Synergistic Effects of Combined Cell Therapy for Chronic Ischemic Cardiomyopathy

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ABSTRACT

BACKGROUND Both bone marrow–derived mesenchymal stem cells (MSCs) and c-kit⁺ cardiac stem cells (CSCs) improve left ventricular remodeling in porcine models and clinical trials. Using xenogeneic (human) cells in immuno-suppressed animals with acute ischemic heart disease, we previously showed that these 2 cell types act synergistically.

OBJECTIVES To more accurately model clinical applications for heart failure, this study tested whether the combination of autologous MSCs and CSCs produce greater improvement in cardiac performance than MSCs alone in a non-immunosuppressed porcine model of chronic ischemic cardiomyopathy.

METHODS Three months after ischemia/reperfusion injury, Göttingen swine received transendocardial injections with MSCs alone (n = 6) or in combination with cardiac-derived CSCs (n = 8), or placebo (vehicle; n = 6). Cardiac functional and anatomic parameters were assessed using cardiac magnetic resonance at baseline and before and after therapy.

RESULTS Both groups of cell-treated animals exhibited significantly reduced scar size (MSCs –44.1 ± 6.8%; CSC/MSC –37.2 ± 5.4%; placebo –12.9 ± 4.2%; p < 0.0001), increased viable tissue, and improved wall motion relative to placebo 3 months post-injection. Ejection fraction (EF) improved (MSCs 2.9 ± 1.6 EF units; CSC/MSC 6.9 ± 2.8 EF units; placebo 2.5 ± 1.6 EF units; p = 0.0009), as did stroke volume, cardiac output, and diastolic strain only in the combination-treated animals, which also exhibited increased cardiomyocyte mitotic activity.

CONCLUSIONS These findings illustrate that interactions between MSCs and CSCs enhance cardiac performance more than MSCs alone, establish the safety of autologous cell combination strategies, and support the development of second-generation cell therapeutic products. (J Am Coll Cardiol 2015;66:1990–9) © 2015 by the American College of Cardiology Foundation.

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Similarly, we showed that cell engraftment and systolic and diastolic recovery were superior with combination therapy. The goal of the present study was to determine whether transendocardial administration of autologous MSCs plus CSCs would similarly produce greater therapeutic potential than MSCs alone in a swine model of chronic heart failure due to post-infarct LV remodeling.

**METHODS**

All experiments were conducted in female Göttingen swine (12). Eighty-eight animals survived a closed-chest ischemic reperfusion MI induced by inflation of a coronary angioplasty balloon in the mid-left anterior descending artery, as previously described (12). Animals were randomized to receive transendocardial injections of either: 1) (12). Animals were randomized to receive transendocardial injections of either: 1) 1 × 10⁶ autologous CSCs co-administered with 200 × 10⁶ MSCs; 200 × 10⁶ MSCs alone; or placebo (Plasma-Lyte, Baxter Healthcare Corporation, Deerfield, Illinois). Each animal underwent an extensive safety evaluation. Noninvasive cardiac magnetic resonance (CMR) was performed (12-14). The study design is outlined in Online Figure 1, and the timeline for serial measurements of LV performance and reduced myocardial scarring to a greater degree than either cell type alone (11).

**IMMUNOHISTOCHEMISTRY.** Twelve slides (4 each per infarct zone [IZ], border zone, and remote zone [RZ]) were randomly chosen from each animal for quantification of phospho-histone H3 (pHH3)-positive nuclei (Online Appendix). Slides were examined by using fluorescence microscopy (Olympus IX81, Olympus Corporation, Tokyo, Japan), and the number of pHH3-positive nuclei was quantified per slide. Representative samples were selected and stained with anti-pHH3 and anti-myosin light chain 2 (Novus Biologicals, Littleton, Colorado). Image acquisition was performed with a Zeiss LSM-710 confocal microscope (Carl Zeiss MicroImaging, Thornwood, New York).

