Homing of intravenously infused embryonic stem cell-derived cells to injured hearts after myocardial infarction

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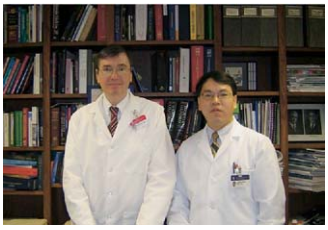

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This work was supported in part by National Institutes of Health grant R01 DA-12774 (Dr Morgan) and P01 HL76540-01 (Dr Oettgen) and National Institute of Aging grant 5P60 AG08814-13 (Dr Min). Dr Xiang is the recipient of a scholarship from the China Scholarship Council and the Foundation of Zhejiang Science and Technology (NO. 2003C34004).

Received for publication July 20, 2005; revisions received Nov 23, 2005; accepted for publication Dec 1, 2005.

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J Thorac Cardiovasc Surg 2006;131:889-97

0022-5223/$32.00

doi:10.1016/j.jtcvs.2005.12.022

Objective: The present study was designed to test whether intravenously infused embryonic stem cell-derived cells could translocate to injured myocardium after myocardial infarction and improve cardiac function.

Methods: Cultured embryonic stem cell-derived cells were transfected with green fluorescent protein. Embryonic stem cell-derived cells were administered through the tail vein (approximately 10^7 cells in 1 mL of medium for each rat) every other day for 6 days in 45 rats after myocardial infarction. Six weeks after myocardial infarction and cell infusion, cardiac function, blood flow, and the numeric density of arterioles were measured to test the benefits of cell therapy. An in vitro Transwell assay was performed to evaluate the embryonic stem cell migration.

Results: Ventricular function, regional blood flow, and arteriole density were significantly increased in rats receiving intravenously infused embryonic stem cell-derived cells compared with control rats after myocardial infarction. Histologic analysis demonstrated that infused embryonic stem cell-derived cells formed green fluorescent protein-positive grafts in infarcted myocardium. Additionally, positive immunostaining for cardiac troponin I was found in hearts after myocardial infarction receiving embryonic stem cell-derived cell infusion that corresponded to the green fluorescent protein-positive staining. The Transwell migration assay indicated that cultured neonatal rat cardiomyocytes with overexpression of tumor necrosis factor α induced greater migration of embryonic stem cells compared with cardiomyocytes without tumor necrosis factor α expression.

Conclusions: Our data demonstrate that intravenously infused embryonic stem cell-derived cells homed to the infarcted heart, improved cardiac function, and enhanced regional blood flow at 6 weeks after myocardial infarction. The in vitro migration assay suggested that such a homing mechanism could be associated with locally released cytokines, such as tumor necrosis factor α, that are upregulated in the setting of acute myocardial infarction and heart failure.

In recent years, cell transplantation has emerged as a potential therapy for heart failure caused by myocardial infarction (MI).[1-6] Initial efforts at cellular cardiomyoplasty have transplanted satellite cells,[1] skeletal myoblasts,[2] bone marrow-derived cells,[5] and fetal cardiomyocytes.[6] Embryonic stem cells (ESCs), which have better plasticity and cardiomyogenic capacity than the cell types listed above, have also been successfully transplanted into infarcted

Abbreviations and Acronyms

cTnI	= cardiac troponin I
DAPI	= 4′-6-diamidino-2-phenylindole
+dP/dtmax	= maximum rate of LV systolic pressure increase
−dP/dtmax	= maximum rate of LV systolic pressure decrease
EDC	= embryonic stem cell-derived cell
ESC	= embryonic stem cell
GFP	= green fluorescent protein
LV	= left ventricular
LVSP	= left ventricular systolic pressure
MI	= myocardial infarction
TNF	= tumor necrosis factor

rodent hearts[3,4] and the left ventricular (LV) wall of dystrophic mice.[7] Most of the previous studies delivered donor cells through intramyocardial injection after cardiac surgery. The advantage of this approach is that it traps implanted cells in selected injured areas of the heart. However, the procedure of intramyocardial injection is invasive and might not be suitable for patients with acute MI or severe congestive heart failure. Recently, Chiu and colleagues[8,9] demonstrated the feasibility of delivering bone marrow stromal cells with coronary infusion and indicated that marrow stromal cells could traffic through the coronary system into injured myocardium and form cardiomyocytes. Mobilized bone marrow cells, stimulated by stem cell factor or granulocyte colony-stimulating factor, have been shown to repair the infarcted mouse heart and improve ventricular function.[10] It appears that somatic stem cells can migrate to heart tissue and further differentiate into cardiomyocytes.[11]

