Allogeneic Mesenchymal Precursor Cell Therapy to Limit Remodeling After Myocardial Infarction: The Effect of Cell Dosage

Hirotsugu Hamamoto, MD, Joseph H. Gorman III, MD, Liam P. Ryan, MD, Robin Hinmon, MS, Timothy P. Martens, MD, Michael D. Schuster, MS, Theodore Plappert, CVT, Matti Kiupel, DVM, PhD, Martin G. St. John-Sutton, MBBS, Silviu Itescu, MD, and Robert C. Gorman, MD

Departments of Surgery and Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; Angioblast Systems Inc, New York, New York; and Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, Michigan

Background. **This experiment assessed the dose-dependent effect of a unique allogeneic STRO-3–positive mesenchymal precursor cell (MPC) on postinfarction left ventricular (LV) remodeling. The MPCs were administered in a manner that would simulate an off-the-self, early postinfarction, preventative approach to cardiac cell therapy in a sheep transmural myocardial infarct (MI) model.**

Methods. **Allogeneic MPCs were isolated from male crossbred sheep. Forty-six female sheep underwent coronary ligation to produce a transmural LV anteroapical infarction. One hour after infarction, the borderzone myocardium received an injection of 25, 75, 225, or 450 × 10^6 MPCs, or cell medium. Echocardiography was performed at 4 and 8 weeks after MI to quantify LV end-diastolic (LVEDV) and end-systolic volumes (LVESV), ejection fraction (EF), and infarct expansion. CD31 and smooth muscle actin (SMA) immunohistochemical staining was performed on infarct and borderzone specimens to quantify vascular density.**

Results. **Compared with controls, low-dose (25 and 75 × 10^6 cells) MPC treatment significantly attenuated infarct expansion and increases in LVEDV and LVESV. EF was improved at all cell doses. CD31 and SMA immunohistochemical staining demonstrated increased vascular density in the borderzone only at the lower cell doses. There was no evidence of myocardial regeneration within the infarct.**

Conclusion. **Allogeneic STRO-3 positive MPCs attenuate the remodeling response to transmural MI in a clinically relevant large-animal model. This effect is associated with vasculogenesis and arteriogenesis within the borderzone and infarct and is most pronounced at lower cell doses.**

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Infarction-induced left ventricular (LV) remodeling remains responsible for most heart failure cases in the United States [1], despite the widespread use of reperfusion therapy. An effective means for limiting infarction-induced remodeling in patients who are not offered reperfusion therapy or have a suboptimal response to it would be of great value. This is especially true for patients who sustain larger infarctions that are associated with an early reduction in global LV function.

During the last decade, extensive interest has developed in cell therapy as a potential treatment for both acute myocardial infarction (MI) and established heart failure. Bone marrow-derived stromal stem cells have shown particular promise in preclinical and some early clinical trials [2–4]; however, the mechanisms by which these salutary effects are achieved remain incompletely understood. Major questions remain, especially about the optimal cell type, cell dose, timing of delivery, location of delivery, and delivery technique. The most appropriate indication (ie, prevention vs reversal of established heart failure) also remains to be established [5, 6].

In this experiment, we studied the effect on infarction-induced LV remodeling of escalating doses of a bone marrow-derived allogeneic, STRO-3–positive, mesenchymal precursor cell delivered into the nonischemic borderzone myocardium within 1 hour after an non-reperfused, transmural MI in sheep. These unique cells possess phenotypic and functional characteristics of vascular pericyte precursor cells and can promote vascular network formation through their capacity not only to differentiate into multiple stromal cell types but also to induce endogenous arteriogenesis by paracrine mechanisms [7, 8].

Material and Methods

STRO-3–Positive Cell Type

The bone marrow contains a small compartment of self-renewing, pluripotent stem cells, known as bone marrow stromal stem cells or mesenchymal stem cells.

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Address correspondence to Dr Robert C. Gorman, Gorman Cardiovascular Research Group, Glenolden Research Laboratory, University of Pennsylvania, 500 S Ridgeway Ave, Glenolden, PA 19036; e-mail: gormanr@uphs.upenn.edu.

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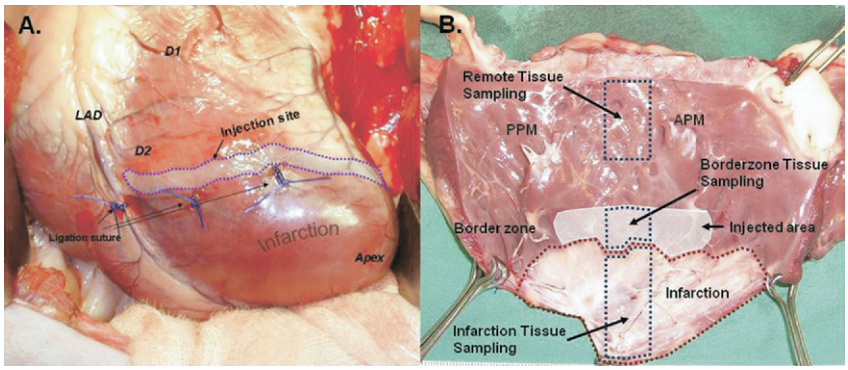

