Intracoronary administration of autologous adipose tissue-derived stem cells improves left ventricular function, perfusion, and remodelling after acute myocardial infarction

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Aims This study was designed to assess whether intracoronary application of adipose tissue-derived stem cells (ADSCs) compared with bone marrow-derived stem cells (BMSCs) and control could improve cardiac function after 30 days in a porcine acute myocardial infarction/reperfusion model.

Methods and results An acute transmural porcine myocardial infarction was induced by inflating an angioplasty balloon for 180 min in the mid-left anterior descending artery. Two million cultured autologous stem cells were intracoronary injected through the central lumen of the inflated balloon catheter. Analysis of scintigraphic data obtained after 28±3 days showed that both absolute and relative perfusion defect decreased significantly after intracoronary administration of ADSCs or BMSCs (relative 30 or 31%, respectively), compared with carrier administration alone (12%, P=0.048). Left ventricular ejection fraction after 4 weeks increased significantly more after ADSC and BMSC administration than after carrier administration: 11.39±4.62 and 9.59±7.95%, respectively vs. 1.95±4.7%, P=0.02). The relative thickness of the ventricular wall in the infarction area after cell administration was significantly greater than that after carrier administration. The vascular density of the border zone also improved. The grafted cells co-localized with von Willebrand factor and alpha-smooth muscle actin and incorporated into newly formed vessels.

Conclusion This is the first study to show that not only bone marrow-derived cells but also ADSCs engrafted in the infarct region 4 weeks after intracoronary cell transplantation and improved cardiac function and perfusion via angiogenesis.

KEYWORDS
Myocardial infarction; Heart failure; Angiogenesis

Introduction
Over the recent years, cellular cardiomyoplasty has been an area of intense research. A variety of cell types have been shown to work beneficially, especially in the ischaemic myocardium of rodents and small animals. Among the cell types used so far, mesenchymal stem cells seem to be highly advantageous for cellular therapy. MSCs are multipotent, might be immune-privileged, and can be expanded easily ex vivo. MSCs isolated from adult bone marrow have shown a great potential for cell therapy because these cells possess multipotent capabilities, proliferate rapidly, induce angiogenesis, and differentiate into myogenic cells. Recently, a multipotent stem cell population with high proliferative potential was isolated from human adipose tissue. Aside from the possible pain and morbidity associated with a bone marrow biopsy, the use of adipose tissue has the advantage of being able to obtain a high number of mesenchymal stem cells (2–10%), whereas bone marrow aspirate typically yields only two to
three mesenchymal cells per 100 000 cells. In most patients, adipose tissues are abundantly available and can be routinely harvested. Thus, adipose tissue appears to be a convenient and preferable source of stem cell recovery for cardiac therapy, compared with bone marrow. The transdifferentiation potential of ADSCs has been shown recently in several studies.8-11 ADSCs have been shown to differentiate into endothelial cells, incorporate into vessels, and promote post-ischaemic neovascularization in nude mice.12,13 Transplantation of monolayered ADSC reversed wall thinning in the scar area and improved cardiac function in rats with myocardial infarction.14 The engrafted sheet formed a thick stratum with newly formed vessels.

To explore the potential therapeutic application of ADSCs in a pre-clinical large animal model using the same instrumentation and standard of care as in humans, we compared the effects of intracoronary administration of ADSCs, BMSCs, or carrier on left ventricular (LV) function, vascular density, and wall thickness (WT) in a porcine model of transmural myocardial infarction.

Methods

Experimental animals

All procedures were performed in accordance with protocols approved by the Institutional Committee for Animal Care and Use of Tulane University and compiled with the guide for the Care and Use of Lab Animals (NIH publication no. 86-23, revised 1985, study protocol 2749). A total of 31 female farm pigs (30.8 ± 5.4 kg) were used in the study.

Experimental design

Thirty-one animals were randomized in the study. Animals that survived the initial infarction induction (21 pigs, three pigs per group) were followed for 28 ± 3 days. Perfusion defect was assessed by standard SestaMIBI evaluation protocols adjusted by our clinical nuclear department to the chest geometry of the pigs. The team who performed the cath procedure was blinded to the randomization protocol. The randomization code was retrieved at the time who performed the cath procedure was blinded to the randomization protocol. The randomization code was retrieved at the time.