**CELL MANUFACTURING AND DELIVERY.** CSCs were isolated from bone marrow obtained from the tibial cavity, as reported elsewhere (11). On the morning of stem cell injection, the cells were thawed, washed, re-suspended in Plasma-Lyte A (Abbott Laboratories, North Chicago, Illinois), counted, and total cell viability determined. The cells (200 × 10⁶ MSCs ± 1 × 10⁶ CSCs) were resuspended in Plasma-Lyte A (total volume 6 ml) before injection. Criteria for release of product were cell viability ≥70% and negative results on sterility testing. The placebo injection consisted of Plasma-Lyte A alone (Online Appendix).

Transendocardial stem cell injection (TESI) was performed 3 months post-MI by using the NOGA system for electroanatomical mapping (Biosense Webster, Inc., Diamond Bar, California) (18). The mapping catheter was advanced through an 8-F introducer sheath and positioned retrograde across the aortic valve into the left ventricle. A complete map of LV geometry and function was generated by collecting local position and electrocardiographic data at >50 points in the endocardium. Cells were injected by using the NOGA Myostar catheter (Biosense Webster, Inc.) directly into the endocardium in approximately 0.5 ml aliquots for each of 10 sites encompassing the infarction and its viable border zone (unipolar voltage ≥6 mV). Injection sites were recorded both in the electroanatomic NOGA map and in 2 orthogonal radiographic projections, and marked on a tracing of the endocardial silhouette.
and ileum to determine the presence of neoplastic tissue at necropsy. CMR was performed by using a 3.0-T clinical scanner (Magnetom, Siemens AG, Munich, Germany).

Swine underwent serial CMR at baseline, 1 and 3 months post-MI, and 1, 2, and 3 months post-TESI. Global and regional function were assessed through the measurement of end-diastolic volume, end-systolic volume, stroke volume, EF, scar size, viable tissue, Eulerian circumferential strain, diastolic strain rate, and perfusion. Endothelial function was measured by the brachial artery flow-mediated dilation (FMD) (19) (Online Appendix).

**STATISTICS.** All data are presented as mean ± SEM. All data points were analyzed by using GraphPad Prism version 4.03 (GraphPad Software Inc., La Jolla, California). For within-group changes, 1-way analysis of variance (ANOVA) was applied with Tukey’s multiple comparison test. For between-group comparisons, an unpaired 2-tailed Student t test and 1- and 2-way ANOVA were applied with Tukey’s multiple comparison test when applicable. A p value < 0.05 was considered statistically significant.

**RESULTS**

Baseline and post-MI conditions for all animals were assessed (Online Table 2). There were no differences between groups for body weight or age at baseline or at scheduled time points (Online Tables 1 and 2). Serum hematology, chemistry, and cardiac enzyme values were measured at several time points throughout the study. There was no evidence of clinically relevant laboratory abnormalities after TESI (Online Figure 2) in any of the groups. TESI was tolerated; there were no sustained arrhythmias and no evidence of ectopic tissue formation (Online Tables 3 and 4).

All study groups had similar infarct sizes, whether evaluated as a percentage of LV mass or absolute scar size 3 months after infarction (Online Table 5).
Stem cell treatment, but not placebo, produced substantially reduced scar size (CSC/MSC – 37.2 ± 5.4%; MSCs – 44.1 ± 6.8%; placebo – 12.9 ± 4.2%; p < 0.0001) and increased viable tissue (CSC/MSC 30.9 ± 7%; MSCs 43.7 ± 13.3%; placebo 13.5 ± 5.9%; p = 0.0002) relative to placebo (Figure 1). Scar size reduction was evident 1 month post-TESI and persisted for 3 months. There was a strong correlation between scar size measured by using delayed-enhancement CMR and scar size measured according to gross pathology sections: r = 0.93; 95% confidence interval: 0.80 to 0.98; p < 0.0001 (Online Figure 3).