Fig 1. (A) In this postinfarction view of the ovine heart as seen through a left thoracotomy, the size and location of the anteroapical infarct are clearly depicted. Cells were injected in the normally perfused borderzone region within 1 cm of the readily identifiable infarction demarcation line (shaded area). (B) Postmortem view of the left ventricle shows ovine left ventricle opened through the intraventricular septum demonstrating the standard infarct size and location as well as the injection site and tissue sampling sites. (APM = anterior papillary muscle; PPM = posterior papillary muscle.)

Perivascular STRO-3 positive mesenchymal precursor cells (MPCs) are a small fraction of these mesenchymal stem cells that demonstrate an extensive capacity for proliferation and differentiation. These noncycling cells lack the phenotypic characteristics of leukocytes and mature stromal cells but maintain the functionality to transfer an active hematopoietic microenvironment in vivo by their capacity to differentiate into multiple stromal cell types, including myelosupportive stroma, smooth muscle cells, adipocytes, osteoblasts, and chondrocytes. These cells have also been shown to foster vasculogenesis and arteriogenesis in animal models [8]. Mounting evidence suggests that mesenchymal stem cells may be able to evade immune detection due in part to direct inhibition of inflammation as well as a lack of cell-surface costimulatory molecules [7–9].

Animal Model and Experimental Design

Animals were treated under experimental protocols approved by the University of Pennsylvania's Institutional Animal Care and Use Committee (IACUC) and in compliance with National Institutes of Health Publication No. 85-23, revised 1996.

A well-characterized ovine model of infarction-induced ventricular remodeling was used. An anteroapical infarction involving slightly more than 20% of the LV mass was produced by ligating the left anterior descending artery and its diagonal branches 40% of the distance from the apex to the base of the heart [10]. After infarction, 46 female sheep were divided into groups that received the following injections:

- control: cell medium, 14 sheep;
- 25M: 25 million male MPCs, 7 sheep;
- 75M: 75 million male MPCs, 7 sheep;
- 225M: 225 million male MPCs, 10 sheep; and
- 450M: 450 million male MPCs, 8 sheep

Male MPCs or cell medium alone were delivered by direct injection into the clearly identifiable borderzone region adjacent to the infarct on the anterior wall of the LV (Fig 1A) within 1 hour of coronary occlusion.

Remodeling and global LV function were determined in all animals using 2-dimensional echocardiography. Echocardiographic studies were performed before infarction, immediately after infarction (and cell or medium injection), and at 4 and 8 weeks after infarction.

Cell Preparation

The STRO-3–positive MPCs used in this experiment were derived from bone marrow aspirates from male crossbred sheep using techniques similar to those previously described [7, 8]. Cells were cryopreserved in 42% Profreeze (Lonza, Mapleton, IL) 50% Alpha minimum essential medium/7.5% dimethylsulfoxide at 450, 225, 75, and 25 $\times$ 10^6 cells/4 mL ampoules. Recovery of the frozen preparations was achieved by rapid thawing. The suspension was then kept on ice until administered; then 100 μL of the suspension was removed and diluted 1:2000 in 0.4% trypan blue/phosphate buffered saline to assess the cell viability and cell number using a hemocytometer. All animals except for the 450M group received 20 middle myocardial (2 to 3-mm depth) borderzone injections of 0.2 mL (4 mL total). The 450M-cell group received 20 injections of 0.4 mL (8 mL total).

Echocardiography Protocol and Data Analysis

Quantitative 2-D echocardiograms were performed before infarction, after infarction (and after injection), and at 4 and 8 weeks after infarction. Images were obtained on a Philips 7500 Ultrasound System (Philips Healthcare, Andover, MA) using a 5-MHz probe. LV end-systolic (LVESV) and end-diastolic (LVEDV) volumes were calculated using a modified Simpson's rule as previously described [11]. Myocardial infarct length (wall motion abnormality length in long axis) was also measured. All image analysis was performed by a blinded analyst.

Hemodynamic Data Collection and Analysis

Arterial blood pressure, LV pressure, central venous pressure, pulmonary capillary wedge pressure, maximum $\pm$ dP/dt, and thermodilution cardiac outputs were measured at the same time points that echocardiographic data were collected.