Cells of the second passage were incubated with 100 μL of the CMVeGFP lentivirus in 10 mL medium per flask (75 cm²) in the presence of 8 μg/mL polybrene (Sigma-Aldrich) at least 2 days prior to transplantation. The proportion of enhanced green fluorescent protein (eGFP)-expressing cells averaged 33 ± 13% (ADSC 31 ± 16% and BMSC 35 ± 10%) upon transplantation as measured by flow cytometry.

Infection of infarct and application of cells

General anaesthesia was induced with intramuscular telazol (4.5 mg/kg), xylazine (3.5 mg/kg), and atropine (0.04 mg/kg) and was maintained with isoflurane (1-2.5%) and telazol/xylazine. Identical regimen was used in all procedure (except the tissue harvest as these procedures were short and no haemodynamic measurement was performed, only telazol/xylazine was used for bone marrow tap and fat tissue excision). We are aware of the influence of isoflurane on the LV ejection fraction (EF); therefore, we kept the dosage on the same level. Surgical cut down approach was used to access the internal carotid artery and LV function was analysed.

Body temperature was monitored during the whole procedure and was kept in normal ranges (97-99°F) by using water circulating blankets placed on the back and on the belly of the pigs. All pigs were treated with oral β-blocker bisoprolol (2.5 mg), once daily, 2 days prior to infarction through day 2 post-infarction.

Baseline LVEF of the LV was assessed by ventriculography in 30° LAO View that was followed by the left coronary angiography to determine the optimal site for the balloon placement in the left anterior descending artery (LAD) and the size of the balloon (visual estimation). Epifibatide was given according to the ESPRIT trial.15

An angioplasty balloon (diameter 2.5 ± 0.4 mm, length 9.4 ± 1.6 mm, Cordis Inc., Miami Lakes, FL, USA) was inflated for 180 min in the mid-LAD artery to induce a large transmural myocardial infarction (Figure 1A and B). Non-life-threatening arrhythmias were treated with amiodarone or magnesium sulfate IV and life-threatening arrhythmias with semi-automatic defibrillation. A dose of 20 mCi Tm⁹⁹mTc Cardiolite® (Bristol-Myers Squibb Medical Imaging, N. Billerica, MA, USA) was injected 20 min prior to the deflation of the balloon to assess the area of myocardium at risk. After 15 min reperfusion of the LAD, either two million autologous cells (BMSC or ADSC) in 4 mL PBS or control solution without cells was injected slowly into the coronary artery in 5 min, at the site of prior infarct induction through the central lumen of the re-inflated balloon. Blood flow was restored after 5 min settlement time for the injected cells and LV angiography was performed subsequently to determine the EF. Gated single photon emission computed tomography (SPECT) was performed and finally the anaesthesia terminated.
Perfusion and functional assessment with $^{99m}$Tc-Sestamibi

SPECT imaging was performed in the anaesthetized animal in a supine position under a single head nuclear gamma camera (Philips Arc-3000, ADAC Laboratories/Philips Medical Systems, Milpitas, CA, USA). SPECT images were acquired with a circular 180° acquisition of 64 projections with an acquisition time of 20 s per image. From the raw data, dedicated software (AutoSPECT + InStill 5.0, Ultra Myocardial Display Version 3.41 and AutoQUANT 5.1, ADAC Laboratories) was used to analyse LV parameters including LV wall motion and wall thickening. The percentage of the LV that was compromised by the initial perfusion defect, the size of the final infarct as percentage of the LV at the time of follow-up, and the degree of myocardial ‘salvage’ as a percentage of the LV was calculated as the size of the initial perfusion defect minus the final size of the infarct (initial perfusion defect or infarct)/(initial perfusion defect) = % salvaged. The salvage index was calculated as the percentage of the LV that was salvaged divided by the percentage that was compromised by the initial perfusion defect (% LV salvaged)/(% of LV initial perfusion defect) = salvage index. The analysis was performed in the scintigraphic core lab by two independent operators blinded to randomization. Mean intra- and interobserver variability in the defect assessment was similar and equal to 2 ± 3% of the LV.

Wall thickness and infarct area

Digital images were taken from heart slices to calculate the WT ratio and the area of infarcted myocardium. LVWT was determined at the centre of the infarct and compared with the thickness of non-infarcted myocardium (LVWT ratio: WT infarct/WT non-infarcted). A line was drawn from the endocardial to the epicardial border in all three areas. For the border zone, two lines were drawn on both sides of the infarct area for a total of four measurement points (infarct, border zones 1 and 2, and non-infarcted myocardium).

