Adipose-Derived Regenerative Cells in Patients With Ischemic Cardiomyopathy

The PRECISE Trial

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Disclosures

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Abstract and Introduction

Abstract

Aims Adipose-derived regenerative cells (ADRCs) can be isolated from liposuction aspirates and prepared as fresh cells for immediate administration in cell therapy. We performed the first randomized, placebo-controlled, double-blind trial to examine the safety and feasibility of the transendocardial injections of ADRCs in no-option patients with ischemic cardiomyopathy.

Methods and results Procedural, postoperative, and follow-up safety end points were monitored up to 36 months. After baseline measurements, efficacy was assessed by echocardiography and single-photon emission computed tomography (6, 12, and 18 months), metabolic equivalents and maximal oxygen consumption (MVO_2) (6 and 18 months), and cardiac magnetic resonance imaging (6 months). We enrolled 21 ADRC-treated and 6 control patients. Liposuction was well tolerated, ADRCs were successfully prepared, and transendocardial injections were feasible in all patients. No malignant arrhythmias were seen. Adverse events were similar between groups. Metabolic equivalents and MVO_2 values were preserved over time in ADRC-treated patients but declined significantly in the control group. The difference in the change in MVO_2 from baseline to 6 and 18 months was significantly better in ADRC-treated patients compared with controls. The ADRC-treated patients showed significant improvements in total left ventricular mass by magnetic resonance imaging and wall motion score index. Single-photon emission computed tomography results suggested a reduction in inducible ischemia in ADRC-treated patients up to 18 months.

Conclusion Isolation and transendocardial injection of autologous ADRCs in no-option patients were safe and feasible. Our results suggest that ADRCs may preserve ventricular function, myocardial perfusion, and exercise capacity in these patients.

Introduction

Stem cell therapy has been shown to be safe and to provide cardiac benefits in patients with chronic ischemic heart disease.[1–5] In most clinical trials, autologous bone marrow mononuclear cells have elicited modest yet significant improvements in left ventricular ejection fraction (LVEF), left ventricular dimensions, or exercise capacity.[1–5] Bone marrow mesenchymal stem cells (MSCs) are also considered an excellent cell type for use in cardiac regenerative medicine and have been examined in preclinical[6] and clinical studies.[7,8] In patients with acute or chronic heart disease, both autologous and allogeneic MSCs have shown safety and consistent benefits in LVEF, cardiac remodeling, and scar size. However, the clinical use of autologous MSCs is hampered by their requirement for ex vivo culture and expansion.

Adipose tissue has emerged as a promising source of stem cells for cell-based therapies. Adipose-derived regenerative cells (ADRCs) can be obtained by liposuction and prepared as fresh cells at the site for immediate autologous transplantation without the need for culture and expansion. The stromal vascular fraction (SVF) of adipose tissue contains a mixed, multipotent population of cells[9] that can differentiate into multiple cell lineages, including cardiomyocytes, endothelial cells, and smooth muscle cells, and that can provide growth factors and cytokines for tissue repair.[10,11] Furthermore, adipose tissue contains 500-fold more MSCs than adult bone marrow.[12,13]

In vitro studies have shown that human ADRCs can differentiate into spontaneously beating cells with cardiomyocyte features[14] and secrete proangiogenic factors.[15] Moreover, the therapeutic efficacy of ADRCs has been suggested in preclinical studies of acute experimentally induced myocardial infarction (MI).[16] In animal models of chronic myocardial injury, adipose-derived MSCs improved cardiac function and survival in animals with scarred myocardium[17] and showed superior benefits to bone marrow mononuclear cells.[18] Furthermore, genetic modifications have enhanced ADRC survival and paracrine effects in preclinical studies, resulting in improved protection and cardiac repair.[19,20]

Here, we report the findings of the first clinical trial of ADRCs in human chronic heart disease. Specifically, we have conducted the first randomized, placebo-controlled, double-

blind study of the safety and feasibility of transendocardial delivery of ADRCs in patients with chronic ischemic cardiomyopathy who have no options for coronary revascularization.