All animals had similar depression of ejection fraction (EF) due to MI (Figure 2A, Online Table 6). EF increased 3 months post-TESI in the combination group by 6.9 ± 2.8 EF units (p = 0.0003), in MSCs by 2.9 ± 1.6 EF units (p = NS), and with placebo by 2.5 ± 1.6 EF units (p = NS; between-group p = 0.0009; CSC/MSC vs. MSC and CSC/MSC vs. placebo, each p < 0.05). EF as a percent change from post-MI improved only in the CSC/MSC group: 20.61 ± 2.11%, 14.37 ± 3.64%, and 13.9 ± 6.2% at 1, 2, and 3 months post-TESI, respectively (between-group p = 0.0004; 3 months post-MI vs. 1, 2, and 3 months post-TESI, each p < 0.05) but was unchanged in the MSC and placebo groups (each p = NS) (Figure 2B).

EF restoration was accompanied by a substantial improvement in stroke volume in the CSC/MSC group, exceeding that of MSCs or placebo (CSC/MSC 47.2 ± 11.1% vs. MSCs 21.2 ± 4.7% vs. placebo 22.4 ± 12.0%; between-group p = 0.008; CSC/MSC vs. MSC, MSC vs. placebo, each p < 0.05) (Figure 2C). Furthermore, cardiac output increased only in the CSC/MSC group: 50.5 ± 11.3%, p = 0.007; MSCs 27.8 ± 13.6%, p = 0.2; placebo: 15.5 ± 9.5%, p = 0.02; between-group comparison p = 0.008) (Figure 2D, Online Table 6).

**FIGURE 2** EF Improvement Post-TESI

Change in ejection fraction (EF) for individual animals for (A) EF units and (B) as a percent change (p = 0.01) post-TESI. Accompanying this EF restoration was a substantial improvement in the CSC/MSC group in (C) stroke volume (p = 0.008) and (D) cardiac output, which increased only in the CSC/MSC group (p = 0.007). Graphs represent mean ± SEM. *p < 0.05 1-way ANOVA, 3 months post-MI vs. 1, 2, and 3 months post-TESI with CSC/MSC; **p < 0.05 CSC/MSC vs. placebo; †p < 0.05 CSC/MSC vs. MSCs. Abbreviations as in Figure 1.
Circumferential strain rate (peak Eulerian circumferential shortening strain), a measure of regional contractility calculated from tagged CMR, exhibited improved regional function (greater negative delta) of the IZ only in the cell-treated groups (CSC/MSC/C0 = 1.2, p = 0.004; MSCs/C0 = 1.1/C6 = 0.9, p = 0.04; placebo delta 0.2/C6 = 0.9, p = NS; between-group comparison p = 0.1) (Figure 3A). TESI did not improve regional function in the border zone compared with placebo (data not shown).

CMR tagging was used to evaluate diastolic performance. Diastolic strain increased only in the combination-treated animals (*p = 0.04), remaining unchanged in the MSC (p = NS) and placebo (p = NS) groups (between-group comparison p = 0.9) (Figure 3B).

The impact of cell therapy on peripheral vascular function was also explored by measuring FMD of the brachial artery, which improved similarly in both cell-treated groups but worsened in the placebo group (CSC/MSC 147.6 ± 74.5%, 1-way ANOVA p < 0.0001; MSCs 142.5 ± 135.4%, 1-way ANOVA p = 0.04; placebo −102.4 ± 106.5%, 1-way ANOVA p = NS; between-group comparison p = −0.01; Tukey’s multiple comparison test p < 0.05, CSC/MSC vs. placebo and p < 0.05 MSCs vs. placebo) (Figure 4).

