Comparative effects of mesenchymal progenitor cells, endothelial progenitor cells, or their combination on myocardial infarct regeneration and cardiac function

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Objective: Recent evidence suggests that the effects of mesenchymal progenitor cell transplantation into the infarcted myocardium might be mediated by local paracrine angiogenesis. We compared the effects of mesenchymal progenitor cell transplantation versus those of a primarily angiogenic cell, the endothelial progenitor cell, in a rat model of myocardial infarction.

Methods: Twenty-one days after left anterior descending artery ligation, rats were injected in their infarcted anterior myocardium with 1×10^6 mesenchymal progenitor cells, 1×10^6 endothelial progenitor cells, 5×10^5 mesenchymal progenitor cells plus 5×10^5 endothelial progenitor cells, or phosphate-buffered saline (n = 6-8 per group). Echocardiography was performed before injection and 4 weeks later, after which rats were killed and immunohistochemical analyses performed.

Results: Connexin43 density was greater in cell-treated groups compared with that seen in the phosphate-buffered saline group (by 91.6% ± 15.2%, $P < .001$), with no observed difference between cell-treated groups ($P \geq .3$). Endothelial progenitor cell treatment increased arteriolar density within the infarct border zone (by 297%, 205%, and 101% vs phosphate-buffered saline, mesenchymal progenitor cell, and mesenchymal progenitor cell/endothelial progenitor cell treatment, respectively; $P < .01$). Postoperative left ventricular ejection fraction (endothelial progenitor cell: 68.3% ± 9.8% vs mesenchymal progenitor cell/endothelial progenitor cell: 55.0% ± 11.1%, mesenchymal progenitor cell: 53.0% ± 6.0%, and phosphate-buffered saline: 49.6% ± 9.5%) and fractional shortening (endothelial progenitor cell: 32.4% ± 5.1% vs mesenchymal progenitor cell: 22.5% ± 5.4% and phosphate-buffered saline: 21.3% ± 5.3%) were greater in endothelial progenitor cell–treated rats versus those receiving other treatments (all $P < .05$). Only endothelial progenitor cells prevented further contractile deterioration compared with baseline values ($P = .8$), whereas other groups had continued loss of function after treatment.

Conclusion: Compared with the use of mesenchymal progenitor cells, cell transplantation with endothelial progenitor cells after myocardial infarction resulted in better neovascularization and contractility. This suggests that angiogenesis is an important mechanism in attenuating the progression of left ventricular dysfunction after myocardial infarction.

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Cell-based myocardial regenerative therapies aim at safely using bone marrow–, blood-, or tissue-derived progenitor cells to restore perfusion and function to chronically ischemic, stunned, hibernating, or scarred myocardial areas and at improving patient quality of life and survival beyond the effects of other available therapeutic modalities. To date, this remains an elusive goal.

Under selected experimental conditions, bone marrow–derived mesenchymal progenitor cells (MPCs) have been reported to generate cardiomyocytes[1,2] and

Abbreviations and Acronyms

EPC = endothelial progenitor cell
FS = fractional shortening
HPS = hematoxylin-phloxine-saffron
LV = left ventricular
LVEF = left ventricular ejection fraction
LVID = left ventricular internal dimension
MI = myocardial infarction
MPC = mesenchymal progenitor cell
PBS = phosphate-buffered saline

therefore constitute a candidate for cell-based therapy. In myocardial infarction (MI) models, transplantation of MPCs into the infarct region has been shown to improve left ventricular ejection fraction (LVEF) and cardiac function.[3] However, studies on large animals and early clinical investigations of MPC therapy after MI have showed only modest regional contractile improvements with little or no global recovery, and underlying mechanisms remain unclear.[4-6] Recent evidence suggests that MPCs do not result in the formation of functional syncytia and that their effects in post-MI scarring might be mediated more by local paracrine angiogenesis than by cardiomyocyte differentiation.[3,7] Hence, angiogenesis (the formation of new blood vessels) might constitute an important mechanism of functional improvement not only among modalities that specifically aim at angiogenesis (ie, for the treatment of myocardial ischemia in the presence of viable heart muscle), but also among modalities aiming at myogenesis to improve cardiac function after an MI (ie, for the treatment of heart failure in the presence of nonviable myocardium).[8,9]