The infarct area and the non-infarcted myocardium were measured in every heart slice (Figure 1C). The basal side of the slices was used, whereas the right ventricle was excluded. After calibration, the areas of infarct and non-infarcted myocardium were delineated and calculated by MetaMorph. The infarct size was compared with the LV size and the ratio expressed in per cent [infarct area/(infarct area + non-infarct myocardial area)].

Immunohistochemistry

Fluorescent immunostaining for eGFP was carried out to identify the transplanted cells in the heart. The following antibodies were used for this study: rabbit anti-von Willebrand factor (vWF) (Dakocytomation), mouse anti-SMA (Zymed, Laboratories Inc., San Francisco, CA, USA), mouse anti-Desmin (Dakocytomation), mouse anti-Ki67 (Zymed), mouse anti-Troponin T (Santa Cruz), and rabbit anti-GFP (Santa Cruz). Samples of infarct tissue, border zones, non-infarcted myocardium from every slice were excised and fixed in formalin, embedded, and cut in 5 µm sections. After deparaffinizing and rehydrating, the slides were subsequently incubated with diluted primary antibody and then secondary antibodies, as described earlier.11

Capillary density

Neoangiogenesis was evaluated in paraffin-embedded sections stained for vWF using 3,3′ diaminobenzidine tetrahydrochloride (DAB) as a chromagen. Sections were treated following the above protocol up to incubation with the secondary antibody which, in this protocol, was biotinylated goat anti-rabbit IgG (Zymed, Laboratories Inc.). After washing in PBS, streptavidin peroxidase (Zymed, Laboratories Inc.) was applied in an additional incubation step for 20 min (37 °C). DAB (Zymed, Laboratories Inc.) was used as a chromogenic substrate and the colour reaction was carried out for
3–10 min and the slides were washed before they were covered with Crystal/Mount (Biomeda, Foster City, CA, USA).

In each pig, a slice from three separate areas of the border zone was counted. Pictures were taken under light microscopy at × 40 magnification in five random fields (0.1 mm² area each) per slide. Only vessels with a diameter equal or below 10 μm on a perpendicular cut were counted. The capillary density was expressed as counts per 0.1 mm².

Statistical analysis

All values are presented as mean ± standard deviations. The differences between the groups were assessed with the use of contingency tables for categorical data and the non-parametric Kruskal–Wallis test for continuous data. A two-tailed P-value of ≤0.05 was considered statistically significant.

Results

Of the 31 animals randomized in the study, 10 died during the induction of myocardial infarction, as it was aimed to induce a large, transmural infarction. In a previously performed pilot study, no reflow was observed in a number of pigs after intracoronary cell injection. The reason was a distal vascular occlusion at the capillary level. The maximal number of cells that could be safely administered was found to be 10 millions (in this disease model in pigs, at this weight). Therefore, in the main study, a total of two million cells (0.06 million cells/kg) was used. We also added an intensified antithrombotic drug regimen (pre-/post-treatment with aspirin and clopidogrel) and administration of low-molecular-weight heparin and eptifibatide during the procedure. We gave enoxoparin 0.7 mg/kg BW IV after the sheath was placed and a second dosage of 0.3 mg/kg BW IV 4 h later and 1.0 mg/kg BW SC at the end of the procedure (day of cell transplantation). In addition, eptifibatide was given two times as a bolus (180 μg/kg BW, first bolus after the first angiography, and second bolus 10 min later) and as an infusion (2 μg/kg BW/min) until the end of the procedure. We did not have any bleeding complications, despite the intensified drug regimen. The remaining 21 pigs (n = 7 per group) were divided into three groups: (i) BMSC, (ii) ADSC, or (iii) control.

Characteristics of mesenchymal stem cells before implantation

The cultured pig ADSCs are positive for CD90 (97.3 ± 0.62%), CD44 (98.27 ± 0.38%), and CD29 (98.2 ± 0.87%) and negative for CD31 (0.03 ± 0.05%), CD45 (0.45 ± 0.41%), and CD11 (0.17 ± 0.17%). These cells were spindle-shaped (Figure 2A) and expressed eGFP after lentiviral transduction (Figure 2B). The proportion of eGFP-expressing cells by flow cytometry averaged 33 ± 13% (ADSC 31 ± 16% and BMSC 35 ± 10%).

