Rebuilding a Damaged Heart
Long-Term Survival of Transplanted Neonatal Rat Cardiomyocytes After Myocardial Infarction and Effect on Cardiac Function

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Background—The long-term effects of cardiac cell transplantation on cardiac function are unknown. Therefore, we tested the survival and functional impact of rat neonatal cardiac myocytes up to 6 months after transplantation into infarcted hearts.

Methods and Results—Cardiomyocytes from male neonatal Fischer 344 rats (1 to 2 days, 3 to 5×10⁶) or medium was injected into the infarcts of adult syngeneic female animals 1 week after left coronary artery ligation. Six months later, implanted cardiomyocytes were still present by quantitative TaqMan polymerase chain reaction and histology. In all treated hearts, discrete lumps of cells were present within the infarct scar, which was not observed in media-injected hearts typified by a transmural infarct scar. Infarct thickness was greater in treated animals versus control animals (909±97 versus 619±43 μm, P<0.02), whereas infarct size and left ventricular volumes were similar. By biplane angiography, left ventricular ejection fractions at 6 months were greater (0.36±0.03 versus 0.25±0.02, P<0.01) and significantly less infarct zone dyskinesis was seen (0.30±0.08 versus 0.55±0.07, P=0.035, lateral projection) in treated animals versus control animals.

Conclusions—Grafted neonatal cardiomyocytes were present in infarcts 6 months after transplantation; they thickened the wall of the left ventricle and were associated with enhanced ejection fraction and reduced paradoxical systolic bulging of the infarct. Therefore, neonatal cardiac cell transplants exhibit long-term survival in a myocardial infarct model and contribute to long-term improved cardiac function. These results suggest that a damaged heart can be rebuilt.

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Key Words: ventricles ■ cells ■ transplantation ■ angiography ■ myocardial infarction

Cardiac tissue is terminally differentiated, and myocardial cells undergo only rare mitoses. Accordingly, regeneration of myocardium after injury is limited.1 Cell transplantation is a new strategy for provision of surrogate cardiomyocytes and for treatment of multiple diseases including myocardial infarction and heart failure. Although some encouraging data have been reported, little is known about the longer-term survival of transplanted cells and their ultimate effect on global and regional left ventricular (LV) function in vivo.

In this study, we used invasive hemodynamics and LV angiography to evaluate rats with myocardial infarcts up to 6 months after the transplantation of neonatal cardiomyocytes or the injection of culture media alone. In addition, LV volumes, infarct sizes, and scar thickness were measured. Cell survival was determined by TaqMan polymerase chain reaction (PCR) (Y chromosome) and histology.

Methods

Cell Isolation and Purification
Cardiomyocytes were isolated 1 to 2 days after birth from Fischer 344 rats.2 Sex was determined by assessing the genito-anal distance, and male cells were selected. Isolated cells (1×10⁶ per heart) were resuspended in MEM containing 5% bovine calf serum, penicillin (100 U/mL), streptomycin (100 μg/mL), and amphotericin B (0.25 μg/mL). Cells were purified by preplating (30 minutes, 37°C) as previously described,2,3 resulting in a high percentage of cardiomyocytes.

Recipient Animals and Myocardial Infarction
Myocardial infarction was induced in female syngeneic adult rats (2 to 3 months old) by ligation of the left coronary artery under ketamine and xylazine anesthesia.4 Rats that survived to 24 hours after occlusion were allowed to recover for 1 week, before they were randomized to receive either injection of cells (3 to 5×10⁶/50 μL) or medium (50 μL).
Cardiomyocyte Transplantation

After overnight incubation (37°C, 95% O₂, 5% CO₂), cells were trypsinized (0.25% Trypsin/1 mmol/L EDTA), pelleted, and resuspended in serum-free MEM. The recipient animals were reanesthetized, and the chest was opened through an intercostal incision. Cells or medium were injected into the center of the infarct area with a 26-gauge needle bent at an angle of 45°, 3 mm from the tip. This configuration was designed to facilitate injection into the infarct. The range in numbers of cells injected was constrained by neonatal tissue availability.

