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Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial

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Summary

Background—Cardiosphere-derived cells (CDCs) reduce scarring after myocardial infarction, increase viable myocardium, and boost cardiac function in preclinical models. We aimed to assess safety of such an approach in patients with left ventricular dysfunction after myocardial infarction.

Methods—In the prospective, randomised CArdiosphere-Derived aUtologous stem CElls to reverse ventricUlar dySfunction (CADUCEUS) trial, we enrolled patients 2–4 weeks after myocardial infarction (with left ventricular ejection fraction of 25–45%) at two medical centres in the USA. An independent data coordinating centre randomly allocated patients in a 2:1 ratio to receive CDCs or standard care. For patients assigned to receive CDCs, autologous cells grown from endomyocardial biopsy specimens were infused into the infarct-related artery 1·5–3 months after myocardial infarction. The primary endpoint was proportion of patients at 6 months who died due to ventricular tachycardia, ventricular fibrillation, or sudden unexpected death, or had myocardial infarction after cell infusion, new cardiac tumour formation on MRI, or a major adverse cardiac event (MACE; composite of death and hospital admission for heart failure or non-fatal recurrent myocardial infarction). We also assessed preliminary efficacy endpoints on MRI by 6 months. Data analysers were masked to group assignment. This study is registered with ClinicalTrials.gov, NCT00893360.

Findings—Between May 5, 2009, and Dec 16, 2010, we randomly allocated 31 eligible participants of whom 25 were included in a per-protocol analysis (17 to CDC group and eight to

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Conflicts of interest

EM and LM are founders and equity holders in Capricor. RRS and LM are employed by Capricor. KM receives consulting fees from Capricor. Capricor provided no funding for this study.

Contributors

EM was the chief investigator for the study, and designed and managed the study with input from RRM, RRS, and GG. RRM, RRS, KC, KM, LEJT, DB, LSCC, LM, AM, PVJ, SDR, KHS, ACL, and GG did the study and collected and/or interpreted data. KM and EM drafted the first and subsequent versions of this manuscript with input and key revisions by all authors, who reviewed and approved the final submission.

standard of care). Mean baseline left ventricular ejection fraction (LVEF) was 39% (SD 12) and scar occupied 24% (10) of left ventricular mass. Biopsy samples yielded prescribed cell doses within 36 days (SD 6). No complications were reported within 24 h of CDC infusion. By 6 months, no patients had died, developed cardiac tumours, or MACE in either group. Four patients (24%) in the CDC group had serious adverse events compared with one control (13%; p=1·00). Compared with controls at 6 months, MRI analysis of patients treated with CDCs showed reductions in scar mass (p=0·001), increases in viable heart mass (p=0·01) and regional contractility (p=0·02), and regional systolic wall thickening (p=0·015). However, changes in end-diastolic volume, end-systolic volume, and LVEF did not differ between groups by 6 months.

Interpretation—We show intracoronary infusion of autologous CDCs after myocardial infarction is safe, warranting the expansion of such therapy to phase 2 study. The unprecedented increases we noted in viable myocardium, which are consistent with therapeutic regeneration, merit further assessment of clinical outcomes.

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Introduction

Myocardial infarction is common, and many patients develop substantial scarring despite optimum treat ment.[1] The presence and extent of myocardial scarring pre-disposes to progressive unfavourable left ventricular remodelling, heart failure, and sudden death.[2,3] Present treatment approaches seek to limit the initial injury and block secondary maladaptive pathways. Conversely, regenerative therapy seeks to shrink scar and regrow healthy heart muscle. Despite more than a decade of clinical trials of cardiac regenerative therapy, this ambitious goal remains elusive. Trials with bone marrow mononuclear cells[4–7] or mesenchymal stem cells in patients after myocardial infarction have shown an excellent safety profile,[8] but efficacy is inconsistent[5,7] and sometimes transient.[6] Most studies have assessed global functional endpoints such as ejection fraction. However, the actual targets of regeneration—scar mass and viable myocardial mass—can be measured rigorously by contrast-enhanced MRI. In the few controlled studies of stem cells that used MRI to assess outcomes, scar size (ie, scar mass normalised by total left ventricular mass) did not change substantially, if at all, after cell therapy, with little or no relation to ejection fraction.[4–6,9–11] Even positive studies have failed to show increases in viable myocardium in addition to shrinkage of scar tissue.[4]

The notion of endogenous mammalian heart regeneration, which has traditionally been viewed as heretical, has gained support recently.[12] Various populations of putative endogenous cardiac progenitor cells have been identified, with widespread preclinical evidence for efficacy in cardiac repair and functional improvement after myocardial infarction.[13] The present study uses a straight forward approach for generation of heart-derived cells as therapeutic candidates. Percu taneous endomyocardial biopsies are used to obtain source tissue and the cardiosphere culture method[14] to yield tens of millions of cardiosphere-derived cells (CDCs) in a timely manner.[15] CDCs are clonogenic, have multilineage potential, can be safely delivered via the intracoronary route, and mediate reductions in scar size in preclinical models of myocardial infarction.[16–19]

In the CArdiosphere-Derived aUtologous stem CElls to reverse ventricUlar dySfunction (CADUCEUS) study, we aimed to assess safety of autologous intracoronary CDCs administered to patients 1·5–3 months after myocardial infarction, and test the hypothesis that CDCs convert scar tissue to viable myocardium.

Methods

Study design and participants

An investigator-sponsored Investigational New Drug Application (number 13930) was granted by the US Food and Drug Administration (FDA) for the CADUCEUS protocol, which involved two sites: the Cedars-Sinai Heart Institute (CA, USA) and The Johns Hopkins Hospital (MD, USA).

Patients with a recent myocardial infarction (≤4 weeks previously) and left ventricular dysfunction (ejection fraction 25–45% by clinically indicated imaging after infarction) were eligible for inclusion if they were aged 18 years or older and had undergone successful percutaneous coronary intervention with stent placement and had resultant TIMI flow of 2 or more in the infarct-related artery. We excluded patients with a life expectancy of less than 3 years, contraindications to MRI, infarction involving the right ventricular endocardium (from which cardiac biopsy samples would be obtained), cardiac tumour, history of sustained ventricular arrhythmias, New York Heart Association (NYHA) class IV heart failure, or tumours visible on screening body CT. The research protocol was approved by the relevant institutional review boards of both institutions and all participants provided written informed consent.