Tissue Collection and Postmortem Analysis

Animals were euthanized 8 weeks after infarction and cell injection. After euthanasia, the LV was opened through the septum and a standardized digital photo was taken (Casio EX-Z850, Tokyo, Japan). All photographs were imported into an image analysis program (Image Pro Plus, Media Cybernetics, Silver Spring, MD), and

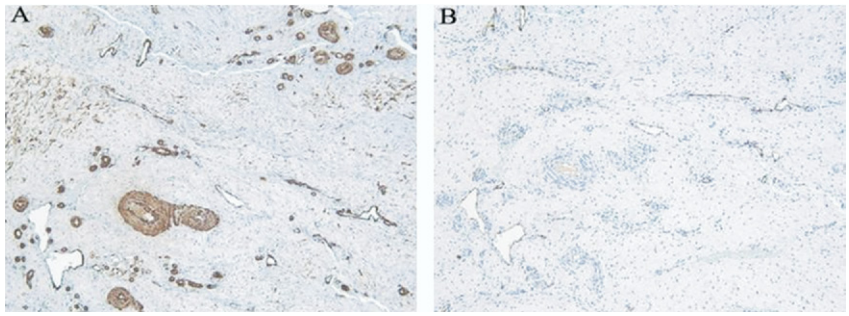

Fig 2. (A) Smooth muscle actin vs (B) CD31 immunohistochemical staining of the same section of sheep infarct tissue. In sheep, CD31 effectively stains the endothelium of thin-walled venules and capillaries but does not reliably stain arterioles and small muscular arteries. Smooth muscle actin staining readily identifies arterioles and small muscular arteries in sheep.

computerized planimetry was performed to assess the size of the infarct (Fig 1B). The infarct area was expressed as a percentage of the LV area.

Representative transmural sections of myocardium from the infarct, borderzone, and remote regions were harvested and fixed in 10% neutral buffered formalin. Myocardial punch biopsies were flash frozen in liquid nitrogen and stored at −80°C.

Vascular Density Analysis

Immunohistochemical staining for the endothelial marker CD31 and smooth muscle actin (SMA) were performed to identify blood vessels in the infarct and borderzone regions. Corresponding CD31 and SMA slides were used to semiquantitatively assess vascular density. Nine matching ×40 fields of view from each slide were examined three different times, and the average number of vessels per ×40 field of view was recorded for CD31 and SMA. In sheep, CD31 effectively stains the endothelium of thin-walled venules and capillaries but does not reliably stain arterioles and small muscular arteries. SMA staining readily identifies arterioles and small muscular arteries in sheep (Fig 2).

Assessment of MPC Engraftment

Genomic DNA was extracted from frozen tissue samples using QIAamp DNA Mini Kit (Qiagen, Valencia, CA). SRY2 and SRY3 primer pairs were selected to amplify genes from the ovine male-specific region [12]. Primers were tested on 1 μg of DNA from male and female sheep using HotStart Taq Master Mix Kit (Qiagen) and an annealing temperature of 58°C. Each primer pair generated a positive signal in the male DNA and a negative signal in the female DNA. A serial dilution of male DNA

Table 1. Hemodynamic Data[a]

Treatment Groups	Sheep No.	CVP (mm Hg)	PCWP (mm Hg)	Mean AP (mm Hg)	CO (L/min)	Max dP/dt (mm Hg/s)
Baseline						
Control	10	6.8 ± 0.7	8.7 ± 0.5	87.0 ± 4.1	3.9 ± 0.4	1093.7 ± 94.8
Cells ($\times 10^6$)						
25	6	5.0 ± 0.9	10.6 ± 0.9	84.3 ± 3.7	3.8 ± 0.4	1083.0 ± 151.9
75	5	7.2 ± 1.7	9.6 ± 1.8	87.2 ± 8.2	3.6 ± 0.4	1101.4 ± 91.5
225	8	6.9 ± 0.9	9.4 ± 1.0	84.8 ± 6.2	4.1 ± 0.2	1071.6 ± 87.4
450	6	5.5 ± 0.8	9.0 ± 1.3	85.3 ± 5.7	3.5 ± 0.5	900.7 ± 63.3
4-wk follow-up						
Control	10	8.8 ± 0.9	11.6 ± 1.0[b]	89.4 ± 4.0	2.7 ± 0.2[b]	
Cells ($\times 10^6$)						
25	6	9.3 ± 1.5	12.2 ± 1.5	100.8 ± 4.6[b]	2.9 ± 0.3	
75	5	10.2 ± 0.6	12.6 ± 0.2	95.4 ± 5.3	3.3 ± 0.4	
225	8	9.5 ± 1.3	11.8 ± 1.2	93.0 ± 3.7	3.3 ± 0.1	
450	6	10.5 ± 0.6[b]	12.8 ± 1.6[b]	83.7 ± 6.7	2.9 ± 0.4	
8-wk follow-up						
Control (n = 10)	10	10.1 ± 0.5[b]	13.1 ± 1.4[b]	81.7 ± 3.1	2.3 ± 0.1[b]	788.2 ± 62.1[b]
Cells ($\times 10^6$)						
25	6	7.3 ± 1.5[c]	12.7 ± 0.6	80.3 ± 3.5[d]	2.4 ± 0.2[b]	994.3 ± 143.0
75	5	8.8 ± 0.5	12.4 ± 0.6	83.8 ± 6.9	2.5 ± 0.2[b]	931.8 ± 156.0
225	8	10.5 ± 0.5[b]	11.9 ± 0.7	78.1 ± 4.9[d]	2.4 ± 0.1[b]	964.6 ± 104.0
450	6	8.2 ± 1.4[b]	14.5 ± 1.5[b]	73.8 ± 4.4[d]	2.4 ± 0.2[b]	719.7 ± 41.4[b]

