ELSEVIER

European Journal of Heart Failure 10 (2008) 525–533

The European Journal of Heart Failure

www.elsevier.com/locate/ejheart

Human angiogenic cell precursors restore function in the infarcted rat heart: A comparison of cell delivery routes

Zhuo Sun [a], Jun Wu [a], Hiroko Fujii [a], Jiang Wu [a], Shu-Hong Li [a], Svetlana Porozov [b], Adina Belleli [b], Valentin Fulga [b], Yael Porat [b], Ren-Ke Li [a,*]

[a] *Division of Cardiovascular Surgery, Toronto General Research Institute, Toronto General Hospital and University of Toronto, Toronto, Ontario, Canada*
[b] *TheraVitae Ltd., Ness Ziona, Israel*

Received 21 January 2008; received in revised form 10 March 2008; accepted 9 April 2008

Abstract

Background: We recently isolated angiogenic cell precursors (ACPs) from human blood, which can induce angiogenesis *in vitro*.
Aims: In the present study, we used a nude rat model of ischaemic cardiomyopathy to compare the efficacy of intramyocardial and intracoronary ACP implantation, and to evaluate effects on cardiac function, scar size and angiogenesis.
Methods and results: Adult nude rats underwent coronary artery ligation. Six days later, ACPs (characterized *in vitro* prior to implantation) or culture media were injected directly into the ischaemic myocardial region or into the coronary artery via the aorta. Cardiac function was measured by echocardiography prior to and at 2 and 4 weeks after implantation. Scar morphology, cell engraftment, and myocardial angiogenesis were evaluated at 4 weeks. Two and four weeks after implantation, cardiac function declined in both of the control groups but improved in both the intramyocardial and intracoronary ACP groups. Significant reductions in myocardial scar area were only observed in the intramyocardial ACP group, while increases in blood vessel density, which were observed in all ACP recipients, were greatest in the intracoronary ACP group.
Conclusions: Human ACPs, delivered via intramyocardial or intracoronary injection, engrafted into damaged cardiac tissue and improved cardiac function within 4 weeks through effects on scar morphology and blood vessel formation.

Keywords: Cell transplantation; Angiogenic cell precursors; Myocardial ischaemia; Heart failure; Angiogenesis

1. Introduction

Physiological organ function requires continuous tissue perfusion. Following a myocardial infarction, neoangiogenesis augmenting the capillary network within the infarct bed is insufficient to keep pace with the extent of tissue growth required for contractile compensation. Most preclinical and clinical interventions for ischaemic tissue revascularization [1–5] involve delivering genes for angiogenic growth factors, such as vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF), into ischaemic tissue to stimulate additional vessel growth. Bone marrow-derived endothelial progenitor cells (EPCs), which are augmented in response to cytokines and/or ischaemic factors, localise to sites of neovascularization and are incorporated into new vessels [6–9], increasing regional perfusion within the ischaemic myocardium and improving cardiac function [10–14].

We recently generated angiogenic cell precursors (ACPs) from human peripheral blood. Similar to EPCs, ACPs simultaneously express Ulex-lectin and uptake of acetylated low density lipoprotein (Ac-LDL), as well as cluster of differentiation (CD): CD34, CD133, CD144, VEGF receptor 2 (KDR), Tie-2, von Willebrand factor (vWF), and $CD31^{Bright}$. They also secrete interleukin-8 (IL-8), VEGF, and angiogenin,

* Corresponding author. MaRS Centre, Toronto Medical Discovery Tower, 3-702, 101 College Street, Toronto, Ontario, Canada M5G 1L7. Tel.: +1 416 581 7492; fax: +1 416 581 7493.
E-mail address: renkeli@uhnres.utoronto.ca (R.-K. Li).

1388-9842/$ - see front matter © 2008 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.ejheart.2008.04.004

and are involved in the formation of tube-like structures *in vitro* [15]. In the present study, we investigated the *in vitro* tube formation and migratory capabilities of ACPs pre-loaded with Ac-LDL, and then evaluated their effects on cardiac function, morphology and histology, in a nude rat model of ischaemic cardiomyopathy. We also compared the effectiveness of two routes of ACP delivery: intramyocardial and intracoronary implantation.

2. Methods

2.1. In vitro cell characterization

2.1.1. Cell culture

Low density synergetic cell populations (SCPs) were isolated from individual healthy adult blood samples obtained from the Israeli Blood Bank as described previously [15]. To generate ACPs, SCPs were cultured at a concentration of $1.5–3.0 \times 10^6$ cells/ml in X-vivo 15 serum-free media (Cambrex, East Rutherford, NJ) supplemented with 10% autologous human serum, 1–10 ng/ml vascular endothelial growth factor (VEGF, R&D Systems, Minneapolis, MN) and 5 IU/ml heparin (Kamada, Beit-Kama, Israel). The investigation conformed to the principles outlined in the Declaration of Helsinki (*Br Med J* 1964;ii:177).

2.1.2. Tube formation assay

To evaluate angiogenic capacity, ACPs ($0.1–0.4 \times 10^6$ cells/ml) were pre-loaded with Ac-LDL-DiO (BTI, Stoughton, MA) using an *in vitro* angiogenesis assay kit (Chemicon), according to the manufacturer's directions. Angiogenic patterns and vascular tube formation were scored as previously described [16].

2.1.3. Flow cytometry

Prior to implantation, the ACPs (5×10^5 cells) were incubated with specific fluorochrome-conjugated CD34-APC (BD Biosciences, San Jose, CA), CD117-APC (Dako-Cytomation) and CD31-FITC (eBioscience, San Diego, CA), or with isotype-matched non-specific controls, and analyzed by flow cytometry. Exclusion of dead cells from the final analysis was performed using 7-AAD (eBioscience) staining. The data were analyzed with CellQuest Pro software (Becton Dickinson).