Randomisation and masking

We randomly allocated patients in a 2:1 ratio to the CDC group or the control group through a central electronic data entry system provided by the data coordinating centre (DCC; The EMMES Corporation, Rockville, MD, USA), stratified by site and ejection fraction (25–35% *vs* 35–45%). We proposed inclusion of a masked placebo group to the FDA but the absence of safety data to support the use of endomyocardial biopsy samples for tissue harvesting after myocardial infarction precluded this option. Thus, controls received routine care while undergoing all protocol-specified safety and efficacy assessments. A preliminary cohort of patients was randomly allocated to receive a low cell dose (12·5 million cells) or routine care. A prespecified safety review by the National Heart, Lung, and Blood Institute (NHLBI) Gene and Cell Therapy Data and Safety Monitoring Board (DSMB) was undertaken after four patients received the low-dose infusion. After this review, the DSMB recommended that the remaining patients could receive the high dose (25 million cells), defined preclinically as the maximum safe dose.[16] One patient received an intermediate dose of CDCs to fit within the prespecified constraint of the delivery window (ie, ≤90 days after myocardial infarction). This patient was included in data analyses in which all patients treated with CDC are grouped together, but not when low-dose and high-dose groups were analysed separately. CDCs were manu factured in a dedicated facility at the Cedars-Sinai Heart Institute.

Procedures

Patients identified within 30 days of myocardial infarction underwent a screening MRI study, and eligible patients were randomly allocated to control or to CDC treatment groups. For patients randomly allocated to receive CDCs, we did an endomyocardial biopsy sampling to harvest tissue; cell infusion was scheduled when CDC dosage was achieved. After a baseline MRI study, CDCs were infused through an over-the-wire angioplasty catheter, with the balloon inflated at the (stented) site of the previous blockage in the infarct-related artery. Cells were infused over 15 min in three boluses, in a saline solution containing heparin (100 U/mL) and nitroglycerin (50 μg/mL).[16] Controls had baseline MRI studies timed to fall within the same timeframe after myocardial infarction (ie, 1·5–3 months). All patients were followed up at 2 weeks and 1, 2, 3, 6, and 12 months after CDC infusion or at corresponding times for controls.

Endomyocardial biopsy samples yielded an average starting tissue mass of 276 mg (SD 177, range 93–891). The process flow for manufacturing CDCs involved mincing the biopsy specimens into about 1 mm explants (figure 1).[14,15] These explants spontaneously yield outgrowth cells, which were harvested and plated in suspension culture to enable the self-assembly of three-dimensional cardiospheres. Subsequent replating of cardiospheres on adherent culture flasks yielded CDCs, which were passaged two to five times until the prespecified dose was achieved (within 36 [SD 6] days of biopsy sampling). As criteria for identity, more than 95% of cells had to express CD105, and fewer than 5% could express CD45 (figure 1). To check for cytogenetic integrity,[20] we verified that every sample of CDCs contained appropriate numbers of chromosomes.[21] Although most CDC batches were euploid, two instances of trisomy 8 were detected; one patient was able to receive a dose of euploid CDCs that had been expanded in parallel in physiological oxygen culture,[22] and the other batch was declared a manufacturing failure. The webappendix provides details of the cell manufacturing process.

The primary safety endpoints at 6 months were death after infusion due to ventricular tachycardia, ventricular fibrillation, or sudden unexpected death, myocardial infarction after cell infusion, new cardiac tumour formation on MRI, or a major adverse cardiac event (MACE), which was defined as the composite of death and hospital admission for heart failure or for non-fatal recurrent myocardial infarction. Secondary endpoints were rates of hospital admission, myocardial injury evidenced by increased cardiac enzymes, TIMI flow after infusion, development of or increased frequency of ventricular arrhythmias, and abnormalities in renal, hepatic, or haematological laboratory criteria. Adverse events were adjudicated by a physician at the DCC and the DSMB. Data were collated and analysed independently by the DCC.

We assessed efficacy in terms of NYHA class, the Minnesota Living with Heart Failure Questionnaire (MLHFQ), 6-min walk tests, and MRI. We did contrast-enhanced MRI studies at baseline, at 6 months for the primary endpoint, and at 12 months to assess longevity of the treatment effects. Images were labelled with a study identification and date of assessment and sent to the imaging core at The Johns Hopkins University, where staff remained masked to treatment-group assignment. MRI assessments measured scar mass and

viable myocardial mass in the left ventricle, scar size, cardiac volumes, global function, and regional function in all patients.

To verify tissue regeneration independent of MRI studies, we did a supplementary study in rats with scar size, scar mass, viable mass from serial sections of hearts stained with Masson's trichrome, and myocyte cross-sectional area as endpoints. Rats underwent 45 min of anterior myocardial ischaemia and 20 min reperfusion followed by intracoronary infusion of syngeneic CDCs or vehicle; hearts were explanted for pathological analysis 3 weeks after this infusion. The webappendix provides more details about the safety and efficacy analyses and details of this supplementary study.

Statistical analysis

This clinical study was designed to assess the safety by 6 months of the administration of CDCs by estimating the CI around the proportion of patients who met the primary endpoint. We based the sample size calculations on a 15% underlying probability (see webappendix for details of the statistical analysis). We calculated exact binomial CIs for a varying number of events on the basis of the sample size. Results are presented as means (SD) in the text and as means (standard error of the mean) in the figures. All reported p values are two-sided and were not adjusted for multiple comparisons. We pooled treatment groups to compare patients who received CDCs with controls.

This study is registered with ClinicalTrials.gov, NCT00893360.