[a] Values are means ± standard error of the mean.
Analysis by repeat measures analysis of variance: [b] $p < 0.05$ vs control; [c] $p < 0.05$ vs baseline; [d] $p < 0.05$ vs 4-week follow-up.

AP = arterial pressure; CO = cardiac output; CVP = central venous pressure; PCWP = pulmonary capillary wedge pressure; dP/dt = time rate of change of arterial pressure.

Table 2. Echocardiographic Data[a]

Group	Sheep, No.	Baseline	Postinfarct[b]	4 Weeks	8 Weeks
Diastolic volume, mL					
Control	10	56.4 ± 3.3	73.5 ± 3.1[c]	98.6 ± 7.8[c,d]	102.8 ± 8.0[c,d]
Cells ($\times 10^6$)					
25	6	61.8 ± 3.8	90.8 ± 3.6[c,e]	69.5 ± 5.3[d,e]	85.0 ± 7.9[c,e,f]
75	5	60.4 ± 3.0	96.7 ± 5.6[c,e]	82.8 ± 6.4[c]	84.6 ± 5.4[c,e]
225	8	63.0 ± 4.5	84.4 ± 3.4[c,e]	92.3 ± 5.2[c,e]	94.0 ± 4.6[c]
450	6	63.3 ± 3.1	93.8 ± 4.4[c,e]	98.3 ± 4.6[c,g]	98.4 ± 8.0[c]
Systolic volume, mL					
Control	10	35.1 ± 2.4	53.4 ± 3.4[c]	84.0 ± 8.1[c,d]	89.0 ± 7.5[c,h]
Cells ($\times 10^6$)					
25	6	35.0 ± 3.1	59.9 ± 3.1[c]	52.4 ± 3.1[c,d,e]	65.0 ± 7.4[c,e]
75	5	34.0 ± 3.6	62.9 ± 5.0[c]	60.8 ± 3.3[c,e]	64.5 ± 4.1[c,e]
225	8	38.9 ± 4.0	54.2 ± 3.1[c]	72.0 ± 5.6[c,d,g]	74.1 ± 3.5[d,c,e]
450	6	38.9 ± 2.5	64.0 ± 5.0[c]	77.9 ± 4.6[c,g,h]	77.8 ± 8.4[c]
Ejection fraction					
Control	10	0.379 ± 0.02	0.276 ± 0.034[c]	0.159 ± 0.016[c,d]	0.137 ± 0.009[c,d]
Cells ($\times 10^6$)					
25	6	0.437 ± 0.017	0.340 ± 0.021[c]	0.240 ± 0.019[c,d,e]	0.238 ± 0.030[c,d,e]
75	5	0.433 ± 0.06	0.352 ± 0.019	0.260 ± 0.026[c,d,e]	0.236 ± 0.024[c,d,e]
225	8	0.386 ± 0.027	0.357 ± 0.032	0.225 ± 0.024[c,d,e]	0.209 ± 0.021[c,d,e]
450	6	0.385 ± 0.022	0.319 ± 0.034	0.209 ± 0.019[c,g]	0.208 ± 0.032[c,e]

[a] Values are means ± standard error of the mean. [b] Postinfarct time point is also postcell/vehicle injection.
Analysis by repeat measures analysis of variance: [c] $p < 0.05$ vs baseline, [d] $p < 0.05$ vs postinfarct, [e] $p < 0.05$ vs control, [f] $p < 0.05$ vs 4-week follow-up, [g] $p < 0.05$ vs 25×10^6 cells, [h] $p < 0.05$ vs 75×10^6 cells.

in female DNA was made to determine the detection limit for male DNA within 1 μg of total DNA. The detection limit for SRY2 and SRY3 was 500 pg, equivalent to approximately 85 cells [13].

Amplification reactions using the SRY2 and SRY3 primer pairs were then set up for each of the DNA extracts of the infarct and borderzone regions of all sheep that survived to the end of study as well as 1 animal that died 1 hour after cell injection of 225 million cells and 1 animal that died 4 weeks after injection of 25 million cells.

Statistical Analysis

Between- and within-group differences in data were assessed for statistical significance. The significance within groups was assessed using repeat measures analysis of variance (ANOVA) and paired *t* tests. Significance between groups was assessed with ANOVA and paired *t* tests. Individual post hoc comparisons were performed using the Tukey honestly significantly different test. Data are presented as means ± standard error of the mean. Difference in survival was assessed using the χ^2 test.