2.1.4. Cell migration assay

Harvested ACPs were loaded with 0.8 mg/ml Ac-LDL-DiO for 15 min at 37 °C. One million pre-loaded ACPs were placed on 8 μm pore size microporous membrane inserts (Nunc, Roskilde. DK); 200 ml media were placed at the bottom of the wells, and the cells were allowed to migrate

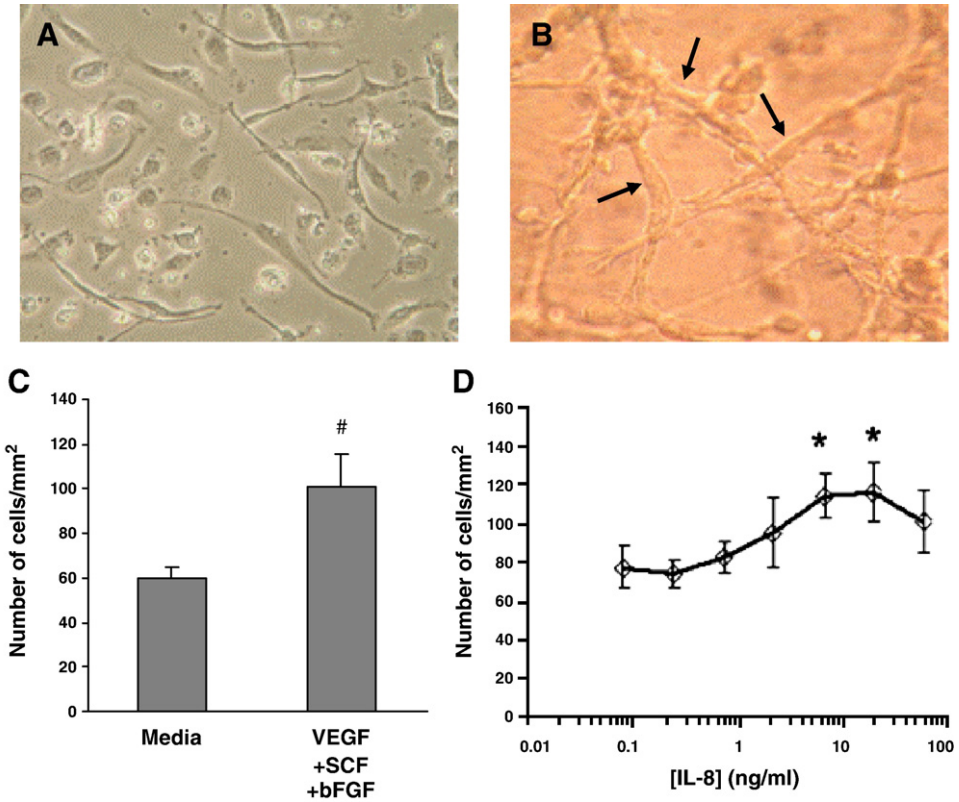

Fig. 1. *In vitro* characteristics of ACPs. A–B: Photomicrographs illustrating the typical elongated, spindle-shaped morphology of cultured ACPs (A), and the angiogenic potential of ACPs indicated by organization into tube-like structures (arrows, B). C: Migration of ACPs pre-loaded with Ac-LDL-DiO toward positive control medium enriched with 20 ng/ml each of VEGF, SCF and bFGF was significantly higher than spontaneous migration toward negative control medium (#$p<0.01$). D: ACP migration toward increasing concentrations of IL-8 (0.08 ng/ml–60 ng/ml; logarithmic scale) was also enhanced at IL-8 concentrations between 6.7 and 20 ng/ml (*$p<0.05$ relative to concentrations outside range).

towards the media. Following 1 h of incubation in the presence of Negative Control (M199; Sigma-Aldrich, St. Louis, MO), Positive Control (20 ng/ml VEGF, 20 ng/ml bFGF, 20 ng/ml SCF), or IL-8 (0.08–60 ng/ml) (PeproTech, Rocky Hill, NJ), the number of labelled migrating cells was automatically counted in 10–15 random fields (0.178 mm^2 each) using a fluorescent microscope and NIH ImageJ software. Cell counts were used to calculate the number of migrating cells per mm^2.

2.2. In vivo experiments

2.2.1. Animals

All experimental procedures were approved by the Animal Care Committee at the University Health Network. The investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Adult male nude rats (200 to 250 g, Charles River, Canada; $n=28$) were used.

2.2.2. Rat myocardial ischaemia model

Myocardial infarction was generated by ligation of the left anterior descending coronary artery as described previously [17], following baseline echocardiographic assessment of cardiac function as described in the "Evaluation of Cardiac Function" section. Electrocardiography was used to monitor the induction of ischaemia and to prevent premature death from excessive ventricular arrhythmia.

2.2.3. Animal selection

Six days after LAD ligation, echocardiography was performed to select animals for the study, according to the following criteria (designed to minimize variations in infarct size and cardiac function): infarct size longer than 0.8 cm but shorter than 1.2 cm; percent fractional shorting (%FS) higher than 20% but lower than 40%.

2.2.4. Cell transplantation

The selected rats were randomly assigned to 4 groups. ACPs (1.5×10^6 cells in 50 μl/rat) or culture media (controls) were injected directly into the infarcted myocardium (M-Cell, $n=9$; M-Control, $n=5$) or into the coronary artery via the aorta (C-Cell, $n=9$; C-Control, $n=5$), as follows:

Intramyocardial cell implantation: Under general anaesthesia, the heart was exposed through a left intercostal incision. The cells were delivered by three injections directly into the ischaemic zone (comprised of scar area and border region). The chest was closed with 3-0 Vicryl sutures.