Role of the funding source

Apart from input from standing committees of the NHLBI (Protocol Review Committee and the Gene and Cell Therapy Data and Safety Monitoring Board), the funding sources had no role in the execution of the study or any role in data analysis or in the preparation of the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between May 5, 2009, and Dec 16, 2010, we screened 436 patients and randomly allocated 31 eligible patients to treatment groups (figure 2). Two patients allocated to receive CDCs withdrew consent before first biopsy sampling and another became ineligible for infusion because of occlusion of the infarct-related artery detected at the time of intended infusion. Four patients received a low cell dose (12·5 million cells), one received an intermediary cell dose (17·3 million cells), and 12 received a high cell dose (25 million cells). After endomyocardial biopsy sampling, the required CDC dose was achieved at a mean of 65 days (SD 14, range 47–90) after myo car-dial infarction. Three technical manufacturing failures occurred: one bacterial contam ination, one cytogenetic abnormality, and one failure to achieve the minimal CDC dose for infusion within the prespecified interval of up to 90 days after myocardial infarction. All patients have been followed up to the primary endpoints at 6 months and 12 month data are pending for four patients. The mean follow-up from time of randomisation was 13·4 months (SD 1·8). MRIs obtained from two patients (both treated

with CDCs) were deemed technically uninterpretable by the imaging core and were excluded from analysis.

The table shows baseline characteristics of study partici pants. 24 (77%) of 31 randomly allocated patients were enrolled at Cedars-Sinai Heart Institute. The mean left ventricular ejection fraction at baseline was 39% (SD 12), and the average scar size was 24% (10). The culprit vessel was the left anterior descending coronary artery or its diagonal branch in 23 (92%) patients. Most participants (75%) had an NYHA functional class of 1 at baseline. Therefore, the CADUCEUS study population seemed to have moderate, but generally presymptomatic, left ventricular dysfunction.

No complications were reported during or within 24 h of biopsy sampling or cell infusion. No events met the stopping criteria. The average serum troponin I was 0·1 ng/mL (SD 0·1) before infusion and 0·1 ng/mL (0·1) 12 h after infusion and the average creatinine kinase concentration (CK-MB) was 2·5 ng/mL (SD 1·1) before infusion and 2·6 ng/mL (1·7) after infusion with equivalent values at 24 h and 48 h after infusion. Within 6 months, five patients had serious adverse events (four in the CDC treatment group [24%] *vs* one control [13%]; p=1·00) and two additional patients who received CDCs had such events by 12 months (p=0·36). In these six patients in the CDC group, serious adverse events included one acute myocardial infarction, two cases of chest pain, one coronary revascularisation, one implantable defibrillator insertion for prophylactic indications, and two other non-cardiac events. One patient in the control group had atypical chest pain. All but one of the serious adverse events were regarded as unrelated or unlikely to be related to the study treatment. The exception was a non-Q wave myocardial infarction in one patient who had received 25 million CDCs 7 months previously; the data and safety monitoring board regarded this event as possibly related to treatment. No patients had ventricular fibrillation or sustained ventricular tachycardia during the monitoring period. One patient from the high-dose CDC group had atrial fibrillation. Incidence of non-sustained ventricular tachycardia did not differ between groups; one patient from the high-dose CDC group had non-sustained ventricular tachycardia of 11 ectopic beats 2 weeks after infusion, one patient who received the high dose level and one patient who received the low dose level had 5–10 ectopic beats, and two controls had 5–10 ectopic beats. We noted no deaths or cases of MACE or tumour formation on MRI. The webappendix shows more details of the safety endpoints.

The proportion of patients in the CDC and control groups in every NYHA class did not change between baseline, 6 months and 12 months. Patients who received CDCs had a mean increase in distance walked in 6 min of 11·4 m (SD 83·3) at 6 months and 33·0 m (58·4) at 12 months compared with a 13·1 m (71·2) increase by 6 months and 9·6 m (89·3) decrease at 12 months in controls. Peak oxygen consumption increased by 2·6 mL/kg per min (SD 5·3) at 6 months in patients treated with CDCs but was stable in controls (–0·5 [6·6]; p=0·07). Total MLHFQ scores decreased for patients who received CDCs (24·9 at baseline to 14·1 at 6 months) and an equivalent decrease was noted in controls (35·4 at baseline to 25·1 at 6 months; webappendix).

Figure 3 shows representative contrast-enhanced MRI acquisitions of hearts in short-axis section at end-diastole. Normal viable myocardium appears dark whereas scar tissue appears

white.[23] In this representative example from a patient who received CDCs, the scar was trans mural and extended from the mid-anterior wall into the septum. 6 months after CDC infusion the scar was visibly smaller in circumference and in thickness and the amount of viable myocardium had increased. Such changes were not apparent in a representative control (figure 3), who had a large, predominantly septal myo cardial infarction at baseline and 6 months, with no evidence of scar shrinkage or myocardial regrowth in the interval.

Figure 3 also shows the pooled changes in scar size (scar mass normalised by total left ventricular mass) between groups from baseline to 6 months and 12 months. Scar size was unchanged in controls (difference of 0·3% [SD 5·4]; p=0·894 within group) but decreased in patients treated with CDCs (absolute difference –7·7% [4·8]; p<0·0001 within group, p=0·001 between groups) in the first 6 months. At 12 months, patients treated with CDCs had a 12·3% (5·0) absolute decrease in scar size (p=0·001 within group), which was greater than was the small change noted in controls (difference –2·2% [7·1]; p=0·452 within group, p=0·007 between groups).

Because scar size is related directly to scar mass and inversely to viable left ventricular mass, we analysed the two components individually. Scar mass decreased in patients treated with CDCs by 8·4 g (SD 5·1; p<0·0001within group) at 6 months and 12·9 g (7·9; p=0·003 within group) and 12 months, but remained unchanged in controls (between-groups p=0·001 at 6 months and p=0·02 at 12 months; figure 4). Mean scar mass decreased in the CDC group by 28% (SD 22) by 6 months and 42% (17) by 12 months. By contrast, viable myocardial mass increased in patients who received CDCs (difference 13·0 g [SD 11·4]; p=0·001 within group) at 6 months, but not in controls (difference 0·9 g [6·2]; p=0·703 within groups, p=0·01 between groups; figure 4). We noted much the same effects at 12 months (figure 4). The noted reductions in scar mass correlate well with the increments in viable myocardium at 6 months and 12 months (r=–0·59, p=0·0007; figure 4). In a comparable patient population, serial MRIs showed about a 14% loss of total left ventricular mass in the first 4 months after myocardial infarction, as (thick) viable myocardium was replaced by (thin) scar.[10] If reversal of injury is operative in patients treated with CDCs, the increase in viable mass should exceed the shrinkage of scar mass. Indeed, viable mass increased on average about 60% more than scar shrunk (figure 4), leading to partial restoration of lost left ventricular mass in patients treated with CDCs.