In Vivo Hemodynamics, Ventriculography, Ejection Fraction, and Segmental Wall Motion Analysis

Hemodynamic and LV angiographic measurements were done 6 months after cell transplantation. Rats were anesthetized as described above, intubated, and ventilated. The right carotid artery was cannulated by a 2F high-fidelity, catheter-tipped micromanometer (model SPR-407, Millar, Inc) and after advancement into the thorax was used to record ascending aortic and LV pressures. Pressure pulses were then digitized and processed. For contrast angiography, 250 µL of nonionic contrast was injected into the jugular vein over a period of 1 to 2 seconds, and video images were acquired on half-inch super-VHS videotape at 30 frames per second under constant fluoroscopy with the XiScan 1000 C-arm x-ray system (XiTec, Inc; 3-inch field of view, anterior-posterior and lateral projections). Later, the interlaced video images were edited and digitally processed off-line (Silicon Graphics R10000 system, Motif 6.5 operating system) with a resolution matrix of 512×512 pixels, 256 shades of gray, 60 fields per second. LV volumes were calculated by means of both the biplane area-length and modified Simpson’s rule methods. Segmental wall motion was examined in anteroposterior and in right lateral views by determining 100 chords around the LV chamber perimeter (Sheehan centerline method) and by sequential decile averaging starting at the free wall end of the mitral annulus and proceeding clockwise to the opposite end of the mitral annulus. The myocardial infarct zone in vivo, as a fraction of the LV perimeter, was estimated angiographically for each animal by sequentially interrogating the centerline chords from 1 to 100; the first and last chords with a fractional shortening of 0.005 were taken as the beginning and end of the infarct zone, respectively. All functional parameters derived from hemodynamic measurements and LV angiograms were analyzed in a blinded manner.

LV Volumes, Infarct Sizes, and Wall Thickness of Explanted Hearts

Left ventricular volumes, infarct sizes, and wall thickness of the explanted hearts were measured as described previously. The LV was pressure-fixed in formalin, and volume was assessed by filling the cavity with water.

Figure 1. A through H, Low-power view of transmural slices of left ventricles stained with hematoxylin and eosin (left column) or picrosirius red (right column). Muscle appears pink in hematoxylin and eosin-stained slides. Muscle appears yellow and collagen red in picrosirius red-stained slides. A and B are from a control heart that received medium injection. Note thin free wall of left ventricle composed primarily of collagen. C and D are from a cell transplant heart. Note two discrete lumps of myocardial cells (c) within the scar that increase wall thickness at the site of the cells. E and F are from a control heart that received medium. Again, note that the free left ventricular wall is thin and composed of collagen. G and H are from a heart that received cell injections into the scar. Note discrete lumps of cells (c) within the scar. Note improved wall thickness.

Figure 2. Staining as per Figure 1. A and B are sections from a control heart. Note thin LV free wall composed of collagen. C and D are from a rat that received cell implantation. Note discrete lump of cells (c) over section of the scar in the bottom half of the photomicrograph.
The hearts were cut parallel to the atrioventricular groove and into 4 slices. Tissue slices were processed for paraffin embedding, sectioned (5-μm thickness), and stained with hematoxylin and eosin. Projected histological images of 2 slices from the center of the infarct were traced, and the circumferential length occupied by noninfarcted muscle and infarct was measured. Infarct size was calculated as the percentage of the LV circumference occupied by infarct in both slices, and the average was determined for each heart. Rats with a myocardial infarct size <15% of the LV circumference were excluded.

Wall thickness was measured from histological sections in the center of each infarct, at the periphery of the infarct (right and left lateral), and in between (right and left mid-lateral), with the use of a calibrated eyepiece reticle and a ×20 objective lens.