Results

Cell Viability

The viability of the cells by group was 25M, 92.4% ± 4.6%; 75M, 92.9% ± 2.0%; 225M, 92.7% ± 2.8%; and 450M, 93.7% ± 1.3%. The actual number of cells ($\times 10^6$) injected by group based on the cell viability analysis was 25M, 22.6 ± 3.3; 75M, 72.1 ± 11.9; 225M, 211.4 ± 27.8; and 450M, 441.2 ± 46.4.

Survival

Ten sheep (21.7%) died prematurely and did not complete the protocol. Three (21.4%) of the 14 control sheep died. The mortality rate was 28.6% (2 of 7) in the 75M group, 20.0% (2 of 10) in the 225M group, 25.0% (2 of 8) in the 450M group, and 14.3% (1 of 7) in the 25M group. The between-group differences in mortality were not statistically significant. All of the deaths occurred suddenly in previously stable animals and were presumably due to arrhythmias. Necropsy results were inconclusive. Seven of the deaths occurred within 6 days of the infarct, and the others occurred at 14, 28, and 31 days after the infarction.

Hemodynamics

Hemodynamic data are summarized in Table 1. Maximum ± dP/dt decreased significantly at 8 weeks after MI relative to baseline in the control group and the 450M group. Pulmonary capillary wedge pressure increased significantly at 8 weeks after MI relative to baseline in the control group and the 450M group. These variables were not significantly different from baseline in the 25M, 75M, and 225M groups at the terminal time point.

LV Remodeling

In the control group, LVEDV and LVESV increased progressively from 56.4 ± 3.3 and 35.1 ± 2.4 mL at baseline to 102.8 ± 8.0 and 89.0 ± 7.5 mL at 8 weeks after infarction. In the 25M group, LVEDV and LVESV increased significantly less than in the control group, from

Table 3. Infarct Size Measurements[a]

	Sheep No.	Infarct Length, cm			Infarct Size, % (Planimetry)
		Postinfarct[b]	4 Weeks	8 Weeks	
Control	10	6.74 ± 0.28	8.70 ± 0.37[c]	9.20 ± 0.32[c,d]	24.4 ± 1.4
Cells ($\times 10^6$)					
25	6	6.73 ± 0.22	7.40 ± 0.33[e]	7.48 ± 0.33[e]	23.9 ± 1.9
75	5	7.46 ± 0.19[f]	7.72 ± 0.35	7.88 ± 0.16[e]	21.4 ± 0.9
225	8	6.41 ± 0.29[g]	7.50 ± 0.23[c,e]	8.23 ± 0.22[c,d,e]	23.7 ± 0.8
450	6	6.97 ± 0.17	8.60 ± 0.30[c,f,h]	8.62 ± 0.27[c,f]	24.0 ± 1.3

[a] Values are means ± standard error of the mean. [b] Postinfarct time point is also postcell/vehicle injection. Analysis by repeat measures analysis of variance: [c] $p < 0.05$ vs postinfarct, [d] $p < 0.05$ vs 4-wk follow up, [e] $p < 0.05$ vs Control, [f] $p < 0.05$ vs 25 $\times$ 10^6 cells, [g] $p < 0.05$ vs 75 $\times$ 10^6 cells, [h] $p < 0.05$ vs 225 $\times$ 10^6.

61.8 ± 3.8 and 35.0 ± 3.1 mL at baseline to 85.0 ± 7.9 and 65.0 ± 7.4 mL at 8 weeks after infarction. The 75M group also demonstrated a significant reduction in remodeling relative to the control group: LVEDV and LVESV were 60.4 ± 3.0 and 34.0 ± 3.6 mL at baseline and increased to 84.6 ± 5.4 and 64.5 ± 4.1 mL at 8 weeks after infarction. LVESV at 8 weeks after infarction was significantly reduced in the 225M group (74.1 ± 3.5) relative to the control group; however, LVEDV was not significantly reduced in this group. The extent of remodeling in the 450M group was not appreciably improved over control. Serial LV volume data are summarized in Table 2.

Global Function

The EF decreased to 0.137 ± 0.09 at 8 weeks after infarction in the control group. EF at 8 weeks after infarction was significantly improved in the 25M, 75M, 225M, and 450M treatment groups with values of 0.238 ± .030, 0.236 ± 0.024, 0.209 ± 0.021, and 0.208 ± 0.032, respectively. Serial EF data are presented in Table 2.