We interpreted MRIs at face value, because of extensive validation of delayed enhancement as a means of quantifying myocardial scar[24] and, in particular, its good reproducibility in serial measurements of scar size after myocardial infarction.[25] Nevertheless, the possibility exists that CDCs distort myocardial architecture and therefore our image interpretation. There were no deaths in this study, so we were unable to verify our conclusions pathologically in human beings. We therefore charac terised hearts from rats mimicking the key features of CADUCEUS (syngeneic CDCs given after a myocardial infarction through the intracoronary route). Figure 5 shows representative Masson's trichrome-stained slices of a vehicle-infused control heart and a CDC-treated heart 3 weeks after intervention. The reduction of scar burden apparent in this CDC-treated heart was representative of pooled volumetric data showing reduced scar size, reduced scar mass, and increased viable mass in CDC-treated hearts relative to controls (figure 5). We noted no hypertrophy within the

infarct border zone in the CDC-treated hearts; myocyte cross-sectional area was lower by about 20% relative to vehicle controls, consistent with restoration of viable myo cardium by new cardiomyocytes. These pathological data support the notion that the CADUCEUS images show regression of scar and tissue regeneration as a result of CDC treatment.

Both controls and patients treated with CDCs had non-significant changes in left ventricular ejection fraction in 6 months (figure 6). Increases in enddiastolic volume and end-systolic volume are typical of adverse remodelling after myocardial infarction. Enddiastolic volume (–7·2 mL [SD 23·0] in the CDC group *vs* 7·3 mL [17·7] in controls; p=0·14) and end-systolic volumes (–7·8 mL [19·2] in the CDC group *vs* 0·2 mL [21·3] in controls; p=0·37) did not differ between groups at 6 months.

Regional contractility, assessed by the negative strain value from MRI tagging analysis (figure 6), was greater in CDC-infused segments at 6 months (–11·8% [SD 7·0]) than it was in controls (–8·5% [6·7]; p=0·02 between groups). Contractility improved in patients treated with CDCs (difference –2·0% [6·3]) and fell in controls (difference 1·5 [7·3]; p=0·009 between groups) by 6 months of follow-up. Systolic wall thickening was also improved in CDC-infused segments at 6 months compared with controls (p=0·015 between groups; figure 6); thickening improved during this interval in patients treated with CDCs but worsened in controls (mean changes of 7·7% *vs* –5·9%, respectively; p=0·045 between groups). Endsystolic wall thickness showed similar changes (data not shown). Thus, CDCs seem to show beneficial functional effects in treated regions of the myocardium.

Discussion

Regeneration is defined as regrowth of lost or destroyed parts or organs.[26] Although nature provides numerous examples of spontaneous regeneration after injury, we have, as physicians, thus far failed in our efforts to achieve therapeutic regeneration. Our study provides an initial indication that therapeutic regeneration might indeed be possible in cardiac tissue.

We report a phase 1 clinical trial of heart-derived cells that reached its prespecified primary endpoints: the controlled proof-of-concept CADUCEUS study showed an increase in viable myocardial tissue as a result of cell therapy. Although two clinical studies of bone marrow mononuclear cells have reported reductions in scar size with cell therapy,[4,9] the effect was attributable only to reduced scar mass. Even when the discussion is restricted to scar reduction, CDC therapy is about 3–5 times more effective than bone marrow mononuclear cells.[4,9] The only other clinical report of a heart-derived cell product, purified c-kit-positive cells, is an interim analysis[27] of a phase 1 single-centre trial targeting coronary bypass patients with ventricular dysfunction. Changes in scar size in that study are difficult to interpret because of the lack of any MRIs in controls. We conclude that, on the basis of the published work (panel), CDCs have an unprecedented ability to reduce scar and simultaneously stimulate the regrowth of healthy myocardial tissue. The basis for the apparently improved efficacy of CDCs remains to be fully elucidated, but we have noted that CDCs outperform bone marrow mononuclear cells, mesenchymal stem cells, and c-kit-positive cells in terms of paracrine potency, anti-apoptotic properties, tissue engraftment,

and regen era tive efficacy when the various cell types are com pared directly in mice after myocardial infarction.[18]

CADUCEUS was not designed to assess how CDCs regenerate the injured heart. Nevertheless, evidence supports the idea that the mechanism of benefit is indirect: both physical contact and paracrine factors stimulate a role model effect and activate endogenous reparative and regenerative pathways.[30] Recent work with allogeneic CDCs further supports the indirect mechanism, as long-term functional benefit and tissue regeneration persist long after all transplanted donor cells have been cleared immunologically.[17] We suggest that the indirect mechanism might result in safer, more durable benefit compared with the paradigm of direct differentiation of transplanted cells, as the new myocardium will be of innate origin and therefore well-integrated into the host heart. However, this hypothesis remains to be tested.

The changes that we noted in scar size in participants who were treated with CDCs were striking, but were not accompanied by clear changes in ejection fraction in this small proof-of-concept study. The reasons for the discrepancy are unclear. In the extreme, complete healing of myocardial injury should result in normal-isation of ejection fraction and reversal of ventricular remodelling. However, we did not report complete healing of myocardial injury: instead, 28% of the scar mass was dissolved and ejection fraction went from 39% to 41% in the patients treated with CDCs by 6 months. This small increment in ejection fraction is entirely consistent with the known relation between scar size and ejection fraction after myocardial infarction, which is quite shallow in terms of the range of scar size in question.[31] Moreover, resolution of ventricular dysfunction in the CADUCEUS population will necessarily be small, as ejection fraction at baseline was only moderately impaired, leaving little room for improvement before it reached the normal range. Notably, ejection fraction is influenced by several confounding variables including afterload, preload, ventricular shape, electrical activation pattern, rhythm, rate, coronary flow, and neurohumoral tone, none of which affects scar size. Nonetheless, the clear increases in regional function in patients treated with CDCs are reassuring of the functional importance of the tissue changes.