Analysis of Cell Survival
The survival of grafted male cells was analyzed by real-time fluorescence TaqMan PCR 3 months and 6 months after cell transplantation.3,7 Tissue from hearts that had been injected with medium only was used as control for specificity of PCR. After the animals were killed, hearts were removed and DNA was isolated.8 The DNA was used as a template for quantitative PCR of the Y-chromosomal Sry gene as an indicator of surviving male cells.3,9 Previously, we have shown that this method is highly sensitive and specific for the detection of viable transplanted cells. Sequences and primers were used, and the number of surviving male cells were calculated as described previously.3

Statistics
Data are presented as mean±SEM. Comparison between groups were made by means of ANOVA, followed by pairwise comparison by Tukey’s test or t tests where applicable. Values were considered to differ at a value of *P*<0.05.

Results
Five animals died within 24 hours of surgery; all others survived without overt heart failure symptoms until they were killed. At 6 months, hemodynamic and LV angiographic analyses were done in 18 rats, histological analysis in 19, and LV volume measurements in 18. PCR was performed in 11 animals at 6 months and 2 at 3 months.

LV Volumes, Infarct Sizes, Wall Thickness, and Histology
All hearts included in the analysis showed large areas of LV scar. Postmortem measurement of LV volume was similar in

Figure 3. A, Appearance of neonatal cell implant (hematoxylin and eosin, ×4 objective) within 24 hours of injection into infarcted rat that died. Plane of myocardial infarct scar is disrupted, and cells occupy central portion of the scar, resulting in what appears to be a thickened LV wall. Endocardium is toward the top and epicardium is toward the bottom of the figure. B, Higher-power view of cells within the first 24 hours of transplantation shows a round, immature appearance and large nuclei (original magnification ×40). C, Polarized light microscopy of picrosirius red–stained section from the same infarct as in A. Note collagen of scar (yellow-green birefringence) along endocardial and epicardial surfaces of ventricle. Neonatal cell implants are devoid of collagen and appear as dark nonbirefringent zones in the center of the ventricular wall. Infarct scar has been bulked up by cell implants (original magnification ×4). D, Infarct scar at 6 months of 2 untreated (media only) rats. In both cases, infarcts are composed of collagen and are transmural, thin, and devoid of myocytes. Visceral pericardium appears thickened in bottom panel of D (hematoxylin and eosin, ×4 objective). E, Transplanted neonatal cardiomyocytes survived in the infarct and form a bulky muscle patch (arrowhead) within the 6-month-old infarct scar. Cell implant is a discrete lump of cells found in the midmyocardium and subepicardium of the scar. We did not observe similar structures in media-treated hearts (hematoxylin and eosin, ×4 objective). F, Magnification of transplant from E. Implanted cells formed a discrete lump of cells. There is some degree of myofiber disarray (hematoxylin and eosin, ×4 objective).
the transplanted (0.42±0.02 mL) and control rats (0.45±0.03 mL, both n=9). By contrast, LV cavity volume in noninfarcted rats was 0.23±0.03 mL. By histological analysis, hearts injected with medium only showed thin-walled collagenous transmural scars devoid of lumps of viable cells on the midmyocardial or epicardial surface (see Figure 1, A, B, E, and F; Figure 2, A and B; and Figure 3D). In contrast, hearts that received cell injections were easily distinguished by the bulk of transplanted cells visible within the scar (see Figure 1, C, D, G, and H; Figure 2, C and D; Figure 3, A, B, C, E, and F; and Figure 4). Accordingly, wall thickness was increased in the cell transplantation group. Whereas the central region of the infarct was only slightly thicker in cell-treated versus medium-injected rats (430±62 versus 305±24 μm, P=0.09), both the thickest region (909±97 versus 619±43 μm, P<0.02) and the mid-lateral regions (531±70 versus 324±24 μm, P<0.02) of the infarcts were significantly thicker in transplanted rats (n=10 treated, n=9 control, Figure 5). The LV circumference (2.39±0.04 cm versus 2.38±0.05 cm), scar length (0.75±0.05 cm versus 0.82±0.08 cm), muscle length (1.64±0.05 cm versus 1.56±0.05 cm), and infarct size (31.5±1.7% versus 34.2±2.8%) were similar in both groups (n=10 transplanted, n=9 control animals). By histology, the cell implants appeared as large confluent clusters of myocar-
dial cells in the scar. Their size was smaller and their nuclei rounder than adult myocardial cells, and in all cases they were less organized than adult myocardial cells of the recipients. These cells typically had striations throughout, lacked large perinuclear spaces, and did not exhibit myocytolysis (Figure 4). Immunostaining for connexin43 was found frequently at junctions between the grafted cells but not between graft cells and host myocardium, which were separated by scar collagen.