Methods

Study Design

This was a prospective, randomized, placebo-controlled, double-blind, safety and feasibility study designed to enroll up to 36 patients at 4 clinical sites (online Appendix Supplementary material). The study was approved by the institutional review boards at all participating sites and was conducted according to the Declaration of Helsinki and ICH E6 Good Clinical Practice Guidelines (ClinicalTrials.gov identifier: NCT00426868). Written informed consent was obtained from all patients before enrollment.

This study was supported by Cytori Therapeutics, Inc, San Diego, CA. The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper, and its final contents.

Patients who met the eligibility criteria and who consented were randomly assigned in a 3:1 ratio to treatment with ADRCs or placebo. The study was originally designed as a sequential dose-escalation study, with up to 3 dosing arms; however, the third dose-escalation arm of the study was not completed because of the practical limitations of isolating higher cell doses. Thus, the study was stopped before enrollment of the high-dose group, and data from cell-treated patients were pooled for final analyses. An interactive voice response system was used for treatment assignments and randomization. A designated hospital staff member who was not involved in patient care made the randomization call to the interactive voice response system. As part of the randomization process, the cell processing technician prepared and clearly labeled 2 syringes, which were then sent for randomization: one syringe contained the ADRCs, and the other contained a visually indistinguishable placebo. The placebo was prepared by diluting approximately 0.4 mL of blood in lactated Ringer's solution. After randomization, the designated syringe (with the identity of the contents concealed) was sent to the Cardiac Catheterization Laboratory for injection, and the contents of the unused syringe were destroyed. The patients, the investigators, all personnel at the

core laboratories, and those involved in data management and in evaluating patient status were blinded to treatment assignments.

Study Population

The study population comprised patients who had coronary artery disease (CAD) not amenable to any revascularization procedure as determined by an interventional cardiologist and a cardiovascular surgeon and who had ischemia in the area supplied by the nonrevascularizable vessel(s). Patients (20–75 years old) were required to have class II to IV Canadian Cardiovascular Society (CCS) angina symptoms and/or class II to III New York Heart Association (NYHA) symptoms of heart failure, an LVEF ?45% as determined by 2-dimensional transthoracic echocardiography within 2 weeks before enrollment, and evidence of a stress-induced reversible perfusion defect on single-photon emission computed tomography (SPECT) within 1 month of enrollment (see online Appendix Supplementary material for full inclusion criteria, exclusion criteria, and screening procedures).

End Points

The primary objective of this study was to assess the feasibility and safety of delivering ADRCs by the transendocardial route in patients with ischemic cardiomyopathy. Feasibility was determined by assessing patients' tolerability of the liposuction procedure and the ability to undergo electromechanical mapping-guided transendocardial injections. Safety was assessed by recording the occurrence of *major adverse cardiac or cerebral events*, which were defined as cardiac death, ST-elevation and non–ST-elevation MI, stroke, emergent coronary artery bypass grafting (CABG), and target lesion revascularization, or any adverse or severe adverse event from enrollment to the 36-month follow-up. The secondary end point of efficacy was examined by measuring LVEF, wall motion score index (WMSI), left ventricular volumes, and total and infarcted left ventricular masses (by echocardiography at baseline and at 6, 12, and 18 months, and by magnetic resonance imaging [MRI] at baseline and 6 months); myocardial perfusion (SPECT at baseline and at 6, 12, and 18 months); and exercise tolerance (metabolic equivalents [METs] and maximal oxygen consumption [MVO_2] testing at baseline and at 6 and 18 months).

Cell Collection and Preparation

Adipose tissue was harvested by liposuction performed under local anesthesia by a plastic surgeon or other appropriately trained medical professional. The ADRCs were isolated by using the Celution System (Cytori Therapeutics Inc, San Diego, CA) (online Appendix Supplementary material).

The 3 cell doses initially planned in the dose-escalating cohorts were 0.4×10^6 ADRCs/kg, 0.8×10^6ADRCs/kg, and 1.2×10^6 ADRCs/kg. The cells were counted, and viability was assessed via trypan blue exclusion before injection.

