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A Combination of Allogeneic Stem Cells Promotes Cardiac Regeneration

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ABSTRACT

BACKGROUND The combination of autologous mesenchymal stem cells (MSCs) and cardiac stem cells (CSCs) synergistically reduces scar size and improves cardiac function in ischemic cardiomyopathy. Whereas allogeneic (allo-) MSCs are immunoevasive, the capacity of CSCs to similarly elude the immune system remains controversial, potentially limiting the success of allogeneic cell combination therapy (ACCT).

OBJECTIVES This study sought to test the hypothesis that ACCT synergistically promotes cardiac regeneration without provoking immunologic reactions.

METHODS Göttingen swine with experimental ischemic cardiomyopathy were randomized to receive transendocardial injections of allo-MSCs + allo-CSCs (ACCT: 200 million MSCs/1 million CSCs, n = 7), 200 million allo-MSCs (n = 8), 1 million allo-CSCs (n = 4), or placebo (Plasma-Lyte A, n = 6). Swine were assessed by cardiac magnetic resonance imaging and pressure volume catheterization. Immune response was tested by histologic analyses.

RESULTS Both ACCT and allo-MSCs reduced scar size by −11.1 ± 4.8% (p = 0.012) and −9.5 ± 4.8% (p = 0.047), respectively. Only ACCT, but not MSCs or CSCs, prevented ongoing negative remodeling by offsetting increases in chamber volumes. Importantly, ACCT exerted the greatest effect on systolic function, improving the end-systolic pressure-volume relation (+0.98 ± 0.41 mm Hg/ml; p = 0.016). The ACCT group had more phospho-histone H3+ (a marker of mitosis) cardiomyocytes (p = 0.04), and noncardiomyocytes (p = 0.0002) than did the placebo group in some regions of the heart. Inflammatory sites in ACCT and MSC-treated swine contained immunotolerant $CD3^+/CD25^+/FoxP3^+$ regulatory T cells (p < 0.0001). Histologic analysis showed absent to low-grade inflammatory infiltrates without cardiomyocyte necrosis.

CONCLUSIONS ACCT demonstrates synergistic effects to enhance cardiac regeneration and left ventricular functional recovery in a swine model of chronic ischemic cardiomyopathy without adverse immunologic reaction. Clinical translation to humans is warranted. (J Am Coll Cardiol 2017;70:2504–15)

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Cell-based therapy reduces scar size and represses adverse remodeling secondary to myocardial infarction (MI) in preclinical models (1-4) and in clinical trials (5,6) of patients with ischemic cardiomyopathy (ICM). We previously showed that in a swine model of chronic MI (4), both autologous (7) and allogeneic (1,2) mesenchymal stem cells (MSCs) improved cardiac function by reducing infarct scar size, reversing negative remodeling, and enhancing endogenous cardiomyocyte proliferation (3). Administration of xenogeneic human c-Kit$^+$ cardiac stem cells (CSCs) to immunosuppressed pigs (8), and autologous CSCs to pigs (9) and humans (10) produces significant improvements in cardiac function. Furthermore, when CSCs are combined with MSCs, regeneration is enhanced (8,11).

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Allogeneic MSCs (allo-MSCs) have distinct advantages over autologous cells in terms of potency and availability (12). MSCs evade and suppress immunologic responses secondary to their lack of class II major histocompatibility complex (13), and their release of immunomodulatory soluble factors (14-18). However, the immunoevasive abilities of other allogeneic cells, in particular CSCs, is unclear and may be dependent on the proportion of allogeneic cells expressing major histocompatibility complex class I and II (12,19). The POSEIDON (Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis) (6,20) and LVAD (Effect of Intramyocardial Injection of Mesenchymal Precursor Cells on Myocardial Function in Patients Undergoing LVAD Implantation) clinical trials (21) demonstrated that allo-MSCs did not induce significant immunologic responses in patients with ICM up to 12 months post-cell treatment, supporting their safety. Furthermore, there is also evidence from clinical trials that allogeneic cell-based therapy may be superior to autologous cell therapy (6,20,22). The immunomodulatory effects of MSCs have driven their clinical use as a primary therapeutic and even as an adjunct to allograft transplant (23). However, whether allogeneic CSCs (allo-CSCs) can be used successfully as an allograft, alone, or in combination with MSCs has never been tested. The objective of this study was to examine the safety and regenerative efficacy of allogeneic cell combination therapy (ACCT), a combination of allo-MSCs and allo-CSCs in a 200:1 ratio.

In this study, Göttingen swine were subjected to MI via ischemia/reperfusion injury, which was allowed to develop into chronic ICM (4). Three months post-MI, swine were administered placebo, allo-MSCs, allo-CSCs, or ACCT to test the hypotheses that: 1) ACCT is safe and does not elicit an immunologic response; and 2) ACCT has greater therapeutic efficacy than either cell type alone.

METHODS

STUDY DESIGN. All animal protocols were reviewed and approved by the University of Miami Institutional Animal Care and Use Committee. Female Göttingen swine were subjected to catheter-induced ischemic reperfusion MI as previously described (4). Swine were randomized for transendocardial stem cell injections (TESI) of 1×10^6 allo-CSCs and 2×10^8 allo-MSCs (ACCT, n = 7), 2×10^8 MSCs (n = 8), 1×10^6 CSCs (n = 4), or placebo (Plasma-Lyte A, Baxter, Illinois) (n = 6). Continuous cardiac monitoring devices were implanted subsequent to stem cell therapy to assess arrhythmogenic events. Measurements of cardiac structure and function were obtained using cardiac magnetic resonance (CMR) imaging, pressure-volume (PV) loops, and histologic analyses of phospho-histone H3 (pHH3)$^+$ cells and CD3$^+$/CD25$^+$/FoxP3$^+$ regulatory T cells (Tregs) in the extracted hearts (Online Appendix, Online Figure 1).

CELL MANUFACTURING PROCESS AND TESI. Heart (right atrial appendage) and iliac crest bone marrow biopsies were attained from male Yorkshire swine. Cells were cultured, amplified, characterized (24-26), and cryopreserved at passage 3. On the day of injection, cells were thawed and aliquoted (total 5.1 ml) (Online Appendix).

TESI were performed at 3 months post-MI using the NOGA injection-catheter system (Johnson & Johnson, New Brunswick, New Jersey). An electromechanical map of the left ventricle (LV) endocardium was generated. The viable border zone of dense scar was determined as a unipolar voltage range of 6 to 12 mV. Cells were injected into 10 sites within the border zone using the Myostar injection catheter (B type, Johnson & Johnson) (Online Appendix).

STATISTICAL ANALYSIS. Parametric values and statistics are presented as mean ± SEM. Normally distributed parameters were evaluated with a repeated measures analysis of variance model including between-group comparisons as well as time and group × time interaction terms. Bonferroni correction was applied for post hoc tests. One pig from the MSC group was excluded from analysis of MRI-derived data due to multiple missing time points. The 1-month post-MI time point was excluded from analysis of

ABBREVIATIONS AND ACRONYMS

ACCT = allogeneic cell combination therapy
BZ = border zone
CMR = cardiac magnetic resonance
CSCs = cardiac stem cells
EDV = end-diastolic volume
EF = ejection fraction
ESPVR = end-systolic pressure-volume relationship
ESV = end-systolic volume
ICM = ischemic cardiomyopathy
IZ = ischemic zone
LV = left ventricular
MI = myocardial infarction
MSCs = mesenchymal stem cells
pHH3 = phospho-histone H3
PV = pressure-volume
RZ = remote zone
Treg = regulatory T cell
TESI = transendocardial stem cell injection