Skeletal Myoblasts Preserve Remote Matrix Architecture and Global Function When Implanted Early or Late After Coronary Ligation Into Infarcted or Remote Myocardium

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Background—The inability of skeletal myoblasts to transdifferentiate into cardiomyocytes suggests that their beneficial effects on cardiac function after a myocardial infarction are mediated by paracrine effects. We evaluated the roles of these factors in the preservation of matrix architecture (in the infarct and remote regions) by varying the timing (postmyocardial infarction) and delivery site of the implanted cells.

Methods and Results—Skeletal myoblasts ($5 \times 10^5$) or control media were injected into the infarct or noninfarcted myocardium at 5 or 30 days after coronary artery ligation in rats. Function was assessed by echocardiography before transplantation and 14 and 30 days thereafter and with a Millar catheter at 30 days after transplantation. Ventricular geometry, remote fibrillar collagen architecture, and changes in the matrix metalloproteinase-TIMP system were evaluated. Myoblast implantation in both sites and at both times preserved matrix architecture (length and width of collagen fibers) in the remote myocardium (in association with some decreases in remote myocardial matrix metalloprotease activity), improved global cardiac function, and attenuated the progressive increase in end diastolic volume ($P<0.05$ for all measures compared with medium controls). Cells delivered into the infarct region preserved scar thickness; cells delivered into the noninfarcted myocardium preserved wall thickness.

Conclusions—Regardless of whether the cells were injected into the infarct or the noninfarcted myocardium early after an myocardial infarction or later, skeletal myoblasts improved cardiac function by preventing ventricular dilation and preserving matrix architecture in the remote region, likely mediated by paracrine effects. (Circulation. 2008;118[suppl 1]:S130–S137.)

Key Words: cells ■ heart failure ■ myocardial infarction ■ remodeling ■ transplantation

More than a decade of preclinical studies and initial clinical trials indicate that the progression of postmyocardial infarction (MI) cardiac failure can be limited by the implantation of either skeletal myoblasts or bone marrow cells.1-4 However, improvements in ventricular function were less impressive in the clinical trials than in animal studies, perhaps due to advanced age and comorbidities in the patients. Identifying the mechanisms responsible for the functional improvements may permit clinicians to adapt cell therapies for elderly patients. Such enhancements could be suitable for therapies involving myoblasts, bone marrow cells, or both.

Considering that significant changes in cardiac geometry and function are affected by relatively few engrafted cells5,6 in the inhospitable environment of the myocardial scar tissue that do not transdifferentiate into cardiomyocytes,7 paracrine effects induced by the implanted cells have been proposed as likely mechanisms for the prevention of cardiac failure. Such effects could include the secretion of factors that stimulate angiogenesis, decrease apoptosis, and/or preserve extracellular matrix structure.8-11

We expect that implanted skeletal myoblasts prevent progressive ventricular dilation by influencing the healing of not only the infarct scar, but also the remote, noninfarcted myocardium. This ongoing remodeling may be difficult to influence, and the optimal timing for cell therapy has not been established.12 Some early clinical trials suggested similar benefits whether cells were implanted early or late after an infarct,13,14 but the effectiveness of the 2 approaches has not been directly compared. The current study evaluated the effects on ventricular remodeling of varying the timing (early or late after MI) and site (into the infarct or noninfarcted myocardium) of skeletal myoblast delivery in rats.

Methods

Experimental Animals

Syngeneic female Lewis rats (Charles River Canada Inc, Quebec, Canada) weighing 250 to 300 g were used in the study. The Animal
Skeletal Muscle Preconditioning, Cell Isolation, and Culture

Bupivacaine (0.5 mL) was injected into rat hind limb tibialis anterior muscles. Forty-eight hours later, preconditioned muscles were harvested and enzymatically dissociated as previously described. The isolated cells were resuspended in culture medium composed of F12 (20%), fetal bovine serum and cultured in humid air with 5% CO2. Skeletal myoblasts were immunohistochemically identified using an antibody against desmin. Myoblasts (5 x 10^6) were harvested and suspended in 150 μL of medium (per rat) just before transplantation.