Despite the small effect of bone marrow mononuclear cell therapy on scar size, substantial benefits for clinical endpoints have been reported. Even though the REPAIR-AMI trial[32] was not powered to detect differences in clinical endpoints, the incidence of the prespecified cumulative endpoint of death, myocardial infarction, or necessity for revascularisation at 1 year was significantly lower after cell therapy. Favourable clinical outcomes were sustained at 2 years of follow up.[33] The fact that positive clinical trends are evident with bone marrow mononuclear cell therapy, with only small underlying changes in scar size and no apparent increase in viable myocardium, gives reason to expect even greater clinical benefits with CDC therapy, although such assessments are beyond the scope of the present proof-of-concept study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Panel: Research in context

Systematic review

We searched PubMed for original research published in any language between Jan 1, 2000, and Jan 1, 2012, with the terms “cardiosphere”, “cardiosphere-derived cell”, “stem cell therapy”, “myocardial infarction”, “left ventricular dysfunction”, “contrast-enhanced magnetic resonance imaging”, “endomyocardial biopsy”, “gadolinium AND scar”, and “therapeutic regeneration”. We identified no studies of cardiosphere-derived cells (CDCs) in human beings, other than our own work[13,15] describing the development of processes to isolate CDCs from human heart biopsies and a report from Mishra and colleagues[28] of similar work with paediatric surgical specimens; all other published studies were undertaken in preclinical models. Clinical trials of relevance to the present topic, and identified with the stated search criteria, were reviewed recently.[13] Since then, a preliminary report of another relevant trial has appeared,[27] as has a full report of a trial with bone marrow-derived cells provided 2–3 weeks after myocardial infarction.[29] We identified no published work providing evidence against the reliability of contrast-enhanced MRI as a means of quantifying scar or viable myocardium in healing or chronic myocardial infarction in humans, but many papers validating the technique.[23–25]

Interpretation

Our trial was a proof-of-concept clinical study of cardiosphere-derived cells that used cells derived from endomyocardial biopsy specimens and focused on patients with convalescent myocardial infarction (1·5–3 months after myocardial infarction), and the report includes all prespecified primary endpoints. The work is conceptually important because it provides early evidence for therapeutic regeneration in a controlled clinical trial. We noted that cardiac scar tissue was reduced and new healthy tissue was generated after treatment with CDCs. This discovery challenges the conventional wisdom that, once established, cardiac scarring is permanent and that, once lost, healthy heart muscle cannot be restored. The work also establishes the feasibility and safety of a novel paradigm for treatment, whereby endomyocardial biopsy samples are used to harvest heart tissue in a minimally invasive manner as starting material for the generation of a treatment option.

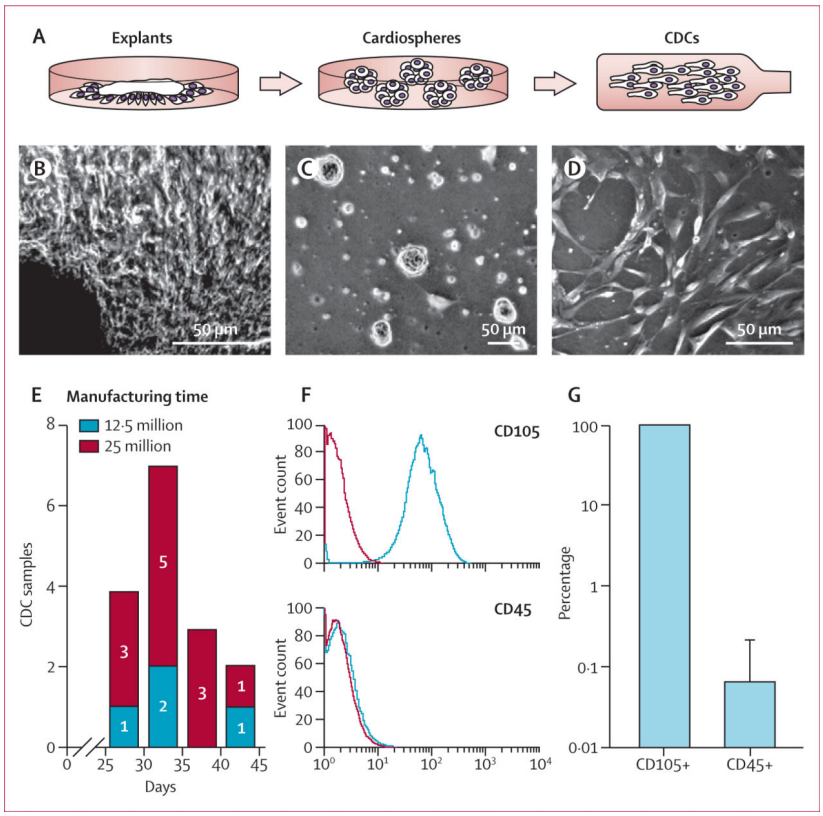

Figure 1. Manufacture and characteristics of CDCs

(A–D) Process flow for manufacture of CDCs. Biopsy specimens are minced into about 1 mm explants that spontaneously yield outgrowth cells (seen budding off the explant in B). These explants are harvested and plated in suspension culture to enable the self-assembly of three-dimensional cardiospheres (C). Subsequent replating of cardiospheres on adherent culture flasks yields CDCs (D). Histogram of time to achievement of the prespecified dose (E). As criteria for identity, representative histograms of flow cytometry data (F) and pooled data (G; logarithmic axis) show that more than 98% of cells expressed CD105, whereas fewer than 0·5% expressed CD45. CDC=cardiosphere-derived cell.

