Bone marrow-derived stromal cells home to and remain in the infarcted rat heart but fail to improve function: an in vivo cine-MRI study

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PIONEERING BASIC STUDIES conducted nearly 10 years ago indicated that murine c-kit+ Lin− bone marrow cells, injected into the heart of animals postinfarction, differentiate into new cardiomyocytes, smooth muscle, and endothelial cells, induce neoangiogenesis, and restore contractile function (38). The striking findings of these early experiments, and the possibility of autologous cell therapy, resulted in the rapid translation of stem cell therapy to the clinic. To date, over 25 clinical trials of autologous cell therapy for the heart, in which stem cells were isolated from the patients’ own blood or bone marrow and subsequently injected into the myocardium or infused via the coronary vessels, have been conducted. Although initial results were promising (6, 14, 58), later randomized clinical trials for acute myocardial infarction, with short-term follow-up, reported only a small (2% to 3%) improvement in left ventricular ejection fractions (EFs) (22, 26, 31, 44). Thus the current methods of cell therapy adopted in the clinic have led to, at best, modest benefits that are likely to result from a paracrine action of these injected cells (42, 55).

Further basic research is needed to explain why the substantial improvements in cardiac function observed in animal models did not translate to improved EFs in many clinical trials. Basic studies have focused on cell homing, engraftment, and survival (35, 38), differentiation (34, 38), neovascularogenesis (23, 35), and/or paracrine (55) mechanisms. However, although the end point of most clinical trials has been an improvement in cardiac function determined using noninvasive imaging modalities, particularly magnetic resonance (MR) imaging (MRI) (14, 22, 26, 31, 58), functional measurements in small animals have been made using M-mode and two-dimensional echocardiography (35, 37, 55, 61). These measurements are less accurate than cine-MRI when applied to asymmetric, infarcted rat and mouse hearts (11, 18, 48). A few basic studies have used MRI to measure mouse heart function (16, 25), but detailed and serial MRI measurements have not been made in rats after infarction and cell therapy. Furthermore, MRI can be used for in vivo tracking of iron-labeled stem cells, permitting injection success and cell retention to be assessed within the live animal (49).

The aims of this study were to use MRI to follow the fate of grafted bone marrow stromal cells (BMSCs) in vivo using micron-sized particles of iron oxide (MPIO) labeling of donor cells and to measure cardiac function repeatedly and noninvasively after infarction in rats that received either a direct injection of BMSCs or underwent intravenous BMSC infusions.

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MATERIALS AND METHODS

Animals

Wistar rats (Harlan UK) were allowed free access to standard rodent chow and water throughout the study. The University of Oxford Animal Ethics Review Committees and the Home Office (London, UK) approved all procedures.

Rat BMSC Isolation, Culture, Characterization, Differentiation, and Labeling

BMSCs were isolated from the tibias and femurs of 8-wk-old male Wistar rats (Harlan UK) and cultured to passage 2 in DMEM containing 10% fetal calf serum and 7% horse serum as described (2). An analysis of BMSC cell surface markers and proliferation was performed in triplicate using flow cytometry. The ability of rat BMSCs to differentiate into adipocytes, osteoblasts, and chondrocytes was tested by plating into differentiation media according to the method of Tholpady et al. (51) and the manufacturer’s instructions (Cambrex Biosciences, Karlskoga, Sweden) and compared with culture in nondifferentiating media. Human p3 BMSCs were used as a positive control. BMSCs were transduced with a green fluorescent protein (GFP) lentiviral vector (a gift from Adrian Thrasher, Institute of Child Health, London, UK) as described (30).

For donor cell reisolation experiments, BMSCs were incubated with chloromethyl-benzamidodialkylcarbocyanine (CM-DiI) cell-tracker dye (Invitrogen, Paisley, UK) for 1 h. For iron labeling, rat BMSCs were incubated overnight with MPIO (2 μl/cm², encapsulated magnetic microspheres; Bangs, Fishers, IN) on the day before transplantation (49).

