Transforming Growth Factor-α Enhances Stem Cell-Mediated Postischemic Myocardial Protection

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Background. **Transforming growth factor-α (TGF-α) has been shown to augment mesenchymal stem cell-mediated cardioprotection during acute ischemia and reperfusion in isolated heart models. To determine whether this pretreatment strategy would be effective in vivo, we hypothesized that the intramyocardial injection of mesenchymal stem cells pretreated with TGF-α after coronary artery ligation would confer greater preservation of cardiac function, reduction in infarct size, and reduction myocardial inflammation.**

Methods. **Sprague-Dawley rats underwent left anterior descending coronary artery ligation. Ischemic border zones were injected 30 minutes later with vehicle (n = 11), 1 million mesenchymal stem cells (n = 9), or mesenchymal stem cells pretreated with TGF-α (250 ng/mL for 24 hours; n = 10). Cardiac function was assessed by echocardiography at 7 and 28 days after ligation. Infarct size was measured using triphenyltetrazolium chloride. Ischemic border zone cytokine expression was measured 30 days after infarction.**

Results. **Myocardial function after ligation was greatest in hearts injected with cells pretreated with TGF-α in association with reduced ventricular remodeling and infarct size compared with vehicle-injected hearts. Myocardial interleukin 1β, interleukin 6, and TNF-α concentrations were lower, and Bcl-2 expression was higher, in hearts injected with either cell type. Vascular endothelial growth factor and matrix metalloproteinase-2 expression were highest in hearts that received pretreated cells.**

Conclusions. **Intramyocardial injection of mesenchymal stem cells pretreated with TGF-α further protects cardiac function and reduces infarct size compared with injection of untreated cells. Pretreating donor cells with TGF-α may be useful for enhancing cell-based therapies for myocardial ischemia.**

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Delivery of autologous bone marrow–derived cells to ischemic myocardium has been shown to improve left ventricular (LV) function, infarct size, and LV remodeling [1]. However, these benefits may have limited duration partly due to barriers to stem cell survival and function after transplantation [2]. Stem cells protect ischemic myocardium partly through the paracrine release of growth factors, which in turn augment myocardial angiogenesis, reduce inflammation, and promote cardiomyocyte survival [3]. Accordingly, strategies designed to augment stem cell paracrine function have been used in an attempt to improve their therapeutic efficacy.

One promising agent for enhancing mesenchymal stem cell (MSC) paracrine function and cardioprotection is transforming growth factor-α (TGF-α), a ligand of the epidermal growth factor (EGF) receptor that is expressed by numerous cell types, including MSCs [4]. After EGF receptor activation, MSCs exhibit increased proliferation, migration, and survival [4, 5].

We have also found that TGF-α directly stimulates MSC production of vascular endothelial growth factor (VEGF) in vitro [6, 7]. VEGF exhibits numerous protective responses in the heart, including promoting cell survival in the acute setting and promoting angiogenesis and increased tissue perfusion during chronic ischemia [8, 9].

Additional putative mechanisms of stem cell-based cardioprotection during ischemia include reducing inflammatory cytokine production [10], promoting upregulation of survival signals such as Bcl-2 [11], and reducing negative remodeling by downregulating fibroblast activity and collagen deposition [12].

Using a model of isolated heart perfusion, we previously observed that MSCs pretreated with TGF-α conferred greater cardioprotection in the acute setting when infused through an intracoronary route before ischemia and reperfusion or injected into the myocardium after left anterior descending artery ligation [7, 13]. However, it remains unknown whether pretreating MSCs with TGF-α may improve their cardioprotective efficacy in vivo. We hypothesized that the intramyocardial injection of MSCs pretreated with TGF-α after coronary artery ligation would be associated with greater myocardial functional recovery, decreased ven-

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Abbreviations and Acronyms

AWTs = anterior wall thickness in systole
FS = fractional shortening
LAD = left anterior descending
LV = left ventricle
LVDP = left ventricular developed pressure
LVEDD = left ventricular end-diastolic diameter
LVEF = left ventricular ejection fraction
LVESD = left ventricular end-systolic diameter
MSC = mesenchymal stem cell
SEM = standard error of the mean
TGF-α = transforming growth factor-α
VEGF = vascular endothelial growth factor

tricular remodeling, reduced infarct size, reduced myocardial inflammation, and greater induction of survival signaling.

Material and Methods

The animal protocol used in this study was reviewed and approved by the Institutional Animal Care and Use Committee of Indiana University. All animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85Y23, revised 1996).