Infarct Expansion

The length of the long axis wall motion abnormality or infarct length (cm) immediately after coronary occlusion was similar in the control (6.74 ± 0.28) and treatment groups (6.73 ± 0.22, 25M; 7.46 ± 0.19, 75M; 6.41 ± 0.29, 225M; and 6.97 ± 0.17 cm, 450M), confirming that the infarct size and remodeling stimulus were comparable in all groups. Control animals experienced progressive infarct expansion as infarct length increased to 8.70 ± 0.37 cm at 4 weeks and to 9.20 ± 0.32 cm at 8 weeks after infarction. Infarct expansion was significantly reduced in the 25M, 75M, and 225M groups (Table 3).

Necropsy Morphometric Analysis

Infarct sizes as determined from planimetry on the digital photographs of the opened heart after death (Fig 1B) were comparable in both the control (24.4% ± 1.4%) and treatment groups (23.9% ± 1.9%, 25M; 21.4% ± 0.9%, 75M; 23.7% ± 0.8%, 225M; and 24.0% ± 1.3 %, 450M) and consistent with published results for this model [10].

Vascular Density

Both CD31 and SMA staining demonstrated a significantly increased number of vessels/×40 field of view in borderzone myocardium of the 25M and 75M groups relative to control sheep. These differences were most pronounced with SMA staining, suggesting that arterioles and small muscular arteries were primarily responsible for the increased vascular density in these two groups. Borderzone vascular density was not significantly different in the 225M or 450M groups relative to

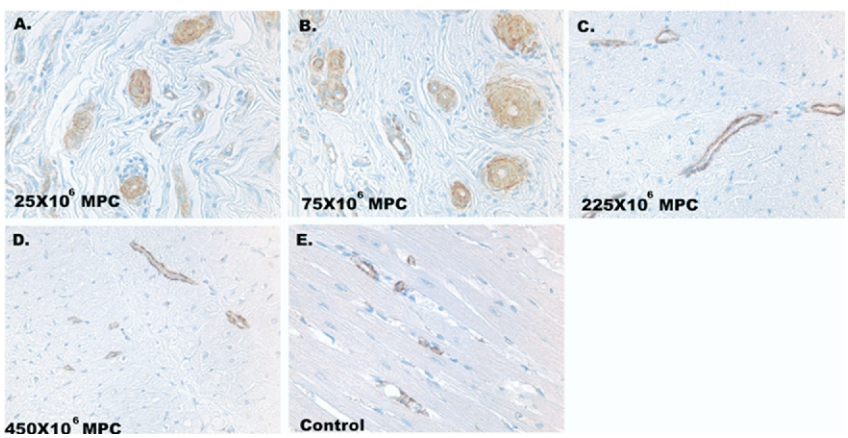

Fig 3. Photomicrographs (original magnification ×40) show results of smooth muscle actin (SMA) immunohistochemical staining of borderzone myocardium 8 weeks after infarction in representative sheep treated with (A) 25 million mesenchymal precursor cells (MPCs), (B) 75 million MPCs, (C) 225 million MPCs, and (D) 450 million MPCs, and the (E) untreated controls. Lower-dose animals had a higher density of muscular arterioles compared with higher-dose animals and control sheep. (MPC = mesenchymal precursor cells.)

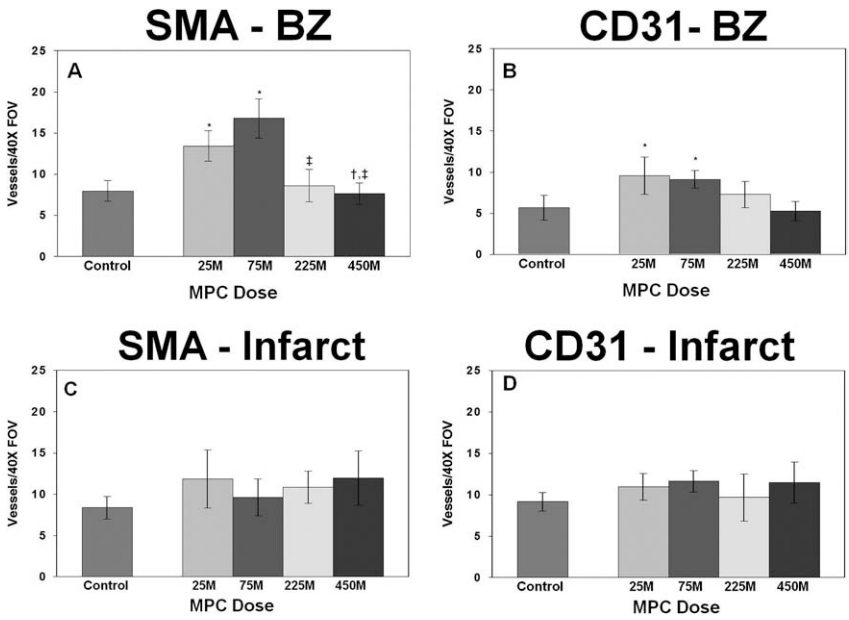

Fig 4. Graphs show quantitative assessment of (A, B) borderzone (BZ) vascular density and (C, D) infarct vascular density using smooth muscle actin (SMA) and CD31 immunohistochemical staining in control sheep and animals treated with mesenchymal precursor cells (MPCs) 8 weeks after infarction ($M = 10^6$). $*p < 0.05$ vs control; $\dagger p < 0.05$ vs 25×10^6 MPCs; $\ddagger p < 0.05$ vs 75×10^6 MPCs. Range bars represent the standard error of the mean.

control sheep (Fig 3). All vascular density data are summarized in Figure 4.