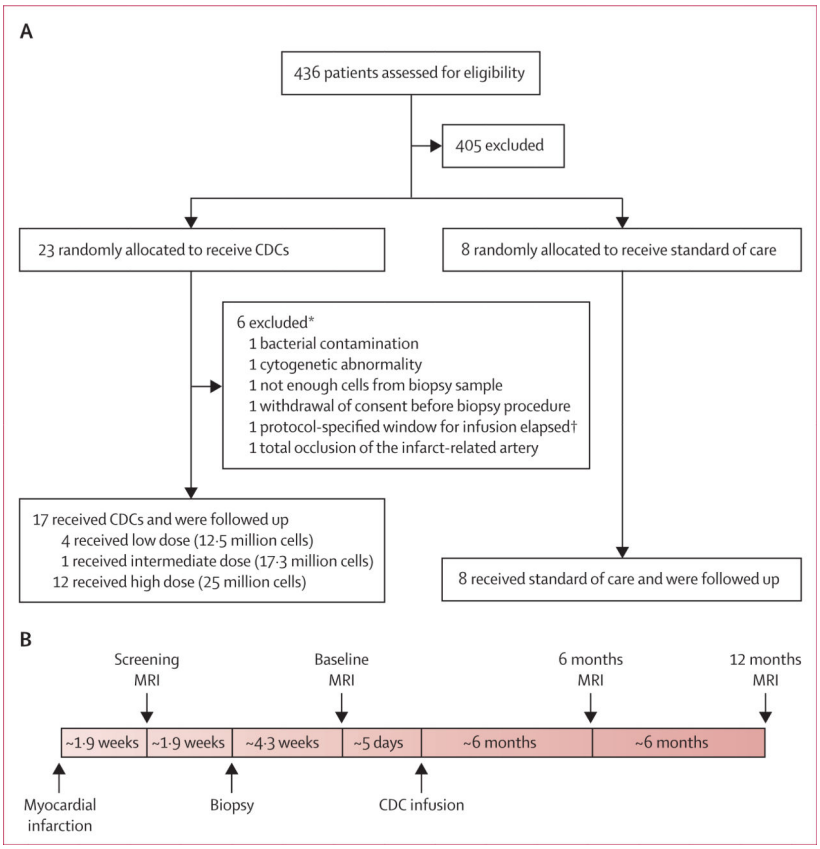

Figure 2. Trial profile and study timeline

(A) CADUCEUS trial profile. (B) Study events and timeline. Major efficacy data are based on comparisons of the baseline MRIs and the 6-month and 12-month MRIs. Study procedures below the timeline apply only to those participants who were randomly allocated to receive CDCs, but all participants underwent the MRI studies shown above the timeline. CDC=cardiosphere-derived cell. *Two patients in the low dose group and four in the high dose group. †Delay due to investigation of contamination.

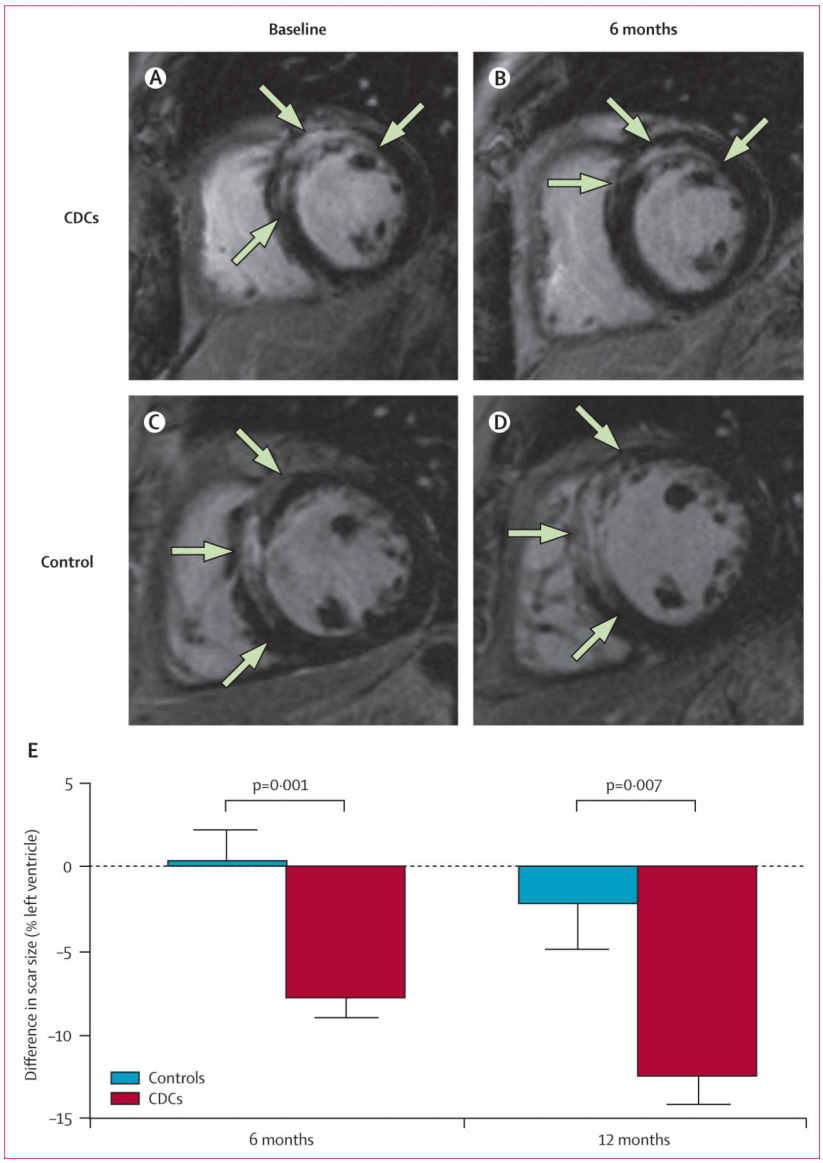

Figure 3. Representative MRI and changes in scar size

Short-axis MRI of heart at baseline (82 days after myocardial infarction; A) and 6 months after CDC infusion (B) in a participant randomly allocated to receive CDCs. Short-axis MRI of heart at baseline (77 days after myocardial infarction; C) and after 6 months (D) in a control. Infarct scar tissue (green arrows) is evident by areas of hyperintensity (white) whereas viable myocardium appears dark. Difference in scar size from baseline to 6 months (E) or 12 months (F). CDC=cardiosphere-derived cell.