Stem cell engraftment and phenotype after implantation

Immunostaining for GFP verified the presence of the intracoronary-administered ADSCs in the LV at day 30 (Figure 2C). The engrafted cells were distributed within and around the vasculature. Most of the GFP⁺ stem cells were stained positively for vWF, alpha-smooth muscle actin, and desmin in the ADSC (Figure 3) and BMSC

(Figure 4) groups, indicating the endothelial and smooth muscle phenotypes of GFP⁺ cells. The co-staining of the GFP⁺ cells with endothelial and smooth muscle markers in the vasculature (Figures 3 and 4A, B and D) indicates that the stem cell transplantation may result in the formation of new vessels. Cell proliferation as evidenced by positive Ki 67 overlay staining was rare in the carrier group (Figure 4C), but was easily detected in the ADSC (Figure 3C) and BMSC (Figure 4C) groups. Despite extensive search, no co-staining for troponin T and GFP was observed in the ADSC and BMSC groups (Figures 3E and 4E).

Capillary density

The capillary vessel density in the infarct border zone was determined by staining for vWF. Positively stained vessels with a diameter ≤10 μm were considered to be capillaries. The capillary count observed per group in 0.1 mm² fields was significantly greater in the ADSC-treated animals [123.0 ± 44.9; confidence interval (CI) 110.0–136.0] than in the carrier group (93.6 ± 32.1; CI 79.0–108.0). The difference in capillary density of BMSC-treated animals (107.0 ± 50.4; CI 93.0–121.0) vs. control was not statistically significant; analysis of variance for all groups: 0.019, post hoc comparison between individual groups: BM vs. ADSC: 0.16; BM vs. sham: 0.19; ADSC vs. sham: 0.002.

Wall thickness

Only a pairwise comparison of ADSC vs. sham therapy resulted in a significant increase in WT (P = 0.003 using t-test), whereas there was no significance level when BMC vs. sham (t-test) or when the group of sham, BMC, and ADSC was compared simultaneously (Figure 6A) (P = 0.14 for all groups, Kruskal–Wallis test).
Figure 3  Phenotypes of engrafted MSCs in border zone of adipose tissue-derived stem cell injected pig. Hoechst 33342 staining for nuclei (a), eGFP<sup>+</sup> cells (b), specific antibodies (c), and overlay image (d) in the following order: (A) vWF, (B) ASMA, (C) Ki67, (D) desmin, and (E) TnT.

Figure 4  Phenotypes of engrafted MSCs in the border zone of bone marrow-derived stem cell injected pig. Hoechst 33342 staining for nuclei (a), GFP<sup>+</sup> cells (b), specific antibodies (c), and overlay image (d) in the following order: (A) vWF, (B) ASMA, (C) Ki67, (D) desmin, and (E) TnT.
Figure 5  Immunostainings in the border zone of sham pigs. Hoechst 33342 staining for nuclei (a), anti-eGFP staining (b), specific antibodies (c), and overlay image (d) in the following order: (A) vWF, (B) ASMA, (C) Ki67, (D) desmin, and (E) TnT.

Figure 6  Wall thickness (A), left ventricular ejection fraction (B), myocardial salvage percentage (C), and myocardial salvage index (D) were compared between the adipose tissue-derived and bone marrow-derived stem cell groups in a 4-week follow-up.
Left ventricular ejection fraction

LVEF at baseline following the induction of the myocardial infarction was similarly reduced by 12.6 ± 7.2% in the control, 14.3 ± 6.1% in the ADSC, and 17.9 ± 4.7% in the BMSC groups (NS). At 4 weeks of follow-up, LVEF improved by 1.9 ± 4.7% in the control, 11.4 ± 4.6% in the ADSC, and 9.6 ± 8.0% in the BMSC groups (P = 0.02 for all three groups by Kruskal–Wallis test). Although there were no significant differences between the treatment groups, both were significantly improved when compared with control (pairwise analysis) (Figure 6B).

Nuclear cardiac imaging

Absolute myocardial salvage was significantly greater in the ADSC and BMSC groups than in the control group (10.3 ± 5.2, 12.0 ± 7.9, and 4.1 ± 2.2, respectively, P = 0.027 for all three groups by the Kruskal–Wallis test). Pairwise analysis also showed similar results (Figure 6C). The myocardial salvage index was also significantly greater in ADSC and BMSC groups when compared with carrier (ADSC 0.30 ± 0.17, BMSC 0.31 ± 0.16, and 0.12 ± 0.07, P = 0.048 for all three groups by the Kruskal–Wallis test). Pairwise analysis also showed similar results (Figure 6D).