ADRC Studies

We analyzed the SVF of freshly isolated adipose tissue from 15 patients who were sequentially enrolled in the first dose cohort (ADRC-treated, n = 12; control, n = 3) by flow cytometry. We excluded lymphocytes (18.61% of the injected cells). We used a fluorescein isothiocyanate–labeled cocktail of antibodies to mature lineage markers (CD45, CD31, CD34, CD71, CD105 [endogline or MAb SH2], and CD184 [CXCR-4]) and vascular endothelial growth factor receptors (BD-Pharmingen, San Diego, CA). Measurements were made in a Coulter flow cytometer (Beckman Counter, Fullerton, CA) and expressed as median and interquartile range because the data were not normally distributed.

Colony-forming unit assays. Clonogenic assays were used to evaluate the frequency of mesenchymal progenitor cells in fresh samples (n = 15) of adipose tissue (online Appendix Supplementary material). Colony-forming unit fibroblast (CFU-F) assay was used to enumerate mesenchymal progenitor cells. The CFU-F activity was expressed as the absolute number of colonies per 10^5 plated cells.

Transendocardial Injection Procedure

The ADRCs were delivered by using the NOGA XP system (Biologics Delivery Systems, Diamond Bar, CA) as described.[21] Cells were injected into areas of the heart identified as having a reversible defect on SPECT and as being viable myocardial tissue as indicated by NOGA criteria (unipolar voltage

of >6.9 mV). In patients in whom SPECT and NOGA showed discordant findings in regard to the ischemic area, coronary anatomy was taken into account. To detect possible pericardial effusion, we performed a transthoracic echocardiogram immediately after injections and on the following day.

Clinical Assessments and Safety Monitoring

Patients underwent standard safety testing in the immediate postoperative period, including electrocardiogram and measurement of cardiac enzymes, and at follow-up visits (1, 3, 6, 12, 18, 24, and 36 months). Holter monitoring was conducted at 6 and 18 months, and recordings were analyzed by a blinded core laboratory (Agility Centralized Research Services, Bannockburn, IL).

MVO_2 and Imaging Protocols

Exercise treadmill tests, echocardiography, SPECT, and MRI protocols are described in the online supplement. All imaging studies were read by blinded, experienced observers at core laboratory facilities.

Statistical Analysis

Continuous variables were presented as mean ± SD or as median and interquartile range if the data were not normally distributed. Categorical variables were compared by using χ^2/Fisher exact tests. Statistical comparisons of continuous variables between initial and follow-up data were performed by using a paired *t* test for intragroup comparisons and a Student *t* test for intergroup comparisons. Comparisons of the changes from baseline to follow-up in the control and treatment groups were made with repeated-measures analysis of variance. The analysis of variance model included control versus treatment and baseline versus follow-up as factors and also included the interaction between the 2 factors. A $P < .05$ was considered significant. Statistical analysis was performed with SPSS for MacIntosh v.20.0 (SPSS Inc, Chicago, IL).

Results

Study Population

Between January 2007 and May 2009, 27 patients (average age, 63.6 ± 7.5 years; 21 men [78%]) were enrolled at the 4 study sites: 21 ADRC-treated patients and 6 control patients. Most baseline characteristics were similar between the groups, but patients in the treatment group were older and had higher diastolic blood pressure (Table I).

Cell Preparation and Injections

Adipose tissue was successfully harvested from all patients (mean volume, 361 ± 122 mL). The ADRCs were prepared successfully from each patient; the average ADRC yield after cell processing was 240,000 cells/g. Cell viability before injection was 86.6% ± 4.9%.

The phenotypic characteristics of ADRCs from the SVF were analyzed by flow cytometry in a subset of 15 sequentially enrolled patients (CD34: 70.4% [range 66.5–73.3], CD45: 21.9% [range 17.3–26.0], CD184: 13.8% [range 6.9–17.1], vascular endothelial growth factor receptors: 10.8% [range 6.7–17.3], CD31: 10.3% [range 8.7–14.5], CD71: 2.8% [range 1.3–5.7], CD105: 1.7% [range 0.6–2.6]) (Figure 1,*A*). The median CFU-F in the SVF was 0.19% (range 0.16–0.25%), and the average CFU-F was 19 ± 9 colonies/10^5 ADRCs. Figure 1, *B* shows the stages of differentiation of ADRCs.