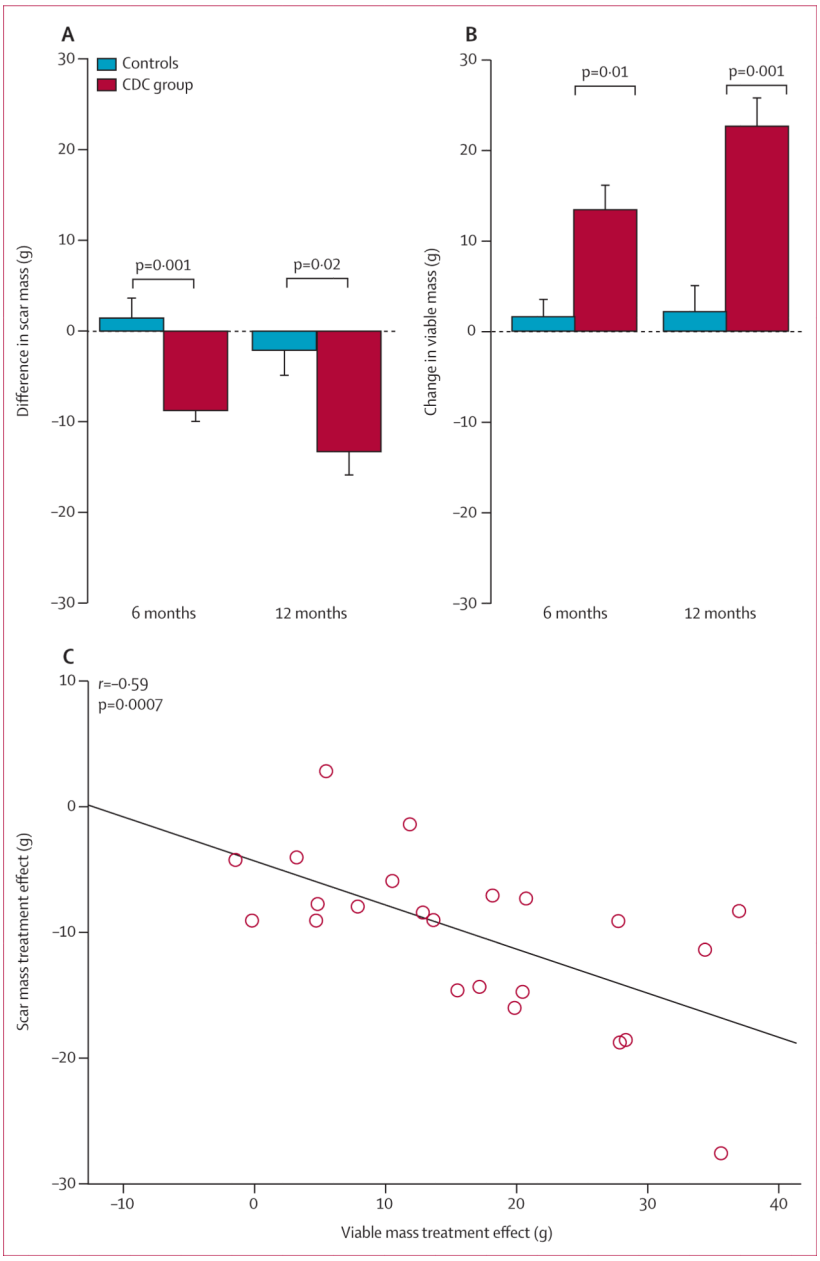

Figure 4. Scar mass and viable left ventricular mass on MRI

We noted decreases in scar mass and increases in viable mass on MRI in patients treated with CDCs but not controls. (A) Differences in scar mass between groups from baseline to 6 months or 12 months. (B) Differences in viable left ventricular mass from baseline to 6 months or 12 months. (C) Correlation between the change in scar mass and the change in viable mass in individual patients at 6 and 12 months compared with baseline. CDC=cardiosphere-derived cell.