Rat Myocardial Infarction and BMSC Administration

The left anterior descending (LAD) coronary artery of female Wistar rats (200–250 g; n = 90) was occluded ~2 mm from its origin as described previously (49). After surgery, rats were assigned to one of seven groups: 1) sham-operated rats (n = 6); 2) intramuscular saline, four direct intramyocardial injections of 10 μl saline into the peri-infarct region, given 10 min after coronary artery ligation (n = 10); 3) intramuscular BMSCs, four direct 10-μl injections of a total of 0.5–2 × 10⁶ GFP-BMSCs (not labeled with MPIO) into the peri-infarct region, given 10 min after coronary artery ligation (n = 7); 4) intramuscular MPIO-labeled BMSCs (Fe-BMSCs), four direct 10-μl injections of a total of 0.5–2 × 10⁶ GFP-BMSCs labeled with MPIO into the peri-infarct region, given 10 min after coronary artery ligation (n = 17); 5) intravenous saline, intravenous tail vein infusion of 1 ml saline given 1 day after coronary artery ligation (n = 8); 6) intravenous Fe-BMSCs at 1 day, tail vein infusion of 4 × 10⁶ GFP-BMSCs in 1 ml saline given 1 day after coronary artery ligation (n = 18); and 7) intravenous Fe-BMSCs at 3 days, tail vein infusion of 4 × 10⁶ GFP-BMSCs in 1 ml saline given 3 days after coronary artery ligation (n = 4). Animals were euthanized after the final MR images were acquired, and hearts were isolated and frozen or fixed in 4% (wt/vol) paraformaldehyde (Sigma UK) in phosphate-buffered saline (pH 7.2).

Cardiac Cine-MRI

Cardiac cine-MRI was performed as described (54). Briefly, rats were anesthetized with 2.5% isoflurane in O₂ and positioned supine in a purpose-built cradle and lowered into a vertical bore 500 MHz, 11.7 T MR system with a Bruker console running Paravision 2.1.1 and with a 60-mm birdcage coil. A stack of eight to nine contiguous 1.5-mm true short axis ECG and respiration-gated cine images [field of view, 51.2 mm²; matrix size, 256 × 256 zero filled to 512 × 512 giving a voxel size of 100 × 100 × 1500 μm; and echo time/repetition time (TE/TR) 1.43/46.4 ms, 17.5° pulse, 25–35 frames/cardiac cycle] was acquired to cover the entire left ventricle. The end-diastolic and end-systolic volumes were measured for each slice using Scion Image (Scion, Frederick, MD) and summed over the whole heart. Stroke volume was calculated as end-diastolic volume minus end-systolic volume. The EF was calculated as the stroke volume divided by the end-diastolic volume. The akinetic region of the myocardium was calculated as the sum of the endocardial and epicardial circumferential lengths of the thinned, akinetic region of all slices, measured at diastole, and divided by the sum of the total endocardial and epicardial circumferences of all slices (36). Long-axis two- and four-chamber images were also acquired. The imaging protocol was performed in ~40 min.

High-Resolution Three-dimensional MR Microscopy

Parafomaldehyde-fixed hearts (intramuscular injection of Fe-BMSCs, n = 9; and 1 day iv infusion of Fe-BMSCs, n = 5) were embedded in 1% (wt/vol) agarose doped with gadolinium diethylene-triamine pentaacetic acid in a 20 mm NMR tube. High-resolution MRI was performed in a 20 mm quadrature-driven birdcage coil (Rapid Biomedical, Würzburg, Germany) using a fast gradient echo sequence (TE/TR = 1.8/30 ms; flip angle, 90°; field of view, 20 × 20 × 20 mm; matrix size, 256 × 256 × 512; voxel size, 78 × 78 × 39 μm; and number of averages, 4).

Immunohistochemical Analysis

Parafomaldehyde (4% wt/vol)-fixed whole hearts were embedded in parafin, and serial 5-μm sections were cut. Cells expressing GFP were detected using a monoclonal mouse anti-GFP antibody, clone GEP-20 (Sigma). Immunoreactive cells were visualized using the EnVision+ System-HRP (diaminobenzidine) kit (Dakopatts) according to the manufacturer’s instructions, and sections were counterstained with hematoxylin. Hearts, taken 10 wk after sham operation (n = 3), intramuscular saline (n = 3), or intramuscular Fe-BMSC (n = 3) injection, were cryosliced into serial 10-μm sections and stained for alkaline phosphatase using 5-bromo-4-chloro-3-indolyl phosphate and nitroblue-tetrazolium (BCIP/NBT kit; Vector). Capillaries were counted in the peri-infarct area, using a light microscope, at ×40 magnification. The results from five high-power fields were averaged and expressed as the number of capillary vessels per squared millimeter.