Figure 7 Nuclear imaging. Bull’s eye visual and semi-quantitative polar plot analysis of perfusion defect (blue zone) in a representative pig injected with tissue-derived stem cell at baseline (A) and follow-up (B). The antero-apical perfusion defect has visually decreased along with a decrease in extent from 45% to 32% of the calculated left ventricular mass on follow-up. Gated short- and long-axis images in control (C) and adipose tissue-derived stem cells (D) at baseline end-diastolic (a) and end-systolic (b) and follow-up end-diastolic (c) and end-systolic frames (d). Little improvement in perfusion defect and thickening is seen in the control pig, whereas modest improvement is seen in both parameters in an ADSC pig (fine arrow: control, bold arrow: adipose tissue-derived stem cells, white arrow: apex at baseline, and yellow arrow: at 4 weeks of follow-up). AutoQuant derived 3D surface-rendered LV end-diastolic and end-systolic contours (Figure 7E and F) showed a significant improvement in LV wall motion in the representative ADSC pigs when compared with a carrier pig at follow-up in both end-diastolic and end-systolic frames. Especially, at end systole, a resumption of inward contraction was clearly identifiable at the antero-apical infarct region (white arrow) compared with the absent or even paradoxical wall movement in the control group at end systole. The end-diastolic volume for the control at baseline (Figure 7Ec) and follow-up (Ec) and that for the ADSC...
pig at baseline (F₀) and follow-up (F_c) are 93.7 and 106.0 and 90.4 and 89.6 mL, respectively. The end-systolic volume for the control at baseline (E_B) and follow-up (E_d) and that for the ADSC pig at baseline (F_b) and follow-up (F_d) are 57.7 and 63.3 and 54.7 and 45.9 mL, respectively.

Table 1 show the absolute values of cardiac functions at baseline and follow-up.

In summary, we found that the transplanted ADSCs significantly improved LV function and expressed endothelial and smooth muscle cell markers and incorporated into newly formed vessels.

Discussion

This is the first study to evaluate the feasibility and effectiveness of adipose-derived stem cells for cellular cardiomyoplasty in an experimental MI model that closely reproduces the current procedural management of MI, including medication and instrumentation used in humans. Four weeks after intracoronary administration of ADSCs into the infarcted myocardium, LV perfusion, function, and remodelling were substantially improved. This improvement was similar to that observed after intracoronary administration of BMSC.

Our FACS data are in agreement with that reported by Miranville et al. and Rehman et al., which freshly isolated ADSCs containing a small percentage of CD45+ cells which diminished after 72 h culture.

Immunohistochemical analysis revealed that the implanted cells differentiated into endothelial cells (ECs) and smooth muscle cells (SMCs), which incorporated into newly formed vessels. Our findings are consistent with previous studies that angiogenesis may contribute to the maintenance of cardiac function by preservation of the remaining, viable hibernating cardiomyocytes and by stem cell-induced neovascularization that may help to meet the greater demands of the residual myocardium in the border zone that would otherwise undergo apoptosis.

It has been shown that the preservation of border zone WT can be achieved by the inhibition of apoptosis through induction of angiogenesis. As most cardiomyocytes within the area at risk are irreversibly damaged after 3 h of occlusion, the salvage effect assumed to be due to preservation of cells in the border zone through stem cell mediated anti-apoptotic and neoangiogenic effects.

Therapeutic enhancement of neovascularization through transplantation of bone marrow-derived and adipose-derived stem cells is associated with long-term salvage and survival of viable tissue. Similarly, ADSCs differentiated into endothelial cells as demonstrated by an abundance of human CD31-positive cells lining regenerated vessels in a nude mouse hindlimbs ischaemia model.

The significant improvement in LV function at 30 days assessed by 99mTc-Sestamibi was apparently not related to a direct transdifferentiation of ADSCs or BMSCs into cardiomyocytes. Our findings are in agreement with the report by