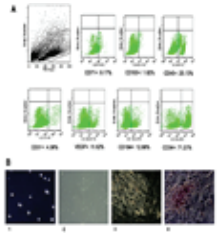

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Figure 1.

A, Flow cytometry analysis of the stromal vascular fraction in 1 patient. CD markers are expressed as percentage of the total SVF population. **B**, ADRCs in different stages of differentiation. **1**, Immediately after isolation (20×); **2**, mesenchymal cells obtained after culturing ADRCs for 7 days (10×); **3** and **4**, fibroblast colony-forming units derived from ADRCs (10×).

Each patient received 15 NOGA-targeted transendocardial injections of either ADRCs or placebo. In cell-treated patients, a mean total of 42×10^6 ADRCs in

a 3-mL volume was delivered into the ischemic myocardium. Control patients received placebo solution in the same volume.

Safety

No complications were related to the liposuction procedure. NOGA mapping of the left ventricle and transendocardial injections were feasible in all patients and were not associated with sustained arrhythmias. In the ADRC-treated group, 1 patient had a mild loculated pericardial effusion after injection that was managed conservatively. One patient in the treatment group had a periprocedural non–ST-elevation MI (Table II); this patient had severe CAD and a mildly stenotic calcified aortic valve and experienced hypotensive episodes during injection. Finally, 1 ADRC-treated patient had transient ischemic attacks 6 and 11 months after treatment.

There were a total of 5 deaths (3 ADRC-treated and 2 control patients), but only 2 were cardiac deaths (1 in each group) (Table II). Of the 3 deaths in the treatment group, 1 patient died of end-stage heart failure 4 months after undergoing cell injections; and 2 noncardiac-related deaths were due to choking at 1 month and to complications related to end-stage renal failure at 16 months after cell therapy. Of the 2 deaths in control patients, 1 died of terminal heart failure at 35 months; and 1 died of ischemic stroke at 23 months after randomization.

No malignant arrhythmias were associated with the procedure or were seen at follow-up. There were no significant changes in laboratory parameters from baseline levels.

Efficacy

Modest improvements were seen in NHYA class and CCS class in both groups (Figure 2).

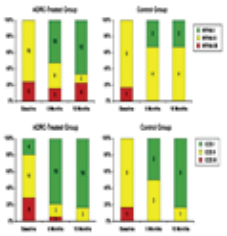

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Figure 2.

NYHA functional and CCS angina classification in control and ADRC-treated patients at baseline and 6 and 18 months after transendocardial injections. *P* = not significant.

On echocardiography, no significant changes were seen in LVEF or left ventricular volumes within either group over time or between groups.

On cardiac MRI, left ventricular total mass increased significantly from baseline to 6 months in ADRC-treated patients (from 128.1 ± 26.0 to 149.5 ± 32.4 g [*P* < .001]) but did not change in the control group (from 144.8 ± 52.7 to 152.6 ± 59.6 g [*P* = .1]) (Figure 3, *A*). Absolute and relative values of left ventricular infarcted mass were significantly higher in the control group over 6 months (from 29.6 ± 15.9 to 39.0 ± 15.4 g [*P* = .01] and from 23.6% ± 15.8% to 28.8% ± 15.3% [*P* = .01], respectively) but were not significantly different in treated patients (from 35.1 ± 20.4 to 34.0 ± 16.5 g [*P* = .8] and from 25.0% ± 10.9% to 22.1% ± 6.3% [*P* = .4], respectively) (Figure 3, *B*). Finally, MRI analysis showed that global WMSI improved in ADRC-treated patients at 6 months (from 2.1 ± 0.6 to 1.7 ± 0.9 [*P* = .04]) but did not change in control patients (from 2.0 ± 0.8 to 1.8 ± 1.0 [*P* = .4]) (Figure 3, *C*).