Intracoronary cell implantation: The right neck and upper sternal area were incised to expose the carotid common artery, brachiocephalic trunk, arch and ascending aorta. 5-0 silks were laid under the arch (between the left common carotid artery and the brachiocephalic trunk) and the brachiocephalic trunk in preparation for the occlusion. A 24 GA 0.75IN I.V. catheter (BD Insyte) was inserted from the carotid common artery into the ascending aorta. The aortic arch and brachiocephalic trunk were occluded by the under-laid silks for 5 s during the cell injection in order to ensure that the cells perfused into the coronary artery.

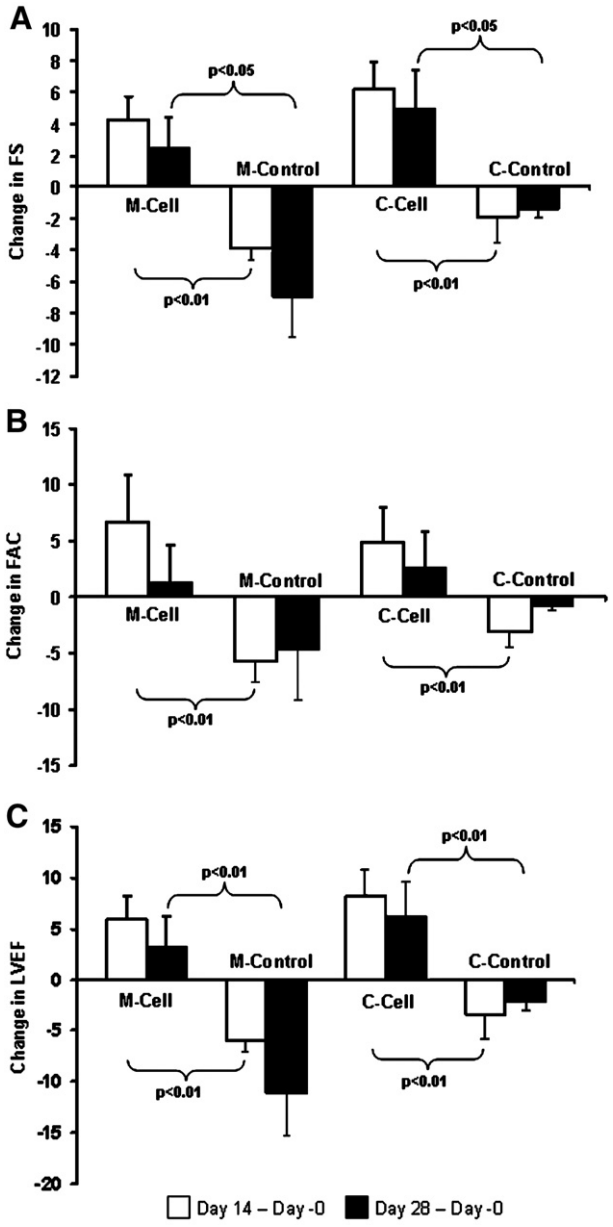

Fig. 2. Changes in left ventricular function evaluated by echocardiography. Differences (compared to pre-transplantation baseline, day 0) in fractional shortening (FS; A) functional area contraction (FAC; B), and ejection fraction (LVEF; C) were calculated at days 14 and 28 after ACP or media implantation in the groups that received ACPs by intramyocardial or intracoronary injection (M-Cell, C-Cell, respectively) and in the respective media control groups (M-Control, C-Control). By day 14, the changes (improvements) in all three measures were significantly greater in the ACP transplant groups than in the control groups ($p<0.01$, white bars). By day 28, the changes (improvements) in FS and LVEF were significantly greater in the ACP transplant groups than in the control groups ($p<0.05$ and $p<0.01$, respectively, black bars). $n=9$/cell group/time point; $n=5$/control group/time point.

The neck and upper sternal incisions were closed with 3-0 Vicryl sutures.

2.2.5. Evaluation of cardiac function

Echocardiography:

Echocardiographic examinations were performed using an ACUSON SEQUOIA C256 System with 15L8 transducer (13 MHz). Left ventricular (LV) end diastolic and systolic diameters and areas were measured, and %FS, functional area contraction (%FAC) and LV ejection fraction (%LVEF) were calculated as described previously [17,18].

Pressure–volume measurement:

End-point functional and morphological assessments were performed using a Millar and conductance catheter (Millar Instruments, USA), as described previously [17]. The left ventricular pressure and volume relationship was analyzed, and real-time pressure–volume loops were constructed. $+dP/dt$ max, $-dP/dt$ min (indices of systolic and diastolic function, respectively), and end diastolic volumes (EDV) were compared between groups.

2.2.6. Morphological study

After functional measurements, hearts were quickly excised. An intraventricular balloon was inserted into the left ventricle of each heart, and intraventricular pressure was maintained at 30 mmHg. The hearts were fixed, and then cut into 2 mm thick slices. Heart slices ($n=5$ per animal) were photographed, and computed planimetry was used to calculate scar area and scar thickness for each heart. Scar areas were presented as a percentage of the left ventricular free wall (LVFW) surface area, calculated as follows: (epicardial scar length)/(epicardial LVFW length) × 100. Scar thickness was presented as an average of wall thickness measurements taken at the middle and at each edge of the scar area at its thinnest point.