Recovery and Quantification of Iron-Containing Cells

In a separate set of animals, 6 wk after intramuscular injection (n = 6) or intravenous infusion (n = 3) of 4 × 10⁶ CM-DiI-labeled Fe-BMSCs, hearts were digested using collagenase, and the MPIO-labeled cells were isolated and concentrated from the digest material using an electromagnet (49).

Statistical Analysis

Results are presented as means ± SE. Differences were considered significant at P < 0.05, determined using ANOVA with repeated measures. A supplemental Materials and Methods section can be found with the online version of this article.

RESULTS

Rat BMSC Characterization

The cell surface marker profile in adherent rat BMSCs is given in Fig. 1A. Over 95% of the cultured rat BMSCs were positive for CD90 and negative for hematopoietic and/or endothelial markers, CD4, CD11b/c, CD31, CD45, CD45R, and CD49d.

Viability of MPIO-labeled Rat BMSCs

More than 95% of the BMSCs endocytosed, on average, 60 MPIOs during overnight incubation (Fig. 1B). Fe-BMSCs had similar rates of proliferation (Fig. 1C) and underwent in vitro
adipogenic and osteogenic differentiation as efficiently as unlabeled cells (Fig. 1D). Rat Fe-BMSCs and the human control BMSCs showed similar potential to differentiate into adipocytes and osteoblasts with 70–90% of cells staining positive for Oil Red O after an induction of adipogenic differentiation and 50–80% of cells staining positive for alkaline phosphatase after an induction of osteogenic differentiation. No evidence of chondrogenesis was detected in the rat BMSCs, with or without MPIO labeling, as has been found by others (20).

MRI Tracking of Fe-BMSCs

After the administration of Fe-BMSCs by intramuscular injection or intravenous infusion, regions of signal void were detected on MRI. A detailed comparison of signal void regions between labeled and unlabeled BMSCs was presented in Figure 1C, showing a significant increase in signal void over time for MPIO-labeled cells compared to unlabeled controls. The results suggest that MPIO-labeled BMSCs can be tracked in vivo using MRI, allowing for precise monitoring of cell distribution and proliferation.
detected in the in vivo cine-MRI images at all times (Fig. 2). These observations were confirmed at the end of the study using ex vivo MR microscopy. When Fe-BMSCs were administered by direct injection into the myocardium, the majority remained in the vicinity of the injection site, although some cells moved to the scar area (Fig. 2, A and B). In animals that received intravenous infusion, Fe-BMSCs were observed throughout the thinned infarct scar and were not detected in viable myocardium (Fig. 2, C and D).

Immunohistochemistry and Recovery of Grafted Cells

With the use of signal voids in MR images to identify cell location within tissue sections, immunohistochemistry revealed 1,063 ± 119 GFP-labeled cells/heart (0.1%; n = 6) in hearts treated with Fe-BMSCs 10 wk earlier. No GFP-labeled cells were detected in saline-treated hearts or in regions of the heart distant from the hypointensities (Fig. 3) (49).

A subgroup of CM-Dil-Fe-BMSC-treated infarcted hearts (n = 9) underwent collagenase digestion, and MPIO-labeled cells were isolated and concentrated from the digest material using an electromagnet. CM-Dil-labeled cells were clearly visible in the digest material and had morphology similar to that of the BMSCs before implantation (Fig. 3). The undigested scar region was densely packed with CM-Dil-labeled cells, confirming the MR images, which indicated Fe-BMSCs within the infarcted myocardium. An estimation of cell numbers in the digest material showed that −0.1% of administered cells remained in the heart 6 wk after infusion.

To assess capillary density, alkaline phosphatase staining was performed on cryosections taken from hearts 10 wk after infarction. No significant difference in the number of capillaries within the peri-infarct region was observed between infarcted hearts treated with BMSCs and those injected with saline (BMSCs = 1,819 ± 57/mm²; and control = 1,757 ± 73/mm²; n = 3 per group; Fig. 3).