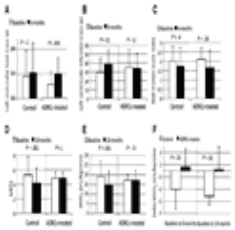

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Figure 3.

A to **C**, Evolution of cardiac MRI parameters from baseline to 6-month follow-up in ADRC-treated and control patients: left ventricular total mass **A**, left ventricular necrotic mass **B**, and WMSI **C**. **D**to **F**, Treadmill exercise results in control and ADRC-treated patients at baseline, 6, and 18 months: METS **D**, MVO_2 **E**, and Δ values of MVO_2 **F**.

The METs values were preserved over time in the ADRC-treated group (from 4.9 ± 0.8 to 4.9 ± 1.4 [*P* = 1]) but decreased significantly in the control group at 18 months (from 5.3 ± 2.5 to 4.2 ± 2.1 [*P* = .001]) (Figure 3, *D*). For MVO_2, no significant differences were seen from baseline to 6 months in the control group (from 18.4 ± 9.1 to 15.8 ± 7.6 mL/[kg min] [*P* = .1]) or the ADRC-treated

group (from 16.9 ± 3.1 to 17.4 ± 4.7 mL/[kg min] [*P* = .4]). However, at 18 months, MVO_2 values were preserved in the ADRC-treated group (from 17.2 ± 3.1 to 17.4 ± 4.7 mL/[kg min] [*P* = .8]) and declined significantly in the control group (from 19.0 ± 8.2 to 14.8 ± 6.9 mL/[kg min] [*P* = .001]) (Figure 3, *E*). The difference in the change in MVO_2 (Δ value) from baseline was significantly lower in control versus cell-treated patients at 6 months (?3.0 ± 3.3 vs 0.6 ± 3.0 mL/[kg min] [*P* = .03], respectively) and at 18 months (?4.1 ± 1.5 vs 0.3 ± 3.7 mL/[kg min] [*P* = .01]) (Figure 3, *F*).

There were no significant differences in SPECT stress and rest total severity scores over time from baseline to 6 or 18 months in either group. Summed stress-rest difference score at 6 months was lower in ADRC-treated patients (from 9.3 ± 7.0 to 5.8 ± 5.8 [*P* = .02]) but remained the same in the control group (from 12.8 ± 5.6 to 9.0 ± 9.2 [*P* = .1]), suggesting less stress-induced ischemia in patients treated with ADRCs. These differences were maintained at 12 months (from 9.6 ± 7.1 to 5.9 ± 6.4 [*P* = .03] vs from 12.8 ± 5.6 to 10.0 ± 9.0 [*P* = .4], respectively) and at 18 months (from 8.2 ± 7.1 to 5.1 ± 3.7 [*P* = .03] vs from 12.8 ± 5.6 to 7.2 ± 8.5 [*P* = .05], respectively). Finally, in accordance with the MRI data, visual summed wall motion score at 6 months was significantly increased in cell-treated patients (from 25.2 ± 11.5 to 27.6 ± 10.8 [*P* = .03]) but showed no differences in the control group (from 35.3 ± 13.5 to 34.0 ± 15.1 [*P* = .5]).

Discussion

In this multicenter, randomized, placebo-controlled, double-blind study, we have shown for the first time that the harvest and transendocardial injection of ADRCs are safe and feasible in no-option patients with ischemic cardiomyopathy. Moreover, cell therapy with ADRCs in these patients may preserve functional capacity over time and could exert a modest beneficial effect on myocardial perfusion, scar size, and left ventricular contractility.

Adipose tissue comprises a mixed cell source containing a high proportion of MSCs. The stromal fraction has been shown to contain multipotent stem cells.[10,11] Our approach allowed us to isolate ADRCs in high numbers in real time (<2 hours), during which time baseline mapping procedures were initiated in patients in preparation for cell injections.