2.2.7. Histological and immunohistochemical study

Single, 2 mm thick ventricular slices from each heart were embedded in paraffin and cut into continuous 10 μm thick sections. Sections were stained with haematoxylin and eosin (Sigma Diagnostics, St. Louis, MO), following the manufacturer's directions, in order to reveal basic histological structures within the myocardial tissue and to identify the ischaemic zone. Within this zone, the scar area and border region were clearly visible, characterized by the appearance of fibrotic tissue. To localize implanted cells within the ischaemic zone and to identify cells positive for contractile or vascular proteins, serial sections were immunohistochemically stained

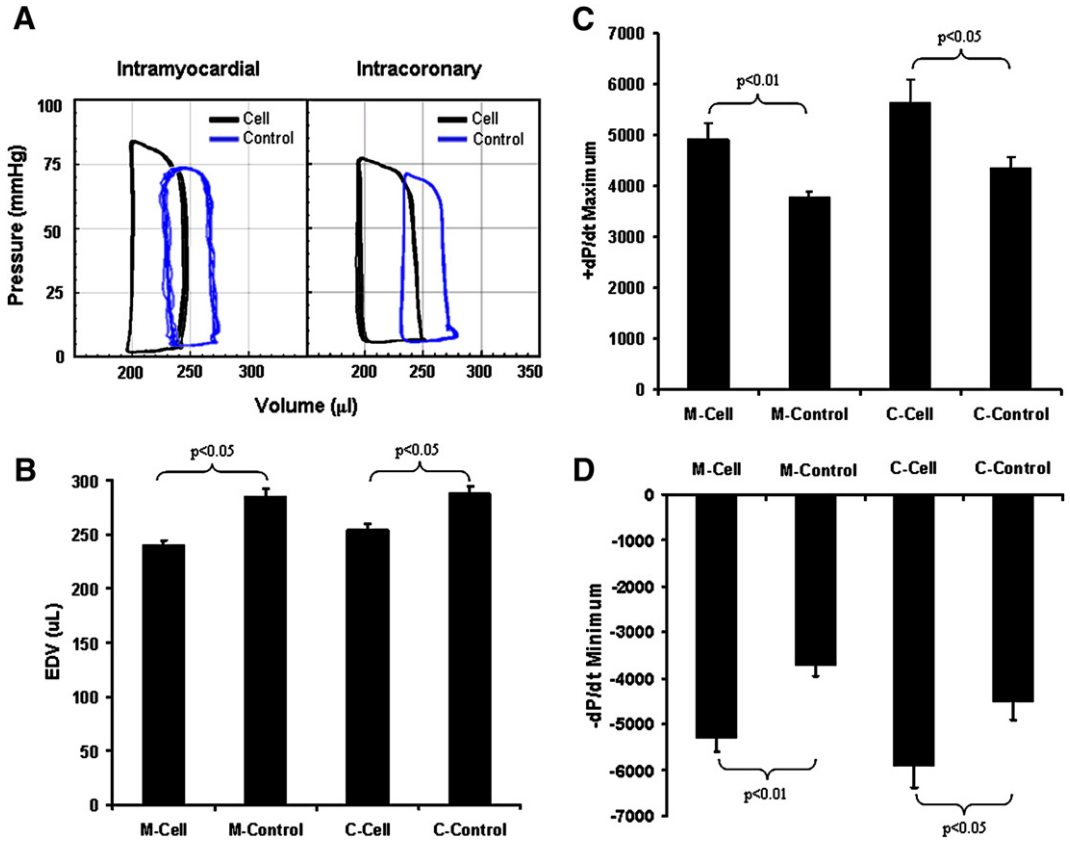

Fig. 3. Left ventricular volumes and function evaluated by Millar catheter at 4 weeks after cell or media implantation. A: Representative pressure–volume loops from rats that received ACPs by intramyocardial or intracoronary injection (Cell) and those in the respective media control groups (Control). Volume (μL) is indicated on the *x*-axis, and pressure (μL) on the *y*-axis. B: End diastolic volume (EDV) was significantly smaller in the ACP transplant groups than in the control groups ($p<0.05$). C–D: Maximum $+dP/dt$ (C) and minimum $-dP/dt$ (D) were significantly higher or lower, respectively, in both ACP transplant groups than in the control groups ($p<0.01$ for M-Cell vs. M-Control, $p<0.05$ for C-Cell vs. C-Control). $n=9$/cell group; $n=5$/control group.

with antibodies against *human mitochondria* (1:100; Chemicon, USA), *myosin heavy chain* (1:400; Santa Cruz, USA), *Troponin I* (1:800; Covance, USA), or *factor VIII* (1:400; DakoCytomation Inc., USA), according to the manufacturer's directions. Blood vessels (factor VIII positive) and surviving (engrafted) ACPs (human mitochondria positive) were counted in 3 randomly-selected high power fields (400×; 0.2 mm^2) in the scar area, and in 4 randomly-selected high power fields (0.2 mm^2) in the border region (two from each side). In each case, quantification was carried out by a blinded observer using a Nikon Eclipse TE200 Microscope. Blood vessel density was expressed separately for the scar area and the border region as the mean vascular density per 0.2 mm^2 in each region. Implanted cell survival was expressed as the mean number of ACPs per 0.2 mm^2 in the myocardial ischaemic zone.

2.2.8. Data analysis

All studies were performed in a blinded fashion, and data were expressed as mean ± SEM (or mean ± SD for *in vitro* tube formation assay and flow cytometry). Between-groups comparisons were made using repeated measures and one-way analyses of variance (ANOVA). Tukey's or least significant difference (LSD) multiple range tests were subsequently performed when the F values were significant. Differences were deemed significant when p values were <0.05.