MRI Measurement of Cardiac Morphology and Function

After direct injection of BMSCs into the infarct periphery. Cine-MRI was used to measure cardiac morphology and function at 1, 4, and 10 wk after myocardial infarction and the direct injection of BMSCs. Infarction resulted in a 33–35% decrease in the left ventricular EF, a 44–56% increase in end-diastolic volume, and a 200–300% increase in end-systolic volume at 1 wk compared with those of sham-operated rats. At no time was there a significant difference in any of the measurements of cardiac morphology and function between infarcted hearts treated with BMSCs and those injected with saline (Fig. 4 and Table 1). In particular, the direct injection of 2 × 10⁶ BMSCs or Fe-BMSCs did not increase EF at any time point (EF at 1, 4, and 10 wk, respectively; BMSC = 53 ± 4%, 58 ± 3%, and 52 ± 4%; Fe-BMSC = 55 ± 3%, 57 ± 2%, and 56 ± 3%; and control = 53 ± 2%, 55 ± 2%, and 55 ± 2%).

Fig. 2. Magnetic resonance imaging (MRI) tracking. Representative in vivo (A) and ex vivo (B) MRI of hearts 10 wk after intramuscular administration of 5 × 10⁶ MPIO-labeled BMSCs (Fe-BMSCs) showing Fe-BMSCs tracking from the point of injection into the scar and in vivo (C) and ex vivo (D) MRI of hearts 4 wk after intravenous administration of 4 × 10⁶ Fe-BMSCs 1 day after myocardial infarction (MI) showing Fe-BMSCs located through the scar but not in the viable myocardium. RV, right ventricle; LV, left ventricle.
The area of the akinetic region of the myocardium, measured in vivo in the cine images, was unaffected by cell therapy over the course of the studies. As can be seen, the lack of beneficial effect of BMSC therapy was not related to MPIO labeling of BMSCs, since all measurements of cardiac morphology and function were similar between the BMSC- and the Fe-BMSC-treated groups.

After intravenous infusion of BMSCs. Cardiac morphology and function was measured at 1 and 4 wk after myocardial infarction and intravenous infusion of BMSCs. Infarction resulted in a 34–37% decrease in left ventricular EF, a 44–67% increase in end-diastolic volume, and a 200–340% increase in end-systolic volume at 1 wk compared with those of sham-operated rats. There was no significant difference at any time in any of the measurements of cardiac morphology and function between rats infused with Fe-BMSCs at 1 or 3 days after myocardial infarction and those infused with saline (Fig. 4 and Table 1). An infusion of $4 \times 10^6$ Fe-BMSCs at 1 or 3 days did not increase EF at any time (EF at 1 wk and 4 wk, respectively; Fe-BMSC at 1 day post-myocardial infarction, EF = 54 ± 2%; and 54 ± 2%; Fe-BMSC at 3 days post myocardial infarction, EF = 54 ± 7% and 56 ± 8%; and saline control, EF = 52 ± 3% and 54 ± 3%). The area of the akinetic region of the myocardium was unaffected by cell therapy.

DISCUSSION

To our knowledge, this is the first in vivo study to track systemically infused stem cells in rats and the first to fully characterize the functional effects of BMSC therapy for the infarcted rat heart using cine-MRI. Cell isolation, culture, and administration were similar to those used in other studies (35, 37, 50, 53). In vivo and ex vivo MRI, histology, and cell reisolation confirmed that donor BMSCs remained grafted.
within the infarcted myocardium for at least 10 wk. However, no BMSC-mediated improvements in cardiac morphology or function were found at any time up to 10 wk after infarction and cell administration compared with those of control animals.

In vivo cell tracking can yield valuable information regarding cell injection success, cell homing, and engraftment (49). BMSCs readily endocytosed MPIO during overnight culture, and labeling did not affect cell viability, proliferation, or differentiation or alter the in vivo therapeutic effect. Iron oxide-labeling of BMSCs has been used to confirm injection success and monitor cell retention for up to 16 wk in the infarcted rat (49), mouse (4), and pig (19) heart after direct intramyocardial injection. The application of this technique to intravenous-infused cells permits the time course of cell migration and the degree of cell homing to be measured in the live animal. BMSCs are able to home to sites of inflammation or tissue damage (40). Mediators, including SDF-1/CXCR4, integrin β1, and VCAM-1, are highly expressed on BMSCs and are involved in BMSC migration and homing (1, 29). Thus the MRI cell-tracking technique described here could improve our understanding of cell homing mechanisms and test the efficiency of different administration techniques, such as intracoronary infusion or left ventricular cavity injection.