Adipose-derived regenerative cells, unlike other cell types such as hematopoietic cells, have no specific cell surface markers. Thus, we used stromal-associated and stem cell–associated markers in our flow cytometric analysis and confirmed that the SVF comprises a heterogeneous cell population. The SVF cell population contained low to medium levels of cells positive for the mesenchymal marker CD105, as previously shown,[22] as well as low to medium levels of cells with endothelial (CD34 and CD31) and hematopoietic (CD45) markers. Although the percentage of $CD34^+$ cells in the SVF varies,[11,22,23] we found a strong positivity for $CD34^+$ cells. In contrast, $CD45^+$ cells were scarce, as previously reported.[24] Our data on cell phenotype should be interpreted cautiously because the SVF is not a homogeneous population, and the final composition can be affected by the methods used to harvest ADRCs and perform flow cytometry. The use of automatic rather than manual isolation may have contributed to our low percentage of $CD105^+$ cells. Finally, the mesenchymal proliferative capacity (CFU-F) of ADRCs in our study supports that reported by Dromard et al.[25]

In the present study, we provide preliminary evidence that harvesting ADRCs and delivering them by transendocardial injection are safe in patients with advanced CAD. No major safety issues were noted, specifically regarding cardiac arrhythmias and clinical adverse events. The safety profile appears to be comparable to that of delivering bone marrow–derived mononuclear cells, and the logistics are suitable for a point-of-care, same-day injection model. The rates of death were similar in our study to those reported in the largest registry of no-option patients.[26] We reported 2 cardiac deaths at 18 months (7.4%), for a total mortality of 18.5%.

Maximal oxygen consumption levels were preserved in the ADRC-treated group at 6 and 18 months as compared with the natural progression of the disease seen in the control group (in whom MVO_2 levels progressed toward eligibility levels for heart transplantation). This finding is interesting, considering that patients received only a single treatment with ADRCs over an 18-month period. Moreover, studies show that MVO_2 has significant prognostic value in patients with severe heart failure[27] and that directional changes in MVO_2 over time have prognostic significance.[28] We believe that monitoring changes in MVO_2 levels may be more meaningful than evaluating LVEF in patients with chronic heart failure.

Our results are comparable to those of previous studies with bone marrow–derived cells[1] and MSCs[29] in no-option patients. Both Losordo et al[1] and Haack-Sorensen et al[29] also showed the safety of transendocardial injections and improvement in exercise tolerance as well as quality of life scores in these patients. However, in contrast to MSCs,[7,8] ADRCs in our study did not reduce scar size or increase LVEF but instead appeared to result in scar stabilization. This may indicate different mechanisms of actions between the 2 cell types.

Recent studies have shown a reduction of scar size after infusion of cardiac stem cells in the subacute and chronic phases of MI.[30,31] Considering that our patients were in advanced phases of ischemic heart disease, a left ventricular mass increase of 21 g and the maintenance of scar size after ADRC injection suggest a beneficial response to cell therapy in these patients. Although mechanistic study of ADRCs is beyond the scope of this study, we could hypothesize that ADRCs have cardiogenic and angiogenic paracrine effects. Accordingly, a recent report showed that ADRC therapeutic function is induced primarily by paracrine-mediated cardioprotection and angiogenesis.[32] Improvements in perfusion and left ventricular mass may support these hypotheses, but confirmation in a larger study is warranted.

This study has several limitations. The small sample size limits the statistical rigor of our findings and our efficacy conclusions. Because baseline MRI and SPECT measurements varied between the 2 groups, imaging results should be interpreted cautiously and considered as hypothesis generating. However, the double-blind nature of the study and the use of a blind core reading of efficacy measures avoid biases in end point assessments. Furthermore, medication use, which is the most important factor that can influence clinical course in these patients, was similar between groups. Age was also significantly different; the treatment group was older, which may have biased them toward a worse outcome. Furthermore, a 3:1 randomization scheme resulted in a smaller control group, thus reducing the power of the study. Finally, MRI data were available only at baseline and 6 months, precluding longer follow-up.

Conclusion

Our findings indicate that obtaining autologous ADRCs via liposuction and delivering them by transendocardial injection are safe and feasible in patients with ischemic cardiomyopathy. Moreover, our trial provides a sound basis for testing the efficacy of ADRC therapy in a larger group of patients with chronic myocardial ischemia