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>ADSC</th>
<th>BMSC</th>
<th>Control</th>
<th>P-value</th>
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<tr>
<td>A LVEF at baseline</td>
<td>33.14 ± 5.54</td>
<td>37.62 ± 4.23</td>
<td>38.36 ± 9.22</td>
<td>n.s.</td>
</tr>
<tr>
<td>LVEF at 4-week follow-up</td>
<td>30.23 ± 4.70</td>
<td>29.31 ± 4.38</td>
<td>27.68 ± 6.29</td>
<td>n.s.</td>
</tr>
<tr>
<td>Change of LVEF from baseline to post-cell transplantation (absolute numbers)</td>
<td>-14.31 ± 6.12</td>
<td>-17.90 ± 6.67</td>
<td>-12.63 ± 7.19</td>
<td>n.s.</td>
</tr>
<tr>
<td>Change of LVEF from post cell transplantation to follow up</td>
<td>11.39 ± 4.62</td>
<td>9.59 ± 7.95</td>
<td>1.95 ± 4.70</td>
<td>Kruskal–Wallis 0.021, t-test (ADSC and BMSC) 0.61, t-test (ADSC and control) 0.002, t-test (BMSC and control) 0.049</td>
</tr>
<tr>
<td>B QPS at baseline</td>
<td>35.71 ± 7.04</td>
<td>39.14 ± 9.63</td>
<td>33.71 ± 4.61</td>
<td>n.s.</td>
</tr>
<tr>
<td>QPS at follow-up</td>
<td>25.43 ± 8.36</td>
<td>27.14 ± 8.84</td>
<td>29.57 ± 4.86</td>
<td>n.s.</td>
</tr>
<tr>
<td>Myocardial salvage</td>
<td>10.29 ± 5.15</td>
<td>12.00 ± 7.85</td>
<td>4.14 ± 2.19</td>
<td>n.s.</td>
</tr>
<tr>
<td>Salvage index</td>
<td>0.30 ± 0.17</td>
<td>0.31 ± 0.16</td>
<td>0.12 ± 0.07</td>
<td>Kruskal–Wallis 0.048, t-test (ADSC and BMSC) 0.8, t-test (ADSC and control) 0.02, t-test (BMSC and control) 0.04</td>
</tr>
<tr>
<td>Infarct area (total)</td>
<td>24.30 ± 4.27</td>
<td>28.49 ± 9.77</td>
<td>29.55 ± 13.80</td>
<td>n.s.</td>
</tr>
<tr>
<td>Infarct area (only LV)</td>
<td>25.27 ± 5.82</td>
<td>31.73 ± 11.10</td>
<td>30.90 ± 12.39</td>
<td>n.s.</td>
</tr>
<tr>
<td>C Wall thickness ratio</td>
<td>0.75 ± 0.05</td>
<td>0.69 ± 0.21</td>
<td>0.58 ± 0.14</td>
<td>Kruskal–Wallis 0.141, t-test (ADSC and BMSC) 0.1, t-test (ADSC and control) 0.003, t-test (BMSC and control) 0.3</td>
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<tr>
<td>Heart rate at baseline</td>
<td>82.3 ± 5</td>
<td>72 ± 7</td>
<td>73.3 ± 14</td>
<td>n.s.</td>
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<tr>
<td>Heart rate after cell transplantation</td>
<td>88.5 ± 5.8</td>
<td>94.3 ± 18.9</td>
<td>89.3 ± 29</td>
<td>n.s.</td>
</tr>
<tr>
<td>Heart rate at follow-up</td>
<td>74.8 ± 11</td>
<td>75.4 ± 6.5</td>
<td>71.5 ± 24</td>
<td>n.s.</td>
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QPS, quantitative perfusion scintigraphy.
Infused cells within 4–5 days after MI. However, it has also shown that ADSCs can express biochemical markers characteristic of cardiomyocytes. Cardiomyocyte differentiation requires a number of crucial growth factors that are released from precursor cells, acting in an autocrine fashion on specific plasma membrane receptors to prime a cardiogenic decision. A better understanding of the interstitial milieu, i.e. the cytokines/growth factors produced by infarcted heart and injected stem cells, may define critical signalling molecules for cardiomyocyte differentiation. Although both ADSCs and BMSCs improved LV function, only ADSCs significantly improved LV remodelling as evidenced by an increase in the LV WT ratio and capillary density. Our data are in line with the report from Moelker et al. that bone marrow-derived cells do not reverse remodelling. Although there is a statistical difference in effects on LV remodelling between the cell groups, this finding needs to be confirmed using a large number of animals in future studies.

