can transplanted cells contract in a Ca^{2+} dependent manner similar to normal myocardium, thus having the potential to re-muscularize the

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damaged region? (2) Can transplanted cells reduce the stiffness of damaged myocardium, thus improving diastolic behavior? (3) Do transplanted cells influence the mechanical properties of myocardium remote from the injury site? (4) Are there potential myofibrillar mechanisms that may explain the mechanical differences observed?

1.1. Materials and methods

More extensive details of methods are provided in the Supplementary data.

1.2. Cell preparation and animal model

These studies were approved by the University of Washington (UW) Animal Care Committee and were conducted in accordance with federal guidelines. Animals were housed in the Department of Comparative Medicine at the UW and were cared for in accordance with the US NIH Policy on Humane Care and Use of Laboratory Animals. Neonatal rat cardiomyocytes (NRCs) were isolated from 1–3-day old newborn syngeneic Fischer 344 rats and Di-I labeled as described in the Supplementary data. The surgical procedure for coronary occlusion of adult rat hearts and graft injection was as previously described [8–10].

1.3. Echocardiography

Echocardiography was performed as described by Laflamme *et al.* [11]. Rats were lightly sedated with isoflurane and monitored by continuous electrocardiography (ECG) via three limb leads. Echocardiographic measurements were taken using a GE Vivid7 echocardiography system with an 11 MHz convex transducer of parasternal long-axis and short-axis images at the mid-papillary muscle level to ensure evaluation of the infarcted region of the hearts. M-mode measurements on the short-axis view were taken to obtain left ventricular end-diastolic (LVEDD) and systolic dimensions (LVESD). Fractional shortening (FS) was calculated as (LVEDD − LVESD) / LVEDD × 100%. Measurements were made on at least three cardiac cycles by two blinded echocardiographers and averaged for each data value.

1.4. Strip dissection and mechanical measurements

Dissected hearts were Vibratome-sliced and demembranated (by Triton-X 100) overnight at −20 °C to remove the sarcolemmal and sarcoplasmic reticulum membranes [12]. Graft regions were identified by visualization of the CM-DiI label under a fluorescent microscope. Strips were dissected manually and connected via aluminum T-clips to pin hooks on a force transducer and linear motor in a mechanical setup similar to one previously described [13] for active and passive force measurements. Following mechanical measurements the samples were processed for histology and western blotting as detailed in the Supplementary data.

1.5. SDS–PAGE, western blotting, histology, and transmission electron microscopy

Primary antibodies used were against α-myosin heavy chain (α-MHC) (ATCC, clone BA-G5, Manassas, VA), β-MHC (ATCC, clone A4.591, Manassas, VA), cardiac troponin I (cTnI) (HyTest, Turku, Finland), slow skeletal TnI (ssTnI) (Santa Cruz Biotechnology, Santa Cruz, CA), TnT (Sigma, St. Louis, MO), and sarcomeric actin as a loading control (Sigma, St. Louis, MO). Secondary antibodies were conjugated to peroxidase for chemiluminescence and were: sheep anti-mouse-IgG (GE Healthcare, Buckinghamshire, UK) for α-MHC, β-MHC, cardiac troponin T (cTnT), and cTnI; bovine anti-goat-IgG (Santa Cruz Biotechnology, Santa Cruz, CA) for ssTnI; and goat anti-mouse-IgM (Sigma, St. Louis, MO) for sarcomeric actin. Pro-Q Diamond staining (Invitrogen, Eugene, OR) was performed as previously

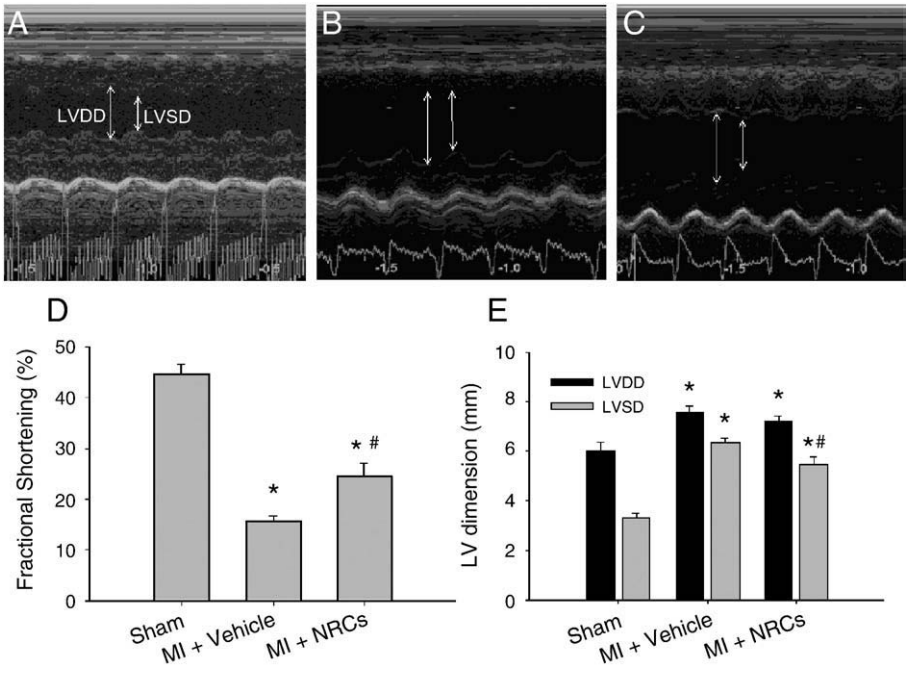

Fig. 1. Assessment of myocardial function after cell transplantation by echocardiography. Representative left ventricular M-mode images from control animals (A, sham, $n=6$), and infarcted animals that received either vehicle (B, non-treated infarcted, $n=9$) or cells (C, grafted, $n=10$). Arrows indicate chamber dimension at end-diastole (LVDD) and end-systole (LVSD). Fractional shortening (D) was greatly reduced with MI, but the functional decline was attenuated by cell transplantation. Both diastolic and systolic LV dimensions increased after MI (E). No difference was found in LV diastolic dimension (E, LVDD) between non-treated infarct or grafted hearts, but LV systolic dimension (E, LVSD) was reduced in grafted hearts. Values are means ± S.E.M.; *$p<0.05$ versus sham; #$p<0.05$ versus MI + vehicle.