3. Results

3.1. In vitro experiments

3.1.1. Characteristics of ACPs

The cultured ACPs showed typical morphology: elongated and spindle-shaped cells along with large, circular cells (~20 μm diameter) (Fig. 1A). Cells pre-loaded with Ac-LDL-DiO generated tube-like structures (Fig. 1B) (Ac-LDL-DiO^+). Three individual batches expressing the stem cell markers CD34 (23.6 ± 1.9% of cells) and CD117 (1.9 ± 1.2% of cells), and the endothelial/angiogenic marker $CD31^{Bright}$ (29.9 ± 15.5% of cells) were used for *in vivo* implantation.

ACPs represent a heterogenic stem/progenitor cell population containing both [1] CD34 and CD117 multipotent haematopoietic cells that can potentially differentiate *in vivo* in response to tissue signals at the site of injection, and [2] linage specific angiogenic precursors. We defined the stemness potential of ACPs as the percentage of multipotent cells expressing CD34 and CD117. According to this definition, the implanted ACP cell population exhibited an average stemness potential of 25.5 ± 0.8%. The angiogenic potential, represented by the average number of angiogenic lineage cells expressing $CD31^{Bright}$, was $44.8 \pm 23.3 \times 10^4$ $CD31^{Bright}$ cells in 1.5 million cells (Supplementary Table E1).

3.1.2. Migratory capability of ACPs

The migratory capability of Ac-LDL labelled ACPs was tested in 5 independent experiments. The migration of ACPs toward positive control medium containing 20 ng/ml each of VEGF, SCF and bFGF was significantly higher ($p<0.01$) than the spontaneous migration of these cells toward negative control medium (Fig. 1C). A dose–response curve assessing the migration of ACPs toward increasing concentrations of IL-8 was also performed. Consistent with the behaviour of endothelial progenitor cells, cell migration peaked at IL-8 concentrations between 6.7 and 20 ng/ml, and was significantly ($p<0.05$) lower at concentrations outside this range (Fig. 1D).

3.2. In vivo experiments

3.2.1. Survival

Two rats died during the LAD ligation procedure, and 2 rats died shortly after the procedure. Three rats were excluded from the study because they did not meet the infarct size criteria for inclusion (2 because scars were too small; 1 because scar was too large). Two rats died following intramyocardial media injection, and 1 rat died following intramyocardial cell injection.

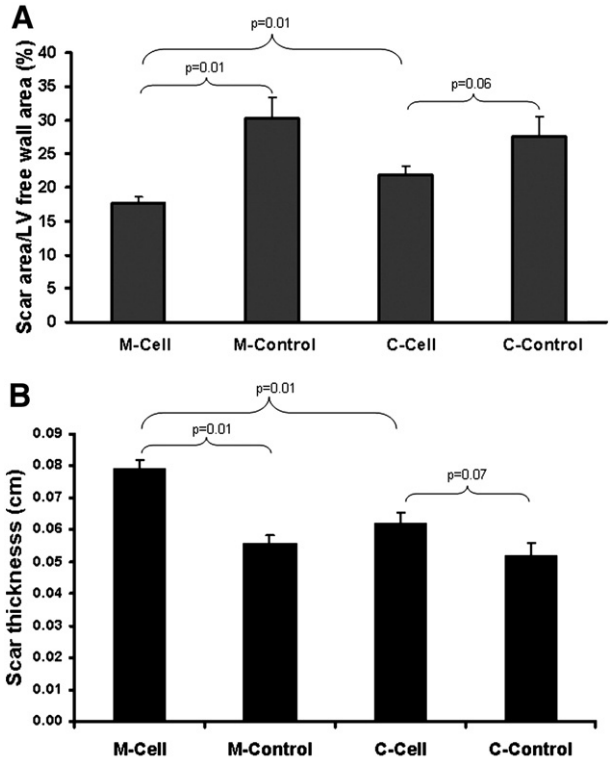

Fig. 4. Scar size and thickness evaluated by computed planimetry at 4 weeks after cell or media implantation. A–B: Scar size (scar area expressed as a percentage of the LV free wall area; A) was significantly reduced and scar thickness (B) was significantly increased in the group that received ACPs by intramyocardial injection (M-Cell) compared to its media control group (M-Control), the group that received ACPs by intracoronary injection (C-Cell), and the intracoronary media control group (C-Control; $p=0.01$ for all comparisons). Differences in scar size and scar thickness in the C-Cell group compared to the C-Control group were not statistically significant ($p=0.06$ and $p=0.07$, respectively). $n=9$/cell group; $n=5$/control group.

3.2.2. Cardiac function

Cardiac function was evaluated by echocardiography (before LAD ligation, before cell/media implantation, and 2 and 4 weeks after implantation) and by pressure–volume catheter measurements (4 weeks after implantation).

Echocardiography:

Two weeks after implantation of cells or media, both groups of ACP-implanted rats exhibited significant increases (relative to pre-implantation) in % FS, FAC, and LVEF, while media-implanted rats (controls) exhibited decreases in heart function by the same parameters. At the 2 week time point, % FAC was significantly ($p<0.05$) increased in the M-Cell and C-Cell groups relative to their respective controls (Supplementary Table E2). Improvements from pre-implantation (day 0) in FS, FAC, and LVEF were significantly greater by 2 weeks after implantation in both ACP-implanted groups than in their respective controls ($p<0.01$ for all groups; Fig. 2, open bars). By 4 weeks after implantation, % FS, FAC and LVEF were increased non-significantly (relative to pre-implantation) in both ACP-implanted groups, but decreased in the control groups (Supplementary Table E2). Improvements from pre-implantation (day 0) in FS and LVEF were significantly greater by 4 weeks after implantation in both ACP-implanted groups than in their respective controls ($p<0.05$ and $p<0.01$, respectively; Fig. 2, black bars).