Myocardial Infarction and Cell Transplantation

MI was generated by ligation of the left coronary artery through a thoracotomy, as previously described. Five days later, selected rats (fractional shortening between 20% and 35% by echocardiography) were randomly distributed into 6 groups, described subsequently. Transplantation was carried out either early (5 days after MI; early transplantation [Tx]) or late (30 days after MI; late Tx). These time points were chosen based on our previous studies: at 5 days, the initial myocardial inflammation that reduced the survival of cells implanted before Day 5 had subsided, and at 30 days, ventricular modulation was underway but not complete. Recipients at each time point were injected with either myoblasts (5 x 10^6) delivered into the infarct (C-I group) or the noninfarcted myocardium (C-M group), contralateral side of the left ventricle [LV] to avoid a direct effect of the cells on the infarcted myocardium), or culture medium (controls; medium group). At 5 days after Tx, a subgroup of rats from each group was euthanized for biochemical assays. The remaining rats from each group underwent functional analyses followed by euthanasia at 30 days after Tx for morphological and histological studies (supplemental Figure I).
Results

Characteristics of Cultured Skeletal Myoblasts

Before Tx, more than 80% of the cultured skeletal muscle cells were skeletal myoblasts (by immunostaining for desmin protein; Figure 1), and more than 90% were viable (by Trypan blue staining).

Myoblast Therapy Enhances Cardiac Function Regardless of the Timing or Site of Cell Injection

Although fractional shortening measured by echocardiography was similar in all groups before myoblast Tx, this measure increased comparably (P<0.05 for all groups) relative to medium controls at both 14 days and 30 days after Tx in all animals that received myoblasts (Figure 2). The magnitude of functional improvement was similar whether the cells were injected early or late after MI and into the scar area or the remote region.

At the end of the study, load-independent indices of LV systolic function calculated using pressure–volume loops generally indicated improvements after myoblast Tx. Specifically, Emax, end systolic elastance, preload recruitable stroke work, and dP/dt-EDV were all increased significantly (P<0.05) relative to medium controls in animals that received an injection of cells into either site at 30 days (late) after MI (Figure 3). Among the groups that received cells at 5 days (early) after MI, all 4 functional measures were significantly improved (P<0.05 versus controls) when the cells were delivered into the remote region.

Myoblast Therapy Attenuates Cardiac Thinning and Dilation and Preserves Remote Collagen Architecture

EDV was evaluated immediately before Tx (5 or 30 days after MI) and 14 and 30 days after Tx in all groups. For comparison with the groups that received Tx early after MI, EDV was also measured at 5 days after MI in the late Tx groups; volumes were similar among all groups at this point. Thereafter, the progressive increase in EDV exhibited by the medium recipients was attenuated by myoblasts delivered into the infarct or the remote region early or late after MI.

Among early-transplanted animals, EDV increased (1.4 times greater than pre-Tx values by 30 days) after medium injection (control) but did not change significantly after the Tx of myoblasts into the infarct or the noninfarcted myocardium (P<0.05 medium versus C-I and C-M at 14 days post-Tx; P<0.01 medium versus C-I and C-M at 30 days; Figure 4A). In late-transplanted animals, EDV again increased (1.6 times greater than baseline values by 30 days) after medium injection (control), but not after the Tx of myoblasts into the infarct or the noninfarcted myocardium (P<0.01 medium versus C-I and C-M at 30 days post-Tx; Figure 4B).

Changes in EDV were independent of rat body weights, which did not increase significantly over the duration of the experiments (mean body weights: early Tx group=269±5 g versus controls).
before Tx, 281±9 g at 30 days after Tx; late Tx group 286±7 g before, 291±6 g after).

Hematoxylin and eosin and Masson’s trichrome staining in histological heart sections demonstrated that the scar area thinned and the heart dilated after medium injection and that cell Tx attenuated the process (Figure 5A–L). Quantitative measurements confirmed that myoblasts injected directly into the infarct at either time point after MI significantly preserved scar thickness (30% to 60% thicker; P<0.05 C-I versus medium) but did not affect remote myocardial wall thickness (Figure 5M–N). On the other hand, cells implanted into the noninfarcted myocardium at either time point did not increase the thickness of the scar tissue, but did increase local wall thickness in the remote myocardium (P<0.05 C-M versus medium; Figure 5O–P).