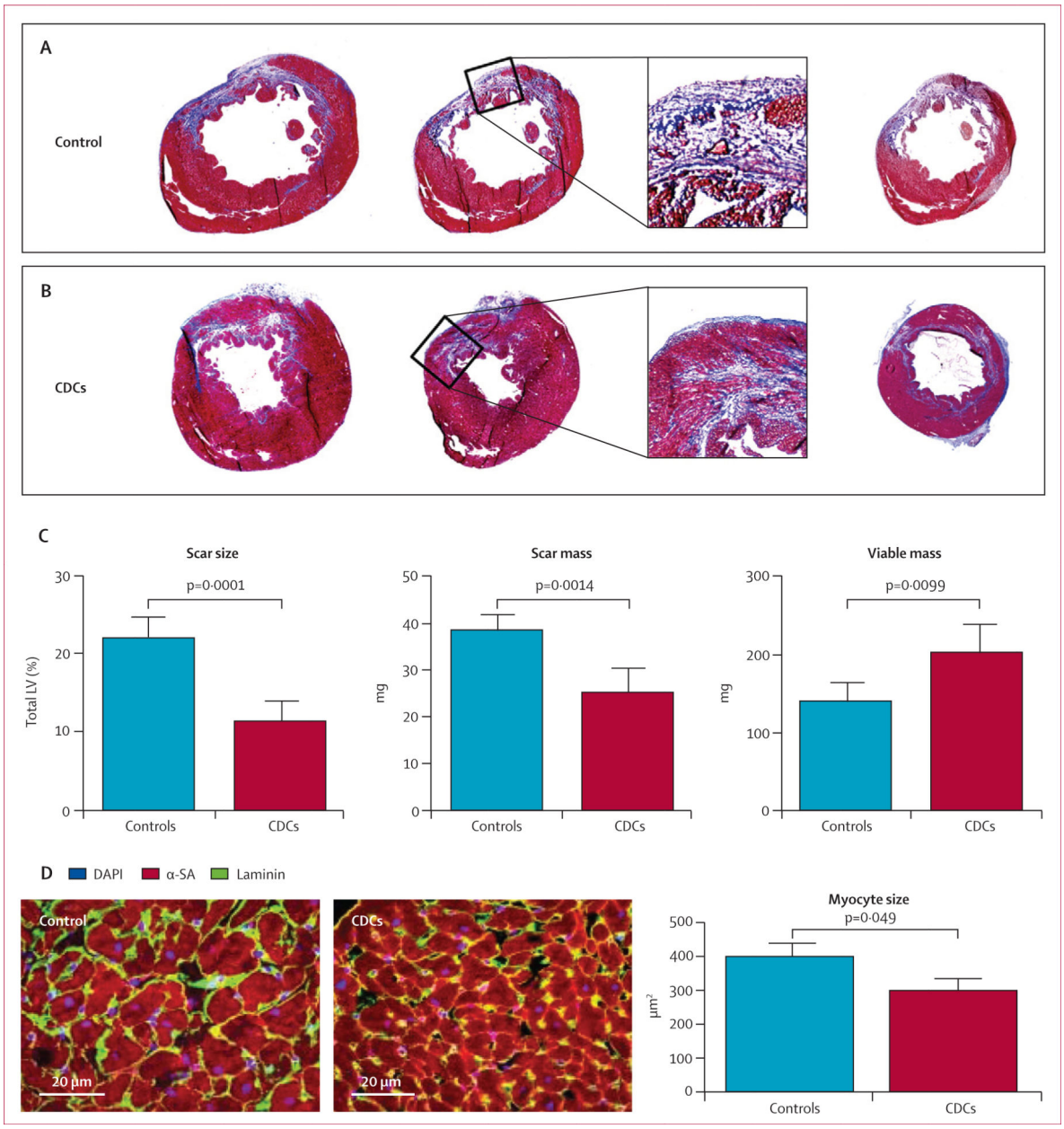

Figure 5. Myocardial regeneration in rats treated with CDCs

Representative images of Masson trichrome-stained sections of vehicle control (A) and intracoronary CDC-treated (B) rat hearts 3 weeks after treatment (scar tissue stained blue and viable myocardium stained red). Enlarged regions show striking differences in transmurality of scar and extent of viable myocardium in the infarcted region. (C) Quantification of scar size, scar mass, and viable mass in CDC-treated and control hearts. (D) Cardiomyocyte cross-sectional area in the peri-infarct area of CDC-treated and control hearts shown no hypertrophy in the CDC-treated hearts. CDC=cardiosphere-derived cell. DAPI=4',6-diamidino-2-phenylindole. α-SA=α-sarcomeric actinin.

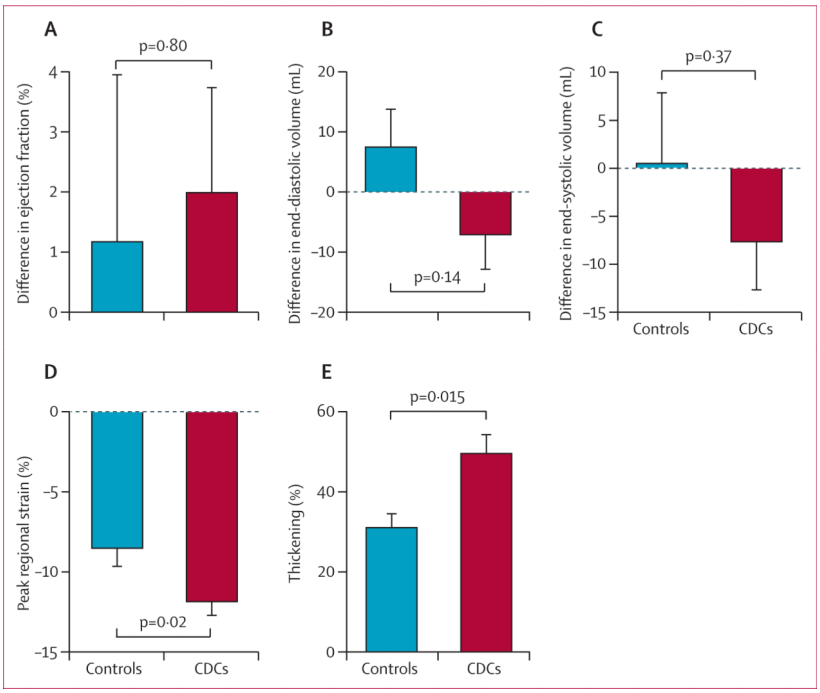

Figure 6. Global function, chamber volumes, and regional function in participants in the CADUCEUS study

(A) Treatment effects (baseline *vs* 6 months) for MRI-derived ejection fraction. (B) Treatment effects (baseline *vs* 6 months) for end-diastolic volume. (C) Treatment effects (baseline *vs* 6 months) for end-systolic volume. (D) Regional strain in infarct-related segments at 6 months. (E) Systolic thickening in infarct-related segments at 6 months. CDC=cardiosphere-derived cell.

Table

Baseline characteristics of patients

	Cardiosphere-derived cell group (n=17)	Control group (n=8)
Sex, male	17 (100%)	8 (100%)
Age, years	54·0 (2.5)	50·9 (5·5)
Race, white	17 (100%)	5 (63%)
History of coronary interventions	5 (29%)	0
History of atrial or ventricular arrhythmia	0	0
History of hypertension	9 (53%)	3 (38%)
History of congestion heart failure	0	0
History of valvular heart disease	0	0
History of smoking	9 (53%)	2 (25%)
History of diabetes	1 (6%)	0
NYHA class		
I	12 (71%)	6 (75%)
II	4 (24%)	1 (13%)
III	0	1 (13%)
Missing	1 (6%)	0
Ejection fraction	38·1% (12·1)	41·0% (11·1)
6-min walk test, m	400.6 (121·9)	421·9 (85·2)
Previous myocardial infarction	4 (24%)	0
Location of index myocardial infarction		
Anterior	9 (53%)	5 (63%)
Anterolateral	4 (24%)	3 (38%)
Inferior	1 (6%)	0
Subendocardial	1 (6%)	0
Transmural anterolateral	1 (6%)	0
Inferolateral	1 (6%)	0
Index myocardial infarction culprit vessel		
LCX	1 (6%)	0
LAD	15 (88%)	8 (100%)
RCA	1 (6%)	0
Drugs		
ACE inhibitors	12 (71%)	5 (63%)
Aspirin	17 (100%)	7 (88%)
Angiotensin II blockers	3 (18%)	2 (25%)
Statins	17 (100%)	8 (100%)

Data are n (%) or mean (SD). NYHA=New York Heart Association. LCX=left circumflex artery. LAD=left anterior descending artery. RCA=right coronary artery. ACE=angiotensin-converting enzyme.