MRI and immunohistochemistry showed that BMSCs were retained within the myocardium for the duration of the experiment. In agreement with others (52), ~0.1% of the administered BMSCs were retained within the heart 10 wk after administration. Cell retention was further characterized using an electromagnet to reisolate the magnetic donor BMSCs from enzymatic digests of grafted hearts. The percentage of retained MPIO- and CM-DiI-labeled cells was similar to that detected using immunohistochemistry (0.1%). We have reported that MPIOs remain within GFP-labeled donor cells for up to 16 wk in vivo, rather than becoming phagocytosed by macrophages (49).

Although we found that BMSCs did not improve morphology or function in the infarcted rat heart, and they did not increase neovascularization, there are numerous reports that bone marrow stem cell therapy improves rat and mouse heart function (23, 35, 37, 55, 61). Yet there are discrepancies between the degree of the functional benefit observed, from near complete restoration (23, 25, 61), via the reduction of detrimental remodeling (4, 16, 37), to no discernable difference in function between bone marrow cell-treated animals and controls (12, 13, 28, 53, 57, 60) and detrimental calcification within the myocardium (59). Reasons for these inconsistencies include different cell isolation and culture protocols (47), variation in the time and method of cell delivery (1), the number of cells administered (46), species and animal age, the different levels of cardiac impairment induced by coronary occlusion, and the use of less accurate measures of cardiac function. Some workers have reported improved cardiac function with very low levels of donor cell retention (7, 21) or when conditioned culture media alone was administered (55). The results were explained by the ability of BMSCs to express and secrete angiogenic cytokines that increased capillary density (32, 53, 60). Even had cytokines been secreted by the BMSCs in our rat hearts, they failed to increase capillary numbers or improve cardiac morphology or function.

The occlusion of the rodent LAD coronary artery results in highly variable infarct sizes, as the coronary network varies considerably between animals, even littermates (27). The identification of myocardial blanching after the tightening of the suture does not confirm a successful ligation. Post-infarction
remodeling is directly related to infarct size, with only hearts with very large infarcts progressing to failure (36, 39). These variations are reflected in the inconsistent results from the infarcted control animals in basic studies of stem cell therapy. After infarction, EFs are reported to either decrease [for example, 55 to 37% between 1 and 8 wk (50), 62 to 54% between 3 days and 3 wk (25), 31 to 16% from 2 days to 2 wk (16)] or remain constant over 4 (4) or 15 (23) wk. Therefore, it cannot