Collagen architecture in the extracellular matrix of the remote (noninfarcted) myocardium was evaluated using an extensive confocal microscopic analysis. We observed fragmentation of the collagen network, characterized by significant reductions (P<0.05 versus cell groups in all comparisons) in perimysial fiber length and width, in the medium-injected controls, whereas collagen architecture was preserved in the remote myocardium of animals that received myoblast implantation into either site at either time point after MI (Figure 6).

**Ventricular Remodeling Is Associated With Biochemical Alterations After Myoblast Therapy**

Gelatinase activities of MMP-2 and -9 were evaluated in tissue harvested from the remote myocardium at 5 days after Tx (Figure 7A–D). In this region, MMP-2 activity was reduced (P<0.05 versus medium) after early (5 days after MI) but not late (30 days after MI) cell injection into either the infarct or the remote region, whereas MMP-9 activity was reduced (P<0.01 versus medium) after early cell injection into the noninfarcted myocardium and increased (P<0.05 versus medium) after late cell injection into the infarct. We

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**Cell Implantation and Matrix Modulation**

**Figure 3.** Cardiac function by pressure–volume loops. Load-independent indices of LV function (Emax, A–B; end systolic elastance, Ees, C–D; preload recruitable stroke work, PRSW, E–F; increase in pressure and time at the end diastolic volume, dP/dt-EDV, G–H) calculated using pressure–volume loops, measured 30 days after rats were injected with either skeletal myoblasts delivered into the infarct (C-I) or the noninfarcted myocardium (C-M), or with culture medium, at 5 days (early Tx) or 30 days (late Tx) after MI. All 4 measures were improved relative to medium after late Tx in C-I and C-M groups and after early Tx in C-I group; only Emax and dP/dt-EDV were significantly improved after early Tx in C-M group. n=6/group; *P<0.05 medium versus C-I and C-M; **P<0.01 medium versus C-I and C-M.

**Figure 4.** Ventricular volumes. Left ventricular EDV measured immediately before transplantation (pre-Tx, 5 or 30 days postinfarction) and 14 and 30 days (D) after rats were injected with either skeletal myoblasts delivered into the infarct (C-I) or the noninfarcted myocardium (C-M), or with culture medium, at 5 days (early Tx; A) or 30 days (late Tx; B) after MI. For comparison with early Tx groups, EDV was also measured at 5 days post-MI in the late Tx groups. The progressive increases in volume in the medium groups were attenuated after early Tx and late Tx in C-I and C-M groups. n=6/group/time point; *P<0.05 medium versus C-I and CM; **P<0.01 medium versus C-I and C-M.
observed no differences among groups in the remote myocardial protein levels of TIMP-3 or -4 (Figure 7E–H).

Together, these data suggest that myoblast therapy contributes to the recovery of ventricular function through varying effects on the elastic properties of the infarcted heart that alter matrix remodeling in the noninfarcted myocardium.

**Discussion**

After an MI, congestive heart dysfunction results from progressive ventricular dilation. In this study, medium injection resulted in scar thinning, ventricular dilation, and dysfunction associated with matrix disruption and collagen fragmentation in the noninfarcted myocardium. Skeletal myoblasts improved ventricular function and limited ventricular dilation whether the cells were injected into the infarct or the noninfarcted region either early (5 days) or late (30 days) after MI. These results suggest that paracrine mechanisms are responsible for the beneficial effects of cell Tx and that matrix preservation in the noninfarcted tissue is an important contributor. However, the structural and functional effects of

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**Figure 5.** Ventricular geometry. Representative macrographs illustrating hematoxylin and eosin (H & E, A–F) and Masson’s trichrome (G–L) staining in tissue sections collected at 30 days after rats were injected with either skeletal myoblasts delivered into the infarct (C-I) or the noninfarcted myocardium (C-M), or with culture medium, at 5 days (early Tx) or 30 days (late Tx) after MI. Areas within black borders in A, C, E, G, I, K shown enlarged in B, D, F, H, J, L; scale bars=100 μm. H & E and Masson’s trichrome staining demonstrates thinning and expansion of the infarct scar in the medium groups that was attenuated in the C-I and C-M groups. Quantification of scar thickness (M–N) and wall thickness in the remote (noninfarcted) myocardium (O–P). Scar thickness was increased relative to medium after early Tx and late Tx in C-I groups; remote myocardial thickness was increased relative to medium after early Tx and late Tx in C-M groups. n=6/group; *P<0.05 versus medium.
implanted myoblasts, several of which are identified in this study, are enacted through different sequences of events after cell implantation at different sites and times after MI.