We analyzed 11 to 12 slides (3-4/zone) from each of 20 pigs for PHH3 and Myosin Light Chain 2 staining (Figures 5A and 5B) and found a greater number of pHH3+ cardiomyocytes in the border zone (TESI site) in the CSC/MSC group (1.0 ± 0.3) compared to the placebo group (0.2 ± 0.1; p = 0.05) but no differences in the infarct or remote zones (Figures 5C to 5E). Similarly in the combination group there were significantly more mitotic pHH3-positive nuclei found within the myocardium in the infarct zone (Figure 5F) per slide compared to the placebo group (CSC/MSC: 1.2 ± 0.2; MSCs: 0.7 ± 0.3; placebo: 0.5 ± 0.3).
Perfusion was assessed in all 3 zones (IZ, border, and remote). In the IZ, there was a borderline trend toward progressively deteriorating tissue perfusion (−0.2 ± 0.1; p = 0.05) but not in the border or remote zones (Figures 5G and 5H).

Vascular density was similar in all 3 groups (Online Figure 4).

**DISCUSSION**

This preclinical animal study was designed to provide a rigorous placebo-controlled and blinded safety and efficacy assessment using autologous MSCs alone or in combination with autologous CSCs in a chronic MI/reperfusion model. There are 3 major findings. First, we established that coinjection of autologous MSCs...
and CSCs is safe and not associated with an increased risk of adverse effects. Second, both cell treatments produced similar antibiotic effects. Third, only the coadministration of cells improved myocardial contractile performance. These findings are the first demonstration that autologous cell combination therapy is superior to MSCs alone in a model of chronic ischemic cardiomyopathy, and they therefore have important implications for future clinical trial design.

The efficacy of autologous MSCs alone has been established pre-clinically and clinically (3,4). MSCs are thought to act primarily via a combination of paracrine signaling (20–22), proangiogenic effects (23,24), and stimulation of endogenous CSC proliferation, differentiation (25–28), and recruitment (29). For the combination of the 2 cell types, efficacy was demonstrated by improvement in both structural and functional parameters. CSCs are located in niches within the heart, and they exert important regulatory and regenerative roles (30) that can be augmented by cell therapy (31). Together, these results suggest that TESI, with a combination of MSCs plus CSCs, provides an advantage over overcoming factors that inhibit or counteract the efficacy of the current single cell-type therapy (25).

The present study illustrates that autologous MSCs alone or in combination with CSCs similarly reduce infarct size and increase viable tissue compared with placebo. We previously showed a 2-fold greater reduction in scar size with combination therapy compared with either cell administered alone in the setting of acute MI and xenogeneic (human) cells 4 weeks post-injection (11). Coadministration of human CSCs and MSCs also significantly increased (7-fold) retention and engraftment compared with either cell type administered alone, suggesting direct cell contribution to myocardial regeneration. One mechanism underlying the beneficial effects of stem cell therapy is endogenous tissue regeneration, including CSC activation and myocyte division (25). We showed that the CSC/MSC combination led to increased cardiomyocyte mitosis 3 months post-TESI and was associated with sustained improvement of cardiac performance and scar size reduction (Central Illustration). Scar size reduction in all cell-treated pigs was accompanied by substantial recovery in cardiac function but was less robust when MSCs were administered alone. Interestingly, significantly improvement in cardiac function was shown only in the combination group, as measured by EF, stroke volume, cardiac output, regional diastolic strain rate, and endothelial function. This discrepancy relative to our earlier study may be due to differences in the 2 models: autologous versus xenogeneic cells (porcine vs. human), timing (chronic vs. subacute), delivery methods (transendocardial vs. direct via thoracotomy), and immunosuppression (absence vs. presence).

The interactions of immunosuppressant drugs with MSCs remain controversial. Buron et al. (32) showed that cyclosporine and other immunosuppressant drugs reduce the ability of MSCs to suppress lymphocyte proliferation. In contrast, other studies found that cyclosporine promotes the lymphocyte-suppressing effects of MSCs (33,34). The longer follow-up time may also play a role, suggesting that the combination of stem cells may help sustain the beneficial effects over a longer period of time. Recently, the POSEIDON (Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis) (1) and TAC-HFT (Transendocardial Autologous Cells in Ischemic Heart Failure Trial) (2) clinical trials reported that injections of autologous MSCs did not improve EF 12 months post-delivery; there was, however, significant improvement in the clinical status of patients, as measured by using the 6-min walk test and Minnesota Living with Heart Failure questionnaire score.