Clinical studies such as the TOPCARE and BOOST trials infused cells within 4–5 days after MI. However, it has also been shown that myocardial interstitial oedema after recanalization of the infarct-related artery persists for at least a week, and the facts that progressive increase of microvascular obstruction within the first 48 h after reperfusion and limited homing after 5 days might favour early cell transfer. Freyman et al. injected bone marrow-derived MSCs 15 min after infarction. They found that the 14-day retention for intracoronary infusion and endocardial injection represented 6 and 3% of the administered dose, respectively. In the intravenous infusion group, none of the infarcts contained a measurable number of cells. As the aim of that study was to compare quantitatively the three most common MSC delivery approaches following infarction in a large animal model with cellular engraftment as the endpoint, they did not report any functional analysis after stem cell administration. Along this line, the present study was primarily designed for efficacy. Furthermore, several groups have suggested that cardiac recruitment of stem cells requires both myocardial injury and expression of stromal-derived factor 1 (sdf-1). Sdf-1 upregulation occurs immediately following myocardial injury. In addition, we were concerned that the capillary bed may become less permeable to injected cells several days after infarction. On the basis of these previous studies, we chose to deliver the cells immediately after infarct revascularization, as this approach might also represent a clinically relevant scenario.

Mimicking the clinical scenario was an important part of this study design, which included the use of an anticoagulation, anti-thrombotic regimen, and accompanying medication (beta-blocker) currently used as state-of-the-art treatment of acute MI patients undergoing percutaneous revascularization. Beta-blocker was not used in other studies of intracoronary delivery of stem cells in experimental animals, which reported the lack of benefit of umbilical cord blood stem cells or BMSC on LV function. The functional improvement observed in our study is likely the result of a synergistic interaction of cells and β-blocker therapy.

There are reports that intracoronary cell injections can cause micro-infarctions when a large number of cells were administered. We compared our study with that reported by Vulliet et al. and Moelker et al. As shown in Table 2, the mean cell size is comparable among the three studies (~20 μm), although the cell number varied considerably. The cell number that we used was much less than that reported by Vulliet et al. and Moelker et al. The particular cell number that we had chosen was based on our pilot study, which showed that the maximal number of cells that could be safely administered was found to be 10 millions. We observed no-reflow phenomenon when more than 10 million cells were administered. Our findings are consistent with Vulliet et al. and Moelker et al. that larger number of cells cause microinfarction. In addition, we used an intensified antithrombotic drug regimen. Vulliet et al. did not report the use of any anticoagulation or antiplatelet therapy. The reported anticoagulation regimen by Moelker et al. was restricted to heparin. These variations might also explain the reported micro-infarction when large numbers of cells were administered.

**Table 2** Comparison of recent studies on intracoronary delivery of stem cells

<table>
<thead>
<tr>
<th>Author</th>
<th>Animal</th>
<th>Cell type</th>
<th>Cell number</th>
<th>Cell size</th>
<th>Anticoagulants</th>
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<tbody>
<tr>
<td>Vulliet et al.</td>
<td>Dog</td>
<td>BMSC</td>
<td>0.5 × 10⁶/kg</td>
<td>19.4 μm</td>
<td>None</td>
</tr>
<tr>
<td>Moelker et al.</td>
<td>Swine</td>
<td>Human USSC</td>
<td>100 × 10⁶/kg</td>
<td>20 μm</td>
<td>Heparin</td>
</tr>
<tr>
<td>Valina et al.</td>
<td>Swine</td>
<td>ADSC or BMSC</td>
<td>2 × 10⁶ (0.06 × 10⁶/kg)</td>
<td>20.8 μm (ADSC)</td>
<td>Aspirin, clopidogrel, heparin, eptifibatide, exoroparin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19.3 μm (BMSC)</td>
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BMSC, bone marrow-derived mesenchymal stem cells; USSC, umbilical cord blood stem cells; ADSC, adipose tissue-derived stem cells.

Silva et al. that bone marrow-derived MSCs co-localized with endothelial and smooth muscle cells, but not with cardiomyocytes in a canine chronic ischaemia model.

Transdifferentiation into cardiomyocytes has been reported previously with BMSCs, although negative studies have also been reported. We and others have shown that ADSCs can express biochemical markers characteristic of cardiomyocytes. Cardiomyocyte differentiation requires a number of crucial growth factors that are released from precursor cells, acting in an autocrine fashion on specific plasma membrane receptors to prime a cardiogenic decision. A better understanding of the interstitial milieu, i.e. the cytokines/growth factors produced by infarcted heart and injected stem cells, may define critical signalling molecules for cardiomyocyte differentiation. Although both ADSCs and BMSCs improved LV function, only ADSCs significantly improved LV remodelling as evidenced by an increase in the LV WT ratio and capillary density. Our data are in line with the report from Moelker et al. that bone marrow-derived cells do not reverse remodelling. Although there is a statistical difference in effects on LV remodelling between the cell groups, this finding needs to be confirmed using a large number of animals in future studies.