Animals

Normal adult (aged 8 to 9 weeks) male Sprague-Dawley rats and male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were fed a standard diet and acclimated in a quiet quarantine room.

Preparation of Bone Marrow MSCs

A single-step purification method using adhesion to cell culture plastic was used as previously described [14]. Mice were euthanized, and bone marrow cells were collected from bilateral femurs and tibias by removing the epiphyses and flushing the shafts with complete media (Iscove's Modified Dulbecco Medium with 10% fetal bovine serum; GIBCO Invitrogen, Carlsbad, CA) using a syringe with a 23-gauge needle. Cells were washed by adding complete media, centrifuged for 5 minutes at 300 rpm at 24°C, and the supernatant was removed.

The cell pellet was resuspended and cultured in 75 cm^2 culture flasks with complete media at 37°C, 90% humidity, and 5% CO_2 in air. MSCs preferentially attached to the polystyrene surface; after 48 hours, nonadherent cells in suspension were discarded. Fresh complete medium was added and replaced every 3 to 4 days thereafter. After three passages, cell surface marker expression was analyzed using flow cytometry. Cells were positive for Sca-1 and CD44 and negative for CD45, CD11b, and CD117 before and after TGF-α treatment [14, 15].

Experimental Groups

After three passages, 1.5×10^6 murine MSCs were transferred to 75 cm^2 culture flasks in complete media, with or without TGF-α (250 ng/mL) 24 hours before infusion. The TGF-α dose and 24-hour dosing period were chosen based on previous work [6]. Cells were recovered using a 0.25% trypsin-ethylenediaminetetraacetic acid solution (GIBCO Invitrogen), washed to remove media containing TGF-α, and resuspended in sterile phosphate buffered saline. Each injection comprised 1×10^6 cells. Groups included (1) sham ligation (n = 12), and injection with (2) phosphate buffered saline vehicle (n = 11), (3) MSC (n = 9), and (4) MSC pretreated with TGF-α (MSC+TGF; n = 10).

Myocardial Infarction Model

Myocardial infarction was induced as previously described [16]. Rats (weight, 250 to 275 g) were anesthetized with 5% inhaled isoflurane. After endotracheal intubation and initiation of ventilation (InspiraASV Rodent Ventilator, Harvard Apparatus, Holliston, MA), the proximal left anterior descending coronary artery was ligated with a 6-0 polypropylene suture 4 mm distal to the bifurcation. Successful ligation was confirmed by visible ischemia and decreased anterior wall motion. Vehicle or cell suspensions (50 μL × 4) were injected into the ischemic border zones 30 minutes after ligation. Three rats in the vehicle group died within 30 minutes of ligation, but no rats died in the cell treatment groups.

Myocardial Function and Dimensions

Parasternal short-axis 2-dimensional echocardiography (Vevo-770, VisualSonics, Toronto, ON, Canada) of the LV at the level of the papillary muscle was performed at baseline and at 7 and 28 days after ligation. LV ejection fraction (LVEF), fractional shortening (FS), LV end-systolic diameter, LV end-diastolic diameter, and anterior wall thickness in systole were measured over 3 adjacent cardiac cycles.

Additional variables of global LV function, including LV developed pressure and +/−dP/dt (representing rate of pressure generation and relaxation), were also evaluated using the Langendorff model of isolated heart perfusion, as previously described [17]. Rats were anesthetized with intraperitoneal sodium pentobarbital (60 mg/kg) and heparinized (500 U). Hearts were rapidly excised through a median sternotomy and placed in a modified 4°C Krebs-Henseleit solution (119mM NaCl, 20.8mM $NaHCO_3$, 11mM dextrose, 12mM $CaCl_2(2H_2O)$, 47mM KCl, 11.7mM $MgSO_4(7H_2O)$, and 11.8mM KH_2PO_4).

The aorta was cannulated and the heart perfused under constant pressure (mean 75 mm Hg) with oxygenated (95% O_2/5% CO_2) Krebs-Henseleit solution (37°C). A water-filled latex balloon was inserted through the left atrium into the LV. The balloon was adjusted to a mean end-diastolic pressure of 5 to 10 mm Hg, and the hearts were allowed to equilibrate for 15 minutes. Pacing wires were fixed to the right atrium and the LV, and the hearts were paced at approximately 6 Hz, 3V, 2 ms (350 beats/min) to ensure a