Pressure–volume catheter:

At 4 weeks after implantation, pressure–volume catheters were used to measure real-time left ventricular pressure and EDV. EDV was reduced in both ACP-implanted groups compared to the control groups ($p<0.05$; Fig. 3A–B). Similarly, both ACP-implanted groups exhibited higher maximum $+dP/dt$ and lower minimum $-dP/dt$ values than their respective control groups ($p<0.01$ for M-Cell vs. M-Control, $p<0.05$ for C-Cell vs. C-Control; Fig. 3C–D), with no significant differences between the two cell delivery methods.

3.2.3. Morphology: left ventricular remodelling

Myocardial scar area and thickness were evaluated by computed planimetry at 4 weeks after cell implantation. Animals that received ACPs by intramyocardial injection exhibited significantly smaller and thicker scars than those that received ACPs by intracoronary injection, or those that

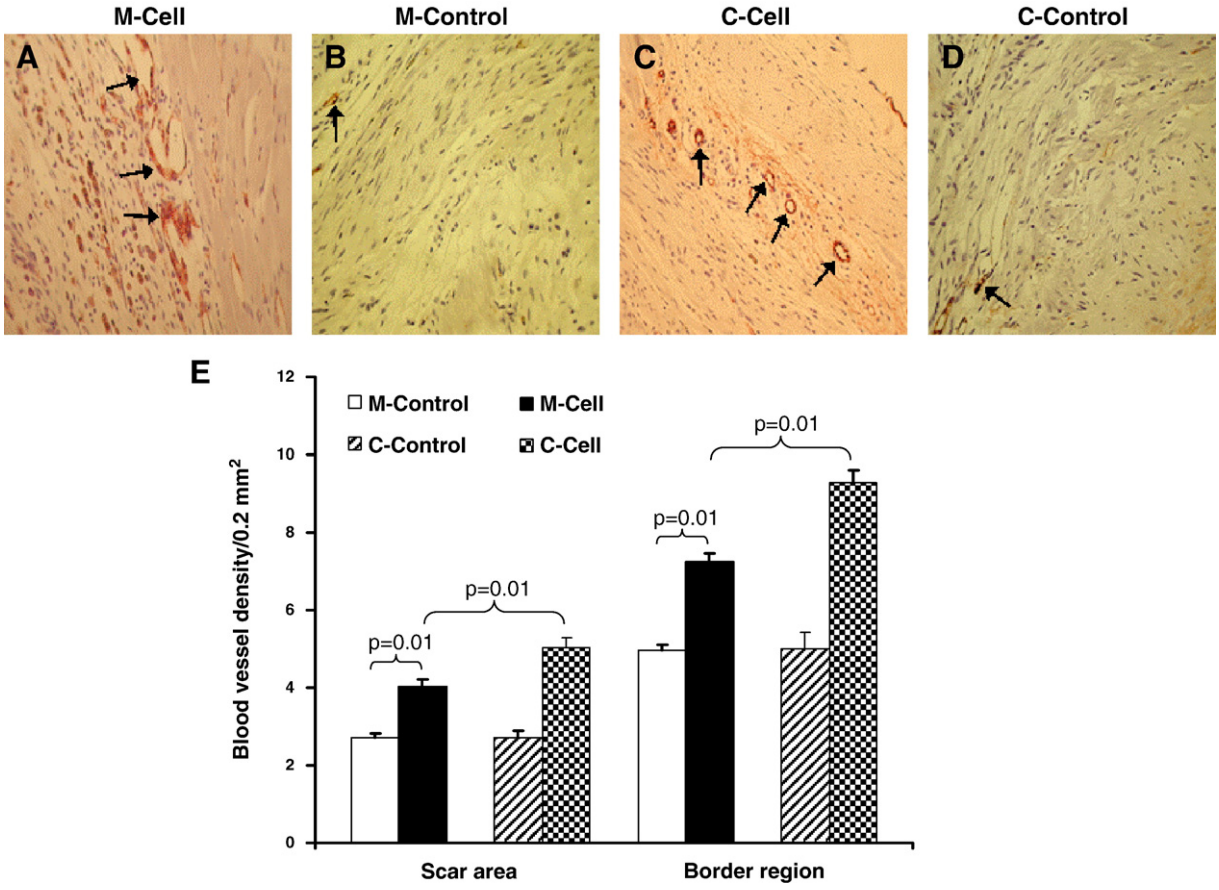

Fig. 5. Histology: angiogenesis. A–D: Representative photomicrographs (X 400) illustrating factor VIII staining to identify blood vessels in the myocardial ischaemic zone of cardiac sections taken at 4 weeks after cell or media implantation in rats that received ACPs by intramyocardial or intracoronary injection (M-Cell, C-Cell, respectively) and in the respective media control groups (M-Control, C-Control). Vascular endothelial cells in the blood vessels stained positive for factor VIII (brown colour, arrows). E: Blood vessel density was significantly greater in the scar area and border regions of the ACP-implanted groups compared to their respective controls ($p=0.01$ for all comparisons), and was greater in the border region compared to the scar area overall. Within each region, vascular density was increased in the C-Cell compared to the M-Cell group ($p=0.01$). $n=9$/cell group; $n=5$/control group.

received media ($p=0.01$ for all comparisons). Scar areas appeared to be reduced and scar thicknesses increased in the C-Cell group compared to the C-Control group, but these differences were not statistically significant ($p=0.06$ and $p=0.07$, respectively; Fig. 4).

3.2.4. Histology and immunohistochemistry

Four weeks after cell implantation, histological and immunohistochemical studies were carried out to identify the implanted cells and determine their location relative to those expressing cardiac or vascular proteins.