MPC Engraftment

DNA extracts from the infarct and borderzone region of all experimental female sheep that survived 8 weeks after MI did not produce a polymerase chain reaction product consistent with the presence of the injected male cells. This was also the case with the 25M sheep that died 4 weeks after MI. The 225M sheep that died 1 hour after cell injection did demonstrate the presence of male cells in the borderzone. The positive control sample generated a polymerase chain reaction product of expected size, and the negative control did not create a signal (Fig 5).

Comment

Aspects of this experiment of cardiac cell therapy include (1) a transmural (non-reperfused) infarct was used as a remodeling stimulus in a clinically relevant large animal model; (2) allogeneic cells were used, simulating an off-the-self, early after MI, preventative approach to cardiac cell therapy; (3) the effect of a wide range of cell dosages was assessed; (4) cells were delivered into the nonischemic borderzone myocardium rather than the infarct region, and (5) a novel new subset of bone marrow stromal stem cell was used.

A moderately sized transmural anteroapical MI was induced in the animals in this study. This is a strong stimulus for ventricular remodeling, which resulted in

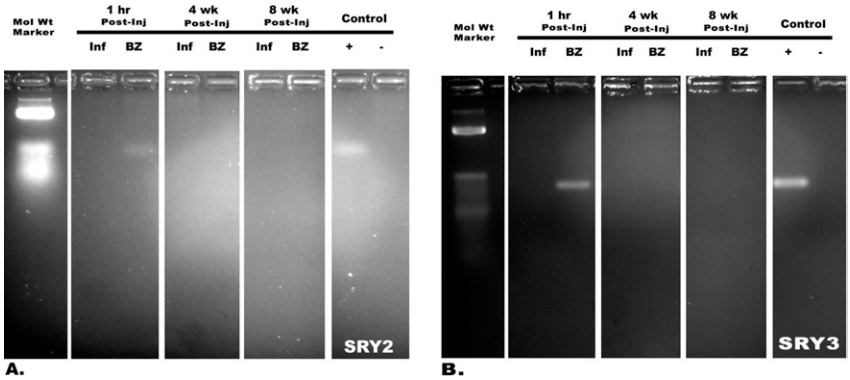

Fig 5. Polymerase chain reaction (PCR) amplification of myocardial DNA from the borderzone (BZ) of representative treatment animals that died 1 hour after cell injection (225 million cells), 4 weeks after infarction (25 million cells), and 8 weeks after infarction (75 million cells) using the (A) SRY2 and (B) SRY3 primer pairs to identify the presence of male DNA. PCR products of expected size were produced in the 1-hour animal with both the SRY2 and SRY3 primers pairs. PCR products from treated animals 4 weeks and 8 weeks after infarction (Inf) were below the detection limit of 500-pg template DNA. Male sheep myocardial DNA was used as the positive control, and female sheep myocardial DNA was used as the negative control.

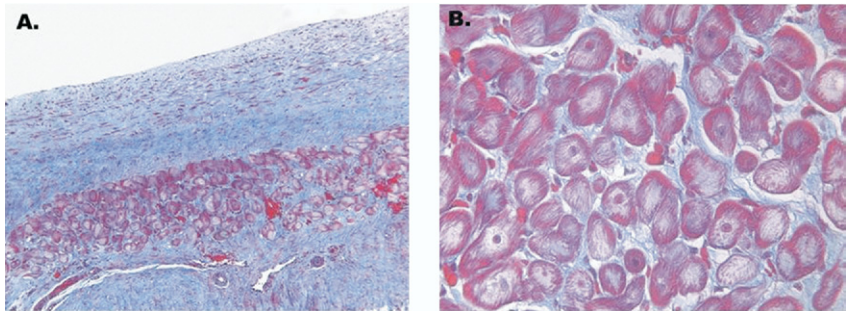

Fig 6. (A) Masson trichrome stain at original magnification ×10 and (B)×40 of sheep infarct tissue 8 weeks after infarction demonstrates an island of myofibrillarlytic myocytes within the scar. These isolated nests of myocytes are commonly seen in transmural infarcts and were not observed with increased frequency in animals treated with mesenchymal precursor cells at any cell dose.

immediate expansion (stretching) of the infarct region, global LV dilatation, and impaired global LV function. This model represents a more profound remodeling stimulus than the reperfused infarct models that have been used to assess other bone marrow stromal stem cells [14, 15]. Although all animals in this study experienced LV remodeling, the MPC injection significantly attenuated global LV dilatation and EF reduction.