MI is associated with inflammatory responses that include upregulation of mast cells, macrophages, and associated inflammatory cytokines. Experimental MI is also associated with activation of a series of cytokines.[12] Mast cell-derived tumor necrosis factor α (TNF-α) released after myocardial ischemia represents an "upstream" cytokine responsible for initiating the inflammatory cascade.[12] Released cytokines from injured myocardium might act as chemoattractants for circulating donor cell migration. Our previous study indicated that mouse ESC-derived cells (EDCs) infused intravenously were able to migrate into injured myocardium caused by encephalomyocarditis virus[13] and increased the survival rate of recipient mice.

The present study was designed to investigate whether intravenously infused EDCs could translocate to injured myocardium in response to locally released cytokines after MI and improve cardiac function. An in vitro culture system was used for testing whether TNF-α, an inflammatory cytokine that is upregulated in the setting of acute MI and heart failure, could facilitate EDC migration responding to cytokine stimulation.

Materials and Methods

EDC Preparation and Transplantation

The mouse ESC line ES-D3 was purchased from the American Type Culture Collection (Manassas, Va) and cultured with the handing drops method, as previously described.[3,4] Before transplantation, cells dissected from beating clusters were transfected with green fluorescent protein (GFP), a marker for identification of infused cells from host myocardium. Plasmids with an hCMV IE promoter/enhancer-driving GFP gene (5.7 kb) and Gene PORTER transfection reagent were obtained from Gene Therapy Systems Inc (San Diego, Calif). GFP-labeled EDCs could be detected under fluorescent microscopy at the second day of transfection, and the transfection efficiency was greater than 90% (Figure 1). Our previous study demonstrated that the action potentials recorded from spontaneously beating EDCs are very similar to those recorded in neonatal mouse cardiomyocytes.[3] Two days after GFP transfection, cultured EDCs were trypsinized and resuspended in Joklik modified medium (Sigma, St Louis, Mo), with a density of approximately 10^7 cells/mL for cell infusion.

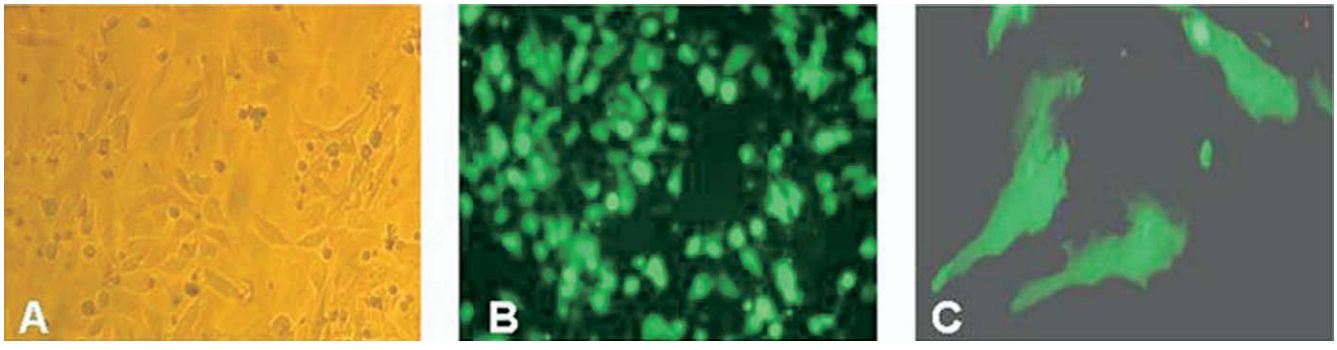

Figure 1. Confluent culture of EDCs under phase-contrast (A, original magnification 40×) and fluorescent (B, original magnification 40×; C, original magnification 400×) microscopy at 3 days after GFP transfection.

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