Human proteins:

Antibodies against human mitochondria protein were used to quantify implanted cell survival and to identify the distribution and engraftment of the implanted human ACPs. At 4 weeks after implantation, cells expressing human mitochondria were observed in the ischaemic myocardium of both ACP-implanted groups, but not in that of the control groups. Overall, the density of surviving ACPs was greater in the myocardial ischaemic zone of M-Cell animals than in that of C-Cell animals (~29 ACPs/0.2 mm^2 in M-Cell; ~20 ACPs/0.2 mm^2 in C-Cell; $p<0.01$). Most cells delivered by intramyocardial injection were retained in clusters in the scar area, while those delivered by intracoronary injection were mainly widely distributed in the border region (Supplementary Fig. E1 A–D).

Cardiac proteins:

Antibodies against myosin heavy chain and Troponin I revealed some muscle-like cells in the myocardial ischaemic zones of both ACP-implanted groups, while non-myogenic cells were observed in the ischaemic zones of the control groups. In the C-Cell group, cells expressing cardiac proteins were more widely distributed than those in the M-Cell group, and were located in closer proximity to the blood vessels (Supplementary Fig. E1 E–L).

Vascular proteins:

Antibodies against factor VIII indicated higher blood vessel density in the myocardial ischaemic zones of both ACP-implanted groups than in those of their respective controls ($p=0.01$; Fig. 5A–D). Comparison of the groups that received ACPs revealed vascular density was significantly greater in the border region (to which the majority of ACPs delivered by the intracoronary route migrated after injection) than in the scar area, and significantly greater in the C-Cell than in the M-Cell group within each region ($p=0.01$ for both regions; Fig. 5E).

4. Discussion

The present study characterizes the *in vitro* features of human ACPs: specifically, the ability of cells pre-loaded with Ac-LDL to form tube-like structures, and their migratory potential. In addition, it evaluates the beneficial effects of human ACPs delivered via intramyocardial or intracoronary implantation in a nude rat model of myocardial ischaemia, and suggests possible mechanisms underlying these effects. We found that both routes of ACP delivery were associated with improved cardiac function following LAD coronary artery occlusion. The functional effects were associated with significant increases in capillary density, with newly formed muscle-like cells, and with decreases in EDV, suggesting that the implanted ACPs induced neovascularization and possibly heart tissue regeneration.

ACPs were chosen as the population for implantation based on a number of considerations. First, ACPs can be produced from human peripheral blood. These cells express the multipotent stem cell markers CD34, CD117 and CD133. They also possess the angiogenic lineage-specific characteristics of concomitant Ulex-lectin binding and Ac-LDL uptake, and express markers such as KDR, Tie-2, CD144 and vWF, as well as high levels of CD31 ($CD31^{Bright}$). Moreover, ACPs secrete angiogenic factors including VEGF and angiogenin [15], and the cytokine IL-8, which is known for its stem cell recruitment, anti-apoptotic and pro-angiogenic capabilities [19]. Our *in vitro* study demonstrated that ACPs have a high angiogenic capacity, and will migrate toward various stem cell attractant chemokines. These capabilities indicate that ACPs posses the characteristics of both multipotent precursor cells and lineage-specific angiogenic progenitors, suggesting a strong potential for effective engraftment, blood vessel formation, and support of tissue survival and regeneration.

The *in vivo* functional analyses demonstrated that ACP implantation significantly enhanced cardiac function after myocardial injury. This effect was associated with greater improvements in FS, FAC and LVEF, and with decreased ventricular dilatation in the ACP recipients relative to the controls following intramyocardial or intracoronary cell delivery. Improved systolic and diastolic function in the ACP recipients was confirmed by greater maximum $+dP/dt$ scores and greater active relaxation during early diastole (represented by lower minimum $-dP/dt$ scores) compared to controls at 4 weeks after cell implantation. Future experiments will clarify whether the functional benefits of the implanted ACPs persist beyond the 4 week end point of this study, but we can speculate on a possible mechanism. We observed in this study that scar areas were reduced and thickened – significantly in the M-Cell group – following ACP injection. Stabilization of the ischaemic zone in the cell-treated animals may have prevented the infarct expansion and adverse left ventricular remodelling that led to systolic and diastolic dysfunction in the control animals.

ACP recipients exhibited a number of cells in the myocardial ischaemic zone that expressed human mitochondrial protein, indicating they were derived from the engrafted human ACPs. Serial slides stained with antibodies against cardiomyocyte markers also revealed the expression of Troponin I in proximity to the engrafted ACPs, raising the possibility that at least some stem/progenitor cells in the ACP population transdifferentiated into myogenic cells within the ischaemic zone. Such post-implantation myogenesis may have contributed to the observed changes in scar

morphology and ventricular volume that were associated with the cardiac functional improvements in ACP recipients.

In this study, ACP-implanted rats, especially those that received the cells by intracoronary injection, experienced significant increases relative to controls in blood vessel density in both the scar area and border region. The implanted ACPs may have increased blood vessel density by exerting a paracrine effect: angiogenic factors secreted by the implanted cells stimulated neovascularization by recruiting angiogenic stem cells to the implanted area. Since we identified angiogenic progenitor cells in the ACP population, it is also possible that the implanted cells became incorporated into the newly-formed blood vessels. An increase in blood vessel density can enhance oxygen diffusion and penetration into hypoxic tissues, thereby restoring oxygen to the mitochondria and maintaining oxidative metabolism. The resultant rescue of hibernating myocardium, reported in human subjects after therapeutic angiogenesis [20], is another contributor to cardiac functional restoration.