If angiogenesis is the underlying mechanism for improved cardiac function after MPC treatment after MI, then transplantation of angiogenic/vasculogenic progenitors might constitute another cell-based approach to achieve post-MI recovery. Among the potential sources of stem/progenitor cells, circulating endothelial progenitor cells (EPCs) and their derivatives have important angiogenic properties.[10] EPCs can be recruited from the blood to sites of angiogenesis, differentiate into endothelial cells, and proliferate to form new vasculature,[11] and might provide mitogenic factors for mature endothelial cells.[12] Animal models have demonstrated a role for EPCs in enhancing vascularization in the infarcted, as well as in the ischemic, heart.[13,14]

Two clinical trials have examined the effects of intracoronary marrow–derived cells versus EPC injections for the treatment of MI, without any observed difference in recovery between the 2 cell-treated groups.[5,15] However, no study to date has compared MPCs versus EPCs by using intramyocardial cell delivery, which is more targeted and effective than intracoronary injection.[16] Furthermore, no study has directly compared the underlying mechanisms of recovery and regeneration after cell therapy by using these 2 widely used cell populations. Therefore we compared and combined the use of MPCs and circulatory EPCs for cell-based therapy in a rat MI model and examined the hypothesis that the EPC, because of its high angiogenicity,[10,11] might elicit an equal or better therapeutic response than the MPC. The data presented herein suggest that focusing on the transplantation of primarily vasculogenic cells, such as the EPC, might be an equally viable or even superior cell-based approach for the treatment of post-MI cardiac dysfunction.

Materials and Methods

Cell Isolation and Culture

MPCs and EPCs were isolated from healthy donor syngeneic Sprague–Dawley rats (Charles River, Wilmington, Mass) weighing 200 to 250 g that did not undergo MI induction or any other manipulation. For EPCs, blood was collected from the aortas of anesthetized rats (2% isoflurane). Total peripheral blood mononuclear cells were isolated and cultured, as described previously.[17] Briefly, cultures were supplemented with endothelial basal medium with EGM-2-MV-SingleQuots (Clonetics, Guelph, Canada), and day 4 adherent cells represented the EPC population. Characteristic of EPCs, these cells stained positive for 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine–labeled acetylated low-density lipoprotein uptake and fluorescein isothiocyanate–labeled *Ulex europaeus* agglutinin 1 binding (not shown). For MPCs, tibias and femurs were dissected from rats after death. Bone marrow was extruded from rat tibias and femurs by using a needle and syringe and flushing with phosphate-buffered saline (PBS). Cells were isolated from the bone marrow extract by means of collagenase treatment (250 μL/mL; Sigma, Oakville, Canada) and then cultured at 1.3×10^5 cells/cm^2 until confluent (approximately 14 days) in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% fetal bovine serum, L-glutamine (2 mmol/L), penicillin (100 U/mL), and streptomycin (100 mg/mL). Nonadherent erythroid progenitor cells were removed with each medium change, yielding a population of adherent MPCs that characteristically expressed the antigens CD29 and CD44 but were negative for the hemopoietic cell marker CD45.[18] Before transplantation, MPCs or EPCs for single cell-type injections were labeled with 4′,6-diamidino-2′-phenylindole (Sigma); in the case of dual cell-type injections, MPCs were labeled as above, and EPCs were labeled with carboxyfluorescein diacetate (Molecular Probes, Eugene, Ore), according to the manufacturer's protocol.

Animal Model

Animal procedures (Figure 1) were performed with the approval of the University of Ottawa Animal Care Committee in accordance with the National Institute of Health's "Guide for the care and use of laboratory animals." Syngeneic Sprague–Dawley rats (200-250 g) were used for the infarction and cell transplantation model. Left coronary artery ligation procedures were performed by Charles River surgical services, and animals were shipped after recovery.

Baseline echocardiography was performed 14 days after ligation (as below). Animals were then randomized to receive injec-