Interestingly, the therapeutic effect of MPCs was most pronounced at the lower cell doses of 25 and 75 million cells. This phenomenon was observed at most end points for which significant differences between therapy and control groups could be demonstrated. Most cardiac cellular treatment strategies have generally assumed that high doses of cells are required to maximize efficacy; a few small-animal studies have supported this hypothesis [16–18], but large-animal and early clinical studies have not assessed wide ranges in cell dose [2–4, 14, 15, 19]. The widely held belief that efficacy is directly related to the number of cells delivered has been further reinforced by studies that have documented rapid cell loss in the hours and days immediately after their delivery into the myocardium [20].

Our data strongly suggest that high cell dosages limit efficacy. This phenomenon has several possible explanations, including:

1. At high cell doses, competition for limited nutrient resources in the highly stressed environments of the infarct and borderzone regions may become intense and limit cell survival or function, or both;
2. Large numbers of injected cells may elicit a more profound inflammatory or immunologic response, or both, that accelerates cell clearance or deactivation.

Although further experiments will be required to more fully elucidate this seemingly paradoxic observation, the implications for future clinical studies of cardiac stem cell therapy are important. These data may also help to explain the variable results that have been reported in recent clinical trials of stem cell therapy in the post-MI period in which the effect of cell dosing was not studied [3, 19].

The method of delivery (ie, intracoronary, transendocardial, epicardial) and the timing of delivery relative to the infarction could influence the dosage required to achieve an efficacious result. Multiple small doses delivered over an extended time period (eg, days to weeks) may represent a technique for optimizing the efficacy of MPC therapy for acute MI.

The beneficial effects on LV remodeling demonstrated in this experiment could not be attributed to regeneration of functioning myocardium within the infarct region. A detailed microscopic examination of the infarct specimens demonstrated isolated nests of myofibrillarlytic myocytes [21] within the infarct region of all animals; however, their distribution and concentration was highly variable and independent of treatment status (Fig 6).

The salutary effects of MPC therapy also could not be attributed to the prolonged engraftment of large numbers cells. Although the long-term survival or differentiation of a small number of cells could not be absolutely ruled out, the number of surviving cells was quite low at 8 weeks after injection. These results suggest that the presence of MPCs in the early post-MI period inherently changes the long-term LV response to infarction.

Our results do provide some important mechanistic insight into how MPC therapy affects the remodeling process. The improved LV remodeling associated with cell treatment observed in this experiment was at least partly due to a change in infarct material properties that acted to reduce infarct expansion (stretching). We and others have identified infarct expansion as an important initiating and sustaining impetus for adverse LV remodeling [22]. We have also demonstrated that mechanical infarct restraint can greatly limit adverse remodeling [23]. The data presented here would suggest that MPC therapy, at optimized doses, is an effective biologic means for limiting infarct expansion and ameliorating the remodeling process.

The reason for the reduced infarct expansion in the therapy animals could not be definitively established, but our results support the hypothesis that MPC therapy improves blood supply within the borderzone regions that may alter infarct healing and tissue remodeling dynamics. Martens and colleagues [8] and Kocher and colleagues [24] have demonstrated a similar effect using bone marrow-derived stem cells in small-animal infarct models.

Interest in regenerative cell therapy to treat cardiac disease continues to grow. Although numerous cell types have been tested in preclinical and clinical studies, allogeneic STRO-3 positive MPCs possess properties that may make them uniquely suited for the early treatment of acute MI. The ease of isolation, ex vivo expansion

potential, and allogeneic use of these cells combined with their biologic plasticity and tolerance for cryopreservation would allow for a readily available off-the-shelf therapy that would not be possible with other autologous cell strategies.

Most importantly, the results of this study would seem to mandate that future clinical trials of cardiac stem cell therapy include multiple dosage arms to establish efficacy and fully define potential risks.

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INVITED COMMENTARY

This article by Hamamoto and colleagues [1] reports the effect of cell dosage of allogenic mesenchymal precursor cell therapy on the post-myocardial infarction left ventricular remodeling in a sheep transmural myocardial infarct model.

Using allogeneic stem cells and off-the shelf availability is the ultimate clinical destination of cell therapy for ischemic heart disease. Cell banks can be built up with in vitro culture of stem cells donated from young and healthy volunteers. This technique provides vast amounts of myocardial cells and enables transplanting cells into any patient at any time in any institute. Hamamoto and colleagues [1] have demonstrated the feasibility of allogeneic mesenchymal precursor cell transplantation with cell viability after cryopreservation and rapid thawing up to 93%. Left ventricular (LV) remodeling was significantly alleviated, and the LV ejection fraction was significantly improved.

There are three major issues regarding myocardial cell transplantation under research and clinical trials (ie, the

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