Our regional analysis of contractility revealed improved peak diastolic strain rate in the targeted areas in the combination-treated animals. Peak diastolic strain rate (35) is a measure of diastolic function that, in impaired myocardial regions, can remain persistently compromised despite complete systolic functional recovery after reperfusion post-MI. FMD,
as a measure of endothelial function that can be imaged and quantified as an index of vasomotor function (36), is an attractive technique because it is noninvasive and allows for repeated measurements throughout the study. Both cell-treated groups demonstrated improved FMD, suggesting that stem cell treatment promotes nitric oxide release by the endothelium with subsequent vasodilation. The improvement in endothelial function due to cell therapy in this study is important in the context of our finding that tissue perfusion was improved in the IZ of cell-treated pigs in the absence of increased vascular density. Recently, we found that MSCs increase endothelial function and release of endothelial progenitor cells in patients with heart failure (37).

Finally, this study suggests that combining cell types can have clinical benefits, including enhancing improvements in myocardial contractile performance (38,39). The strategy tested here supports the feasibility of clinical trials, as both cell types have been tested in early-stage clinical trials. Admixtures of cell types that complement each other’s capabilities seem to provide synergistic benefits that enhance the short-term (11) and long-term therapeutic outcomes compared with 1 type of cell alone.

**STUDY LIMITATIONS.** The present study was rigorously designed but lacked a CSC-alone group. However, in early-phase clinical trials (40), autologous CSCs had a successful safety profile, including increased EF, regional wall motion, New York Heart Association functional class, and quality-of-life scores on the Minnesota Living with Heart Failure questionnaire. Although in vitro and rodent studies show that CSCs are necessary and sufficient for functional cardiac regeneration and repair (41), others have found that endogenous CSCs may produce new cardiomyocytes at low levels (42). There is growing consensus (43) that CSCs alone may be insufficient to repair the failing myocardium, and strategies are being developed to address this issue (44,45). Adding MSCs may overcome these shortfalls by providing the needed paracrine effects.

**CENTRAL ILLUSTRATION** Combination Stem Cell Therapy for Heart Failure

**A** 3m MI Regional Contractility
IZ Peak Ecc -4.5%

**B** 3m Post TESI Regional Contractility
IZ Peak Ecc -12.8%

Tagged harmonic phase cardiac magnetic resonance strain maps show significantly depressed regional function according to peak Eulerian circumferential shortening strain (Ecc) at **A** 3 months post-myocardial infarction (white arrows). **Red/white** indicates weak contractility (more positive Ecc) and **green/blue** indicates vigorous contractility (more negative Ecc) in harmonic phase strain maps. **B** At 3 months after cell injection, infarct zone contractility has improved (less, **red/white**; more, **green/blue**). **TESI** = transendocardial stem cell injection.
factors, stromal support, and cell-to-cell contact that contribute to cardiac niche reconstitution. Interestingly, Konfino et al. (46) showed that the type of injury, resection, or infarction dictates the mode of repair in the neonatal and adult murine heart. They suggested that MI and subsequent inflammation might inhibit complete regeneration. Thus, the immunomodulatory properties of MSCs render this cell type an indispensable component for effective cell combinations.

Another limitation is that the present study lacked dose escalations of each stem cell therapy. However, there are many contradictory reports relating therapeutic efficacy with higher (6) or lower (1) doses of MSCs, and there is still no defined efficacious dose range for CSCs. In addition, using autologous cells precluded quantification of engraftment.

CONCLUSIONS

Transendocardial injection of autologous MSCs plus autologous CSCs produced scar size reduction, increased viable tissue, and restored contractile performance 3 months post-MI. These findings showed, for the first time, that important interactions between these stem cells produce substantial enhancement in cell-based therapy for at least 3 months after treatment. The current and previous cell combination studies have produced excellent safety and highly encouraging efficacy profiles, supporting the conduct of clinical trials.

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