Cell transplantation to restore cardiac function after myocardial infarction has been studied extensively in preclinical studies and early phase clinical trials [21–28]. In all these studies, donor cells were implanted either by intramyocardial or by intracoronary infusion. However, few studies have directly compared the effectiveness of these two cell delivery routes, and the optimal route for cell transplantation remains unclear. Accordingly, the current study provides a useful comparison of intramyocardial and intracoronary cell delivery, employed here to implant ACPs into the ischaemic nude rat myocardium: When equal doses of ACPs were given by two different routes, intracoronary delivery resulted in less efficient ACP engraftment than did intramyocardial delivery, but produced similar improvements in cardiac function. In the M-Cell group, greater number of cells concentrated in the implanted scar area increased scar thickness and limited scar expansion, while in the C-Cell group, the comparatively smaller number of ACPs that migrated and engrafted into the border region stimulated more blood vessel formation.

The current data suggest that human ACPs generated from peripheral blood may represent a novel therapeutic approach to treat myocardial ischaemic disease. Further studies measuring the cell dose–responses for both intramyocardial and intracoronary cell delivery would allow us to quantitatively compare the outcomes achieved at the optimal dose associated with each route, further elucidating which is the optimal cell delivery method to restore function in the ischaemic myocardium. It would also be useful to examine the effects of administering the cells using both routes in the same animal, and to track the distribution of the implanted cells within the body using a molecular imaging technique. Finally, comparing the transcriptomes expressed by ACP populations engrafted into different regions of the damaged myocardium would help to clarify how these cells could exert different effects within the tissue depending on the route by which they are implanted.

Funding

TheraVitae Ltd., Israel to R.L.

Conflict of Interest

This work was supported by a grant to R.L. from TheraVitae Ltd., Israel. Co-authors Svetlana Porozov, Adina Belleli, Valentin Fulga, and Yael Porat are employed by TheraVitae Ltd.

Acknowledgement

We thank Heather McDonald Kinkaid for her assistance with manuscript preparation and editing.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejheart.2008.04.004.

References

[1] Baumgartner I, Pieczek A, Manor O, et al. Constitutive expression of phVEGF165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. Circulation 1998;97:1114–23.
[2] Harada K, Grossman W, Friedman M, et al. Basic fibroblast growth factor improves myocardial function in chronically ischemic porcine hearts. J Clin Invest 1994;94:623–30.
[3] Risau W. Mechanisms of angiogenesis. Nature 1997;386:671–4.
[4] Takeshita S, Zheng LP, Brogi E, et al. Therapeutic angiogenesis. A single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hind limb model. J Clin Invest 1994;93:662–70.
[5] Yla-Herttuala S, Alitalo K. Gene transfer as a tool to induce therapeutic vascular growth. Nat Med 2003;9:694–701.
[6] Asahara T, Masuda H, Takahashi T, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 1999;85:221–8.
[7] Isner JM, Asahara T. Angiogenesis and vasculogenesis as therapeutic strategies for postnatal neovascularization. J Clin Invest 1999;103: 1231–6.
[8] Shi Q, Rafii S, Wu MH, et al. Evidence for circulating bone marrow-derived endothelial cells. Blood 1998;92:362–7.
[9] Takahashi T, Kalka C, Masuda H, et al. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med 1999;5:434–8.
[10] Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964–7.
[11] Kalka C, Masuda H, Takahashi T, et al. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. Proc Natl Acad Sci U S A 2000;97:3422–7.
[12] Kawamoto A, Gwon HC, Iwaguro H, et al. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. Circulation 2001;103:634–7.
[13] Kawamoto A, Tkebuchava T, Yamaguchi J, et al. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. Circulation 2003;107:461–8.
[14] Kocher AA, Schuster MD, Szabolcs MJ, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med 2001;7:430–6.

[15] Porat Y, Porozov S, Belkin D, et al. Isolation of an adult blood-derived progenitor cell population capable of differentiation into angiogenic, myocardial and neural lineages. Br J Haematol 2006;135:703–14.
[16] Kayisli UA, Luk J, Guzeloglu-Kayisli O, Seval Y, Demir R, Arici A. Regulation of angiogenic activity of human endometrial endothelial cells in culture by ovarian steroids. J Clin Endocrinol Metab 2004;89: 5794–802.
[17] Kan CD, Li SH, Weisel RD, Zhang S, Li RK. Recipient age determines the cardiac functional improvement achieved by skeletal myoblast transplantation. J Am Coll Cardiol 2007;50:1086–92.
[18] Mayo Clinic Cardiology Review. 2nd Ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2000. p. 775.
[19] Schomig K, Busch G, Steppich B, et al. Interleukin-8 is associated with circulating CD133+ progenitor cells in acute myocardial infarction. Eur Heart J 2006;27:1032–7.
[20] Vale PR, Losordo DW, Milliken CE, et al. Left ventricular electromechanical mapping to assess efficacy of phVEGF(165) gene transfer for therapeutic angiogenesis in chronic myocardial ischemia. Circulation 2000;102:965–74.
[21] Assmus B, Schachinger V, Teupe C, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). Circulation 2002;106:3009–17.
[22] Fuchs S, Baffour R, Zhou YF, et al. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. J Am Coll Cardiol 2001;37:1726–32.
[23] Kang HJ, Kim HS, Zhang SY, et al. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. Lancet 2004;363:751–6.
[24] Perin EC, Dohmann HF, Borojevic R, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation 2003;107:2294–302.
[25] Shintani S, Murohara T, Ikeda H, et al. Augmentation of postnatal neovascularization with autologous bone marrow transplantation. Circulation 2001;103:897–903.
[26] Stamm C, Westphal B, Kleine HD, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet 2003;361: 45–6.
[27] Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 2002;106:1913–8.
[28] Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. Lancet 2003;361:47–9.