The cardiac response to injury alters the size and shape of the heart and consequently its function. This process, cardiac remodeling, is characterized by well-defined clinical, physiological, and anatomic hallmarks. The myocardial microenvironment changes with time after an MI, and so the interlocking effects of myocardial stiffness, inflammatory cytokine release, and myocardial apoptosis explain why the heart fails months or years after the original insult. The potential for cell Tx to alter the course of remodeling through processes such as myogenesis, angiogenesis, stem cell homing, and extracellular matrix stabilization has been demonstrated in animal models. Although the mechanisms are unclear, preventing matrix modulation and ventricular dilation could be critical for functional improvement.

Ventricular modulation is progressive and the optimal timing (after MI) to administer cell therapy may be an important factor in optimizing the functional benefit of implanted cells. Clinical trials of cell therapy reported similar results when cells were injected early or late after an infarction.

Here, we demonstrated that implanted myoblasts preserved matrix collagen architecture when they were injected into the infarct or the noninfarcted myocardium either at 5 days after an MI (before ventricular remodeling had progressed significantly) or at 30 days (during the later stages of heart failure).

Cell Tx into the infarct prevented scar thinning, whereas cell Tx into the noninfarcted myocardium increased the wall thickness in that region. Still, considering that cardiac function and ventricular volumes were similarly preserved after cell injection into either site at either time, increased scar thickness (and presumably scar elasticity) is only one of many factors responsible for the functional benefits of cell therapy. Equally important are paracrine influences of the implanted cells, the effects of which on the cytokine milieu may be more important than the exact timing of cell delivery.

The structure of the remote extracellular matrix was adequately preserved in the cell-transplanted groups compared with the medium controls, suggesting a possible mechanistic contributor to the preserved wall thickness and ventricular volumes in groups that received cell therapy. In fact, relative to all measures assessed in response to cell therapy, the pattern of changes in the remote matrix architecture most closely matched that for global ventricular function. We previously found that the effect of cell Tx on matrix components occurred not only in the border zone of the infarct (the region into which cells were injected), but also in the wall opposite this site. The mechanisms responsible for matrix remodeling, particularly at a distance, are not obvious. The close interactions between MMPs and their natural inhibitors have a role, but TIMP-3 activity occurs attached to the matrix components in response to integrin signaling and may not be reflected in measured levels. Therefore, matrix remodeling...
can be achieved by a variety of stimuli without significant increases or decreases in the measured activities of MMPs or TIMPs.

A limitation of the current study is the fact that we evaluated only some of the multiple factors involved in the functional effects of cell therapy. We did not evaluate angiogenesis, marrow-derived progenitor cell recruitment, or myofibroblast activity in the heart, each of which is believed to be affected by cell Tx. A more detailed understanding of the mechanisms responsible for the benefits of implanted cells in animal models of cardiac injury may eventually permit more directed cell therapies for patients with acute or chronic ischemic injury who are at high risk for progressive congestive heart failure because they cannot be adequately revascularized using conventional interventions. To that end, this study demonstrated that improved ventricular function in response to cell Tx (whether early or late after MI, into the infarct (C-I) or the noninfarcted myocardium (C-M), or with culture medium, at 5 days (early Tx) or 30 days (late Tx) after MI. MMP-2 activity was reduced relative to medium after early Tx, but not late Tx, in C-I and C-M groups. MMP-9 activity was reduced relative to medium after early Tx in the C-M group and increased after late Tx in the C-I group. n=4/group; *P<0.05 versus medium; **P<0.01 versus medium.

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Disclosures
None.

References


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