Survival of Transplanted Cells
By TaqMan PCR analysis, the Y chromosomal Sry gene was identified in 3- and 6-month-old infarcts. After 3 months, 1.9×10⁶ cells (survival rate 62%, n=1) carried the Y-chromosomal Sry gene DNA within the infarct, and after 6 months, between 1.6×10⁶ and 3.3×10⁶ cells (mean 2.5×10⁶±3.0×10⁵, survival rate 62±11%, n=6) were Y-chromosome positive (3 to 5×10⁶ injected). Few male cells (701±2181, n=5) were detected when remote cardiac tissue (from right ventricle and atria) was analyzed. The background signals with DNA from animals injected with medium corresponded to 3150 cells (3 months, n=1) and 244±167 cells at 6 months (n=5), demonstrating the high specificity of this technique.

**Hemodynamics, LV Angiography, and Regional Wall Motion**
Hemodynamic parameters and LV angiographic parameters were measured at 6 months after cell implantation. Heart rate tended to be lower in the treated group; LV pressures, peak positive dP/dt, and peak negative dP/dt were similar in both groups (Table 1).

By LV angiography, LV ejection fraction was significantly higher in cell-treated animals irrespective of the calculation method (area-length or Simpson’s rule). There were trends for smaller LV end-systolic volume and larger stroke volume in the cell transplantation group (Table 1). The angiography

**Table 1. LV Angiogram and Hemodynamics 6 Months After Cell Transplantation or Medium Injection**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=9)</th>
<th>Cell Transplantation (n=9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF area-length</td>
<td>0.25±0.02</td>
<td>0.36±0.03</td>
<td>0.009</td>
</tr>
<tr>
<td>EF Simpson</td>
<td>0.25±0.2</td>
<td>0.35±0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>ED volume, μL</td>
<td>286.0±26.6</td>
<td>268.7±20.4</td>
<td>0.6</td>
</tr>
<tr>
<td>ES volume, μL</td>
<td>214.8±20.5</td>
<td>172.0±18.4</td>
<td>0.14</td>
</tr>
<tr>
<td>SV, μL</td>
<td>71.1±8.5</td>
<td>96.6±11.4</td>
<td>0.09</td>
</tr>
<tr>
<td>Ejection time, ms</td>
<td>129.6±8.7</td>
<td>135.3±7.0</td>
<td>0.61</td>
</tr>
<tr>
<td>HR</td>
<td>237.3±5.0</td>
<td>216.5±11.1</td>
<td>0.096</td>
</tr>
<tr>
<td>MaxP, mm Hg</td>
<td>110.8±7.5</td>
<td>105.0±3.9</td>
<td>0.52</td>
</tr>
<tr>
<td>EDP, mm Hg</td>
<td>9.4±1.6</td>
<td>11.5±2.4</td>
<td>0.46</td>
</tr>
<tr>
<td>+dP/dt, mm Hg/s</td>
<td>6319.7±554.7</td>
<td>6319.4±426.1</td>
<td>0.9997</td>
</tr>
<tr>
<td>−dP/dt, mm Hg/s</td>
<td>3995.6±243.9</td>
<td>3755.5±231.0</td>
<td>0.49</td>
</tr>
<tr>
<td>τ</td>
<td>25.7±2.2</td>
<td>27.8±3.7</td>
<td>0.62</td>
</tr>
</tbody>
</table>