Table 1. Cardiac morphology and function measured using in vivo cine-MRI

|            | Sham | Intramuscular | Fe-BMSCs | Intravenous |
|------------|------|--------------|----------|-------------|
|            |      | Saline       | BMSCs    | Fe-BMSCs    |
| n          | 6    | 10           | 7        | 17          |
| Body weight, g |      |              |          |             |
| 1 wk       | 235 ± 9 | 204 ± 4 | 218 ± 6 | 218 ± 4 |
| 4 wk       | 245 ± 7 | 221 ± 4 | 235 ± 8 | 232 ± 4 |
| 10 wk      | 261 ± 8 | 245 ± 4 | 254 ± 9 | 248 ± 4 |
| Ejection fraction, % |      |              |          |             |
| 1 wk       | 82 ± 1  | 53 ± 2      | 53 ± 4  | 55 ± 3      |
| 4 wk       | 80 ± 2  | 55 ± 2      | 58 ± 3  | 57 ± 2      |
| 10 wk      | 82 ± 1  | 55 ± 2      | 52 ± 4  | 56 ± 3      |
| End-diastolic volume, mm³ |      |              |          |             |
| 1 wk       | 242 ± 23 | 348 ± 24 | 378 ± 31 | 370 ± 24 |
| 4 wk       | 290 ± 14 | 388 ± 27 | 455 ± 35 | 419 ± 30 |
| 10 wk      | 304 ± 12 | 434 ± 26 | 453 ± 40 | 490 ± 33 |
| End-systolic volume, mm³ |      |              |          |             |
| 1 wk       | 44 ± 6  | 165 ± 16    | 184 ± 29 | 174 ± 20    |
| 4 wk       | 58 ± 7  | 179 ± 21    | 198 ± 29 | 188 ± 24    |
| 10 wk      | 54 ± 5  | 199 ± 22    | 222 ± 36 | 230 ± 30    |
| Stroke volume, mm³ |      |              |          |             |
| 1 wk       | 197 ± 19 | 183 ± 11 | 194 ± 9  | 196 ± 9     |
| 4 wk       | 232 ± 12 | 210 ± 10 | 257 ± 11 | 231 ± 9     |
| 10 wk      | 250 ± 9  | 234 ± 9    | 230 ± 17 | 261 ± 10    |
| Heart rate, beats/min |      |              |          |             |
| 1 wk       | 402 ± 18 | 362 ± 12 | 404 ± 5  | 390 ± 10    |
| 4 wk       | 383 ± 16 | 360 ± 17 | 375 ± 9  | 393 ± 9     |
| 10 wk      | 400 ± 11 | 381 ± 12 | 399 ± 11 | 402 ± 7     |
| Cardiac output, ml/min |      |              |          |             |
| 1 wk       | 79 ± 7  | 66 ± 4      | 79 ± 4  | 76 ± 4      |
| 4 wk       | 88 ± 7  | 75 ± 5      | 99 ± 4  | 91 ± 4      |
| 10 wk      | 100 ± 5 | 89 ± 3      | 91 ± 5  | 105 ± 5     |
| Area of akinetic myocardium, % of LV wall |      |              |          |             |
| 1 wk       | 0       | 19 ± 3      | 19 ± 3  | 17 ± 2      |
| 4 wk       | 0       | 17 ± 4      | 17 ± 2  | 15 ± 2      |
| 10 wk      | 0       | 16 ± 3      | 17 ± 3  | 14 ± 2      |
| LV mass, mg |      |              |          |             |
| 1 wk       | 441 ± 28 | 474 ± 19 | 468 ± 18 | 485 ± 17 |
| 4 wk       | 446 ± 23 | 497 ± 15 | 526 ± 21 | 519 ± 16 |
| 10 wk      | 480 ± 23 | 543 ± 22 | 549 ± 24 | 607 ± 20 |

Values are means ± SE; n, number per group. There were no significant differences between cell- and saline-treated hearts. Differences between sham and infarcted animals are not shown for clarity. MRI, magnetic resonance imaging; BMSC, bone marrow stromal cell; Fe-BMSC, micron-sized particles of iron oxide-BMSC; LV, left ventricle.

Table 2. Average change to ejection fraction reported by the largest clinical trials

| Trial   | Reference                  | Year | Change in Ejection Fraction, % |
|---------|----------------------------|------|--------------------------------|
|         |                            |      | Controls | Stem Cells | Treatment Effect |
| TOPCARE-AMI, Assmus et al. | 6     | 2002 | 2.5    | 8.5   | 6.0* |
| Fernandez-Aviles et al.    | 14    | 2004 | 0.0    | 5.8   | 5.8* |
| TOPCARE-CHD, Assmus et al. | 5     | 2006 | -1.0   | 1.0   | 2.0  |
| Janssens et al.             | 22    | 2006 | 2.2    | 3.3   | 1.1  |
| ASTAMI, Lunde et al.        | 26    | 2006 | 4.5    | 1.4   | -3.1 |
| BOOST, Meyer et al.         | 31    | 2006 | 3.1    | 5.9   | 2.8  |
| REPAIR, Schachinger et al.  | 43    | 2006 | 3.0    | 5.5   | 2.5* |
| Mean                          |      |      | 2.0 ± 0.8 | 4.2 ± 1.1 | 2.4 ± 1.3 |

Values are means ± SE. Change in ejection fraction that occurred from baseline to end of study and the treatment effect between the control and cell-treated groups are shown. Data was compiled from the latest seven stem cell clinical trials that included 20 or more patients and a control group. Note that Fernandez-Aviles et al. (Ref. 14) did not report ejection fraction in controls. Assmus et al. (Ref. 5) enrolled chronic heart failure patients. MRI data are reported from Lunde et al. (Ref. 26) and Meyer et al. (Ref. 31) studies. *P < 0.05, significantly greater improvement in stem cell-treated group vs. controls.
be assumed that the average EF of two small groups of infarcted hearts before cell therapy was the same. Many publications report a beneficial effect of cell therapy within the first few days of cell delivery with little or no further benefit (4, 25, 37). Another interpretation may be that cardiac function was better in the cell-treated group before cell injection and that cell therapy had no effect on function.