Clinical studies such as the TOPCARE and BOOST trials infused cells within 4–5 days after MI. However, it has also been shown that myocardial interstitial oedema after recanalization of the infarct-related artery persists for at least a week, and the facts that progressive increase of microvascular obstruction within the first 48 h after reperfusion and limited homing after 5 days might favour early cell transfer. Freyman et al. injected bone marrow-derived MSCs 15 min after infarction. They found that the 14-day retention for intracoronary infusion and endocardial injection represented 6 and 3% of the administered dose, respectively. In the intravenous infusion group, none of the infarcts contained a measurable number of cells. As the aim of that study was to compare quantitatively the three most common MSC delivery approaches following infarction in a large animal model with cellular engraftment as the endpoint, they did not report any functional analysis after stem cell administration. Along this line, the present study was primarily designed for efficacy. Furthermore, several groups have suggested that cardiac recruitment of stem cells requires both myocardial injury and expression of stromal-derived factor 1 (sdf-1). Sdf-1 upregulation occurs immediately following myocardial injury. In addition, we were concerned that the capillary bed may become less permeable to injected cells several days after infarction. On the basis of these previous studies, we chose to deliver the cells immediately after infarct revascularization, as this approach might also represent a clinically relevant scenario.

Mimicking the clinical scenario was an important part of this study design, which included the use of an anticoagulation, anti-thrombotic regimen, and accompanying medication (beta-blocker) currently used as state-of-the-art treatment of acute MI patients undergoing percutaneous revascularization. Beta-blocker was not used in other studies of intracoronary delivery of stem cells in experimental animals, which reported the lack of benefit of umbilical cord blood stem cells or BMSC on LV function. The functional improvement observed in our study is likely the result of a synergistic interaction of cells and β-blocker therapy.

There are reports that intracoronary cell injections can cause micro-infarctions when a large number of cells were administered. We compared our study with that reported by Vulliet et al. and Moelker et al. As shown in Table 2, the mean cell size is comparable among the three studies (~20 μm), although the cell number varied considerably. The cell number that we used was much less than that reported by Vulliet et al. and Moelker et al. The particular cell number that we had chosen was based on our pilot study, which showed that the maximal number of cells that could be safely administered was found to be 10 millions. We observed no-reflow phenomenon when more than 10 million cells were administered. Our findings are consistent with Vulliet et al. and Moelker et al. that larger number of cells cause microinfarction. In addition, we used an intensified antithrombotic drug regimen. Vulliet et al. did not report the use of any anticoagulation or antiplatelet therapy. The reported anticoagulation regimen by Moelker et al. was restricted to heparin. These variations might also explain the reported micro-infarction when large numbers of cells were administered.

**Conclusion**

After intracoronary administration into acutely infarcted myocardium, ADSCs transdifferentiate into endothelial and vascular smooth muscle cells and improve LV function, remodelling, and over time, perfusion in a porcine MI model that closely reproduces the current clinical management of the human disease. In this experimental MI model, ADSCs and BMSCs exert similar beneficial effects on LV function but not on LV remodelling. The beneficial effects of ADSCs on LV function and remodelling documented in this experimental large animal model warrant further investigation of the therapeutic usefulness of intracoronary injection of ADSC at the time of revascularization in patients with acute MI.
Limitations
The optimal time point for MSC delivery is still controversial. Although clinical studies injected cells 4–5 days after MI, pre-clinical studies on large animals injected cells immediately after MI. Ideally, this issue will be investigated thoroughly in large animals prior to clinical trials. Future studies designed to compare the beneficial effect at various time points are certainly warranted. Furthermore, the numbers of injected cell reported in the literature varies greatly; the optimal cell number, the choice of pre-cultured vs. freshly isolated cells also warrants further investigation.

The current study is a first feasibility, safety, and efficacy assessment and comparison of the use of non-bone marrow cells in a clinically relevant model of infarction. This proof of concept is an important step for further studies. We acknowledge, however, that harvesting and culturing of the ADSCs reflect only limited clinical merits as the patients with infarction typically will not report for stem cells harvest 2 weeks prior to infarction, unless someone is willing to freeze their cells. However, such an approach would be compromised by considerable logistical limitations.

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Conflict of interest: K.P. is currently an employee of Cytori Therapeutics. R.J.C. is on Bristol-Myers Squibb speakers’ bureau, for which he receives honoraria in conjunction with presentations that involve Tc-99m Septambibi.

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