EF indicates ejection fraction; ED, end-diastolic; ES, end-systolic; SV, stroke volume; HR, heart rate; MaxP, maximal LV pressure; EDP, end-diastolic pressure; +dP/dt, maximal rate of rise in LV pressure; −dP/dt, maximal rate of decay in LV pressure; and τ, monoexponential rate constant for pressure decay during period of isovolumic relaxation.
cally determined myocardial infarct zone, as a fraction of the perimeter of the left ventricle, was similar in both groups for both anteroposterior and lateral projections (Figure 6A and Table 2).

Regional wall motion within this myocardial infarct zone differed between the two groups. Cell-treated animals manifested a small but significant improvement in average chordal shortening and a marked reduction in paradoxical systolic expansion (dyskinesis) of the anterior wall and apex (Figure 6, B and C; and Table 2). These differences were most striking on the lateral projection of the LV angiogram, where the apical movement was seen in profile.

**Discussion**

The major findings of this study were that (1) neonatal cardiac cells implanted 1 week after myocardial infarction in the rat demonstrated long-term 6-month survival, documented by quantitative TaqMan PCR and histology; (2) discrete clusters of cells in the wall of the scar thickened the infarcted ventricular wall; (3) there was less dyskinesis in the infarcted zones that received cells; and (4) ejection fraction assessed by LV angiography was improved. For the first time, the long-term effect of neonatal cardiac cells transplanted into an infarct was examined, resulting in improvements of both global and regional in vivo cardiac function.
TABLE 2. LV Angiogram Regional Wall Motion Analysis 6 Months After Cell Transplantation or Medium Injection

<table>
<thead>
<tr>
<th></th>
<th>Control (Media) (n=9)</th>
<th>Cell Transplantation (n=9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI zone A-P (fraction of perimeter)</td>
<td>0.420±0.038</td>
<td>0.393±0.030</td>
<td>0.588</td>
</tr>
<tr>
<td>MI zone lateral (fraction of perimeter)</td>
<td>0.440±0.038</td>
<td>0.414±0.036</td>
<td>0.633</td>
</tr>
<tr>
<td>Dyskinesis—A-P (fraction of MI zone)</td>
<td>0.400±0.085</td>
<td>0.372±0.083</td>
<td>0.816</td>
</tr>
<tr>
<td>Dyskinesis—lateral (fraction of MI zone)</td>
<td>0.5512±0.073</td>
<td>0.295±0.063</td>
<td>0.035</td>
</tr>
<tr>
<td>Dyskinesis—A-P (fraction of perimeter)</td>
<td>0.177±0.041</td>
<td>0.140±0.026</td>
<td>0.460</td>
</tr>
<tr>
<td>Dyskinesis—lateral (fraction of perimeter)</td>
<td>0.244±0.039</td>
<td>0.111±0.033</td>
<td>0.018</td>
</tr>
<tr>
<td>Average chordal shortening: MI zone-A-P</td>
<td>0.0003±0.001</td>
<td>0.002±0.002</td>
<td>0.533</td>
</tr>
<tr>
<td>Average chordal shortening: MI zone-lateral</td>
<td>−0.002±0.001</td>
<td>0.002±0.001</td>
<td>0.020</td>
</tr>
</tbody>
</table>

A-P indicates anterior-posterior projection of LV angiogram; lateral, lateral projection of LV angiogram; and dyskinesis, paradoxical systolic movement of endocardium. MI zone: See text.