A further explanation for the inconsistencies between studies is that different methods have been used to measure cardiac function. Invasive techniques, including isolated heart perfusion (28, 53) and in vivo hemodynamics (10, 35), have identified improvements to heart function after bone marrow cell therapy, but measurements can only be performed at a single time point. Noninvasive methods permit multiple measurements of heart function, allowing the progression of post-infarction remodeling to be followed and cell-mediated improvements to be identified. Echocardiography has been regularly used to measure rodent heart function. However, the accuracy and reproducibility of the technique is still controversial (3, 48), especially when low-frequency transducers are used and data are acquired in M-mode (11, 18) as in most studies (35, 37, 55, 61). Furthermore, the calculation of the EF from echo data requires geometric assumptions that are incorrect in the asymmetrical infarcted heart (11, 18). The gold standard for the in vivo measurement of cardiac function in humans (18, 41) and rodents (36, 45, 54) has been set by cine-MRI. MRI has excellent interstudy reproducibility (18, 48, 54), is routinely used in the clinic (56), and has been used in many clinical stem cell trials (14, 22, 26, 31, 58). MRI is not commonly used to study heart function in rodent hearts because of their small size and high heart rate (54). The high spatial resolution of the cine-MRI method used here, coupled with excellent contrast between myocardium and blood, resulted in high-definition, volumetric images from which left ventricular cavity volumes could be accurately measured. The high temporal resolution of the cine-MRI method (4.6 ms) permitted the accurate identification of end diastole and end systole, the duration of which is ~3–5 ms. These could be missed by two-dimensional echocardiography with a temporal resolution typically greater than 10 ms. Further, the ability to acquire data in any plane, using computerized slice positioning, allowed a contiguous stack of true short-axis images to be acquired covering the entire left ventricle with no geometric assumptions. Hence, MRI studies should provide the most accurate and reproducible results regarding the benefits of stem cell therapy for the infarcted heart (8, 9, 15, 48).

The results of this study are in conflict with earlier studies that reported beneficial effects of bone marrow cell therapy (4, 10, 23, 25, 35, 37, 50, 61). The end point of other studies was the fate of grafted cells (35, 38), the ability of cells to home, engraft, and survive within the myocardium (4, 35), form new vasculature (23, 35), and differentiate into (38) or fuse with (34) cardiomyocytes and/or secrete paracrine factors (55). Extensive cardiac functional measurements have rarely been made, and it has often been assumed that the injection or infusion of most cell types, including macrophages (24), can improve function (33). This is not the case, as demonstrated not only by our study but also by clinical trials of stem cell therapy, which focused on cell-mediated improvements in global heart function (22, 26, 31, 44), especially EF.

Clinical trials of cell therapy for the heart have been initiated worldwide on the basis of basic research results. These carefully designed double-blind trials generally included large numbers of patients per group (compared with those of many basic studies) and measured heart function at multiple points using accurate imaging methods. Although initial clinical studies were promising (6, 14, 58), more recent studies have found only a limited degree of stem cell-mediated improvement in heart function. Table 2 shows that the average increase in left ventricular EF was 2.4 ± 1.0% in the seven largest clinical trials (20 or more patients in the treated group) that included control patients. Our results are in agreement with these clinical findings, since our study (or any other basic study to our knowledge) was not powered to identify a 2% to 3% improvement in EF.

In summary, BMSCs administered by direct injection or systemic infusion homed, survived, and remained viable in the contracting myocardium for up to 10 wk after infarction. However, no BMSC-mediated improvement in morphology or function was observed. We suggest that cultured BMSCs are not the ideal population for treatment of the infarcted heart.

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