Effect of Cells on Cardiac Function

By thickening the LV wall, the degree of dyskinesia was reduced on LV angiography. The cells prevented the outward motion of the infarct that occurs during systole (paradoxic systolic bulging). In control animals, this paradoxical systolic bulging was most pronounced in the apical region, which is typical of this pathological process. In contrast, in treated hearts, the systolic endocardial contour remained inside the diastolic contour, reflecting the attenuation of systolic bulging by transplanted cells. As a consequence, there was a trend toward lower end-systolic volume and larger stroke volume in the treated group even though end-diastolic volumes were the same in both groups. Chordal shortening in the infarct zone was improved in treated hearts, and some degree of direct contraction of the transplanted cells may have contributed to systolic function. In support of this notion is the fact that the morphology of these cells, although somewhat different from remote host myocytes (thinner with rounder nuclei), was very different from that described for noncontracting, hibernating myocardium (myofilaments limited to the periphery and large pools of mitochondria and glycogen surrounding the nucleus). The physiology underlying the improved systolic function remains speculative because histology did not show any cell-cell contacts between graft and host myocytes. Potential mechanisms may be (1) that some rare gap junctions may have triggered contraction of the grafted cells, although they were missed by histology; (2) that stretch may have triggered the contraction of grafted cells; (3) that grafted cells may have contracted independently from host myocardium; or (4) that grafted cells did not actively contribute to contractility, and the effect seen on ejection fraction and chordal shortening was a passive effect from scar stiffening.

Cell implantation did not significantly affect end-diastolic LV volumes, postmortem volumes, LV circumference, infarct length or length of noninfarcted tissue by histology, or perimeter of infarct zone by angiography. Therefore, the cells did not significantly influence LV infarct expansion or global LV remodeling, although wall stress within the infarct region probably was reduced by increased scar thickness. It is likely that the primary benefit from transplanted cells was thickening of the wall, preventing dyskinesia, and that a secondary factor was direct contribution to systolic contraction.

Recent experimental and clinical trials have shown early promise for cell transplantation into damaged hearts. Skeletal muscle cells, bone marrow stem cells, and fetal cells have all resulted in potential improvements of LV function. However, most studies to date have analyzed ventricular function after a relatively short period of time. We chose neonatal cardiomyocytes for transplantation because they are most differentiated toward adult myocytes while still resilient in a hypoxic environment and assessed longer-term function. Six months after implantation into infarcts, neonatal cardiomyocytes were viable in large numbers, thickened the scars, prevented LV dyskinesia, and improved global ejection fraction assessed by angiography. These results suggest that a damaged heart can be rebuilt.

Cell Survival

In a previous study, we observed that 15% of neonatal cardiomyocytes survived for at least 12 weeks when transplanted into healthy rat myocardium. The observed cell loss may have been due to physical strain during and after injection, hypoxia, or cell wash-out through the vasculature or lymphatics. In the present study, based on quantitative TaqMan PCR analysis, we estimated that ~60% of injected neonatal cardiac myocytes would have survived. However, the actual number will be lower because >10% of the injected cells were noncardiomyocytes such as fibroblasts or endothelial cells, which may be more resistant to the physical strain of injection and hypoxia and may also have proliferated. The PCR analysis does not allow distinction between cell types. Still, by histology, it was clearly observed that adequate numbers of myocytes were injected and survived to “bulk-up” the thin-walled scar of the infarct. This is supported by our previous study, in which fetal cardiomyocytes were identified by immunostaining (α-actin) 65 days after being grafted into infarcted rat hearts. Apparently, cell transplantation was more efficient in the present study than in our previous study, when cells were injected into healthy rat hearts. In infarcted hearts, cellular transplantation may have been aided by injection of the cells into healing scar because it may have been more difficult for the injected cells to escape beyond the fibrous capsule through vascular or lymphatic channels. It is also possible that lack of active contraction within the infarct zone prevented implanted cells from being killed or squeezed out of the region. Growth factors, released within or around the injured myocardium, may have contributed to graft survival or proliferation, although this theory remains speculative.
Acknowledgments
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References
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