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Stem Cells for Myocardial Revascularization and Regeneration

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Early reperfusion of the infarct-related artery (IRA) improves both early and late outcomes in patients with acute myocardial infarction (MI). However, adverse left ventricular (LV) remodeling and the subsequent development of heart failure (HF) remain important causes of morbidity and mortality, as well as significant cost to the healthcare system. As the pathogenetic mechanisms of LV remodeling (Table 1) and the associated neurohormonal activation are only partially mitigated by timely revascularization and aggressive medical therapy with angiotensin-converting enzyme (ACE) inhibitors, beta-blockers, and aldosterone antagonists, new strategies for myocardial preservation are needed. The recent identification and characterization of circulating bone marrow-derived stem cells capable of myocardial revascularization and regeneration is revolutionalizing our understanding of vascular biology and has important clinical implications for the treatment of a wide spectrum of cardiovascular disorders.

A brief history

Unlike the lower vertebrates, the human heart has been traditionally viewed as having little regenerative potential in response to injury. In addition, adaptive angiogenesis and collateral vessel formation is often limited in patients with coronary disease as a consequence of chronic hypertension, diabetes, hypercholersterolemia, and older age.[1] The presence of circulating bone marrow-derived mononuclear cells capable of forming endothelial cells and new blood vessels (vasculogenesis) in human adults was first described by Asahara and colleagues in 1997.[2] These cells possess the capacity for self-renewal and were termed endothelial progenitor cells (EPCs). This represented a major paradigm shift in vascular biology, as previous dogma had asserted that vasculogenesis occurred only during fetal development, while angiogenesis (new blood vessel formation from preexisting vessels through local endothelial proliferation and migration) was thought to predominate in the adult.

Stem cells can be classified on the basis of cell surface markers and functional characteristics (Table 2). EPCs and circulating EPCs (CPCs) are types of bone marrow-derived hematopoietic stem cells that differentiate primarily into endothelial cells and contribute

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Table 1: Mechanisms of post-MI left ventricular remodeling.

- Infarct expansion with LV dilatation and thinning
- Scar formation, interstitial collagen deposition
- Altered calcium handling
- Left ventricular hypertrophy
- Cardiomyocyte apoptosis
- Local production of neurohormones

to neovascularization of ischemic tissue.[3,4] Moreover, recent data suggest that EPCs can also differentiate into cardiomyocytes.[5] In contrast, bone marrow-derived mesenchymal stem cells (MSCs) are considered to be of a non-hematopoietic lineage and capable of differentiating into smooth muscle cells and cardiomyocytes, as well as endothelial cells. Strikingly, animal models suggest that MSCs are also capable of myocardial repair after MI.[6] Although the past 6 years have witnessed an explosion in new information about stem cells, our understanding of stem cell biology is still at a very early stage, and the above classification will likely evolve as new data becomes available.

Biological roles

Stem cells play a fundamental role in the response to vascular injury, as illustrated by the following observations:

- In a canine study of bone marrow transplantation, Dacron grafts implanted in the thoracic aorta were found to be colonized exclusively with CD34+ endothelial cells of donor origin.[7]
- In a rabbit model of carotid angioplasty, transplanted EPCs were found to home to the region of balloon injury, restoring the endothelial lining and limiting neointimal hyperplasia.[8]
- Piechev *et al* found that the surface of implanted left ventricular assist devices in patients with endstage HF were colonized with CD133+/VEGFR-2 and EPCs.[9]

These studies underline the role of stem cells in re-endothelialization of damaged vessels, thereby reducing the risk of thrombosis.

Table 2: Classification and function of bone marrow-derived stem cells

Endothelial progenitor cells: Hematopoietic precursors

- Cell surface antigens: CD34+, CD133+, VEGFR-2
- Vasculogenesis, cardiomyocyte regeneration

Mesenchymal stem cells: Non-hematopoietic precursors

- Cell surface antigens: CD34-, c-kit, CD90, CD117
- Myocardial repair, cardiomyocyte regeneration, vasculogenesis

A number of studies have demonstrated that stem cells migrate to regions of tissue ischemia, where they directly promote vasculogenesis, as well as angiogenesis through the local secretion of vascular growth factors (eg, vascular endothelial growth factor [VEGF] and angiopoietins).[3,4] Experimental models of MI employing transplanted human *ex vivo* expanded EPCs or CD34+ cells demonstrated that these cells home to and incorporate into ischemic myocardium, with an associated enhancement of capillary density and collateral vessel formation. Significantly, animals that received EPCs or CD34+ cells had reduced left ventricular remodeling and preserved left ventricular function when compared to control animals. The exact mechanism(s) of these beneficial effects is an area of active investigation.

Recent data have led to the unexpected finding that stem cells are capable of myocardial repair and regeneration. In a seminal study by Orlic and colleagues, mice with MI induced by coronary ligation underwent intramyocardial injection of $Lin^{-}/c\text{-}kit^{+}$ MSCs 3-5 hours post-MI, and hearts were examined 9 days later.[6] In mice that received MSCs, 68% of the infarct zone was found to be composed of regenerating myocardium of transplanted stem cell origin. These new cardiomyocytes were found to express cardiac-specific proteins such as cardiac myosin and formed functional gap junctions. Robust neovascularization was also observed, with stem cell-derived smooth muscle and endothelial cells lining new blood vessels. These struc-

tural changes translated into significant functional benefits, with a 36% reduction in LV end-diastolic pressure and 35%-40% increases in LV developed pressure and +dP/dt.

Stem cells and vascular risk factors

It is well known that vascular risk factors (VRFs) contribute directly to endothelial dysfunction. Vasa and colleagues noted an inverse correlation between the number of VRFs and number and migratory activity of circulating EPCs in patients with stable coronary artery disease (CAD).[10] Smoking was found to be independently associated with EPC apoptosis, while hypertension was associated with impaired EPC migration.

These observations were further extended by Hill *et al*, who examined EPCs in asymptomatic men with VRFs, but no overt vascular disease.[11] The number of circulating EPCs was found to inversely correlate with the Framingham Risk Score (FRS), and was a better predictor of endothelial dysfunction (measured as forearm blood flow) than the FRS itself. Interestingly, patients with a higher FRS had a higher rate of EPC senescence *in vitro*, which the authors suggested may indicate VRF-induced accelerated aging and bone marrow exhaustion. Although the underlying mechanisms are presently unclear, preliminary data suggest that these effects may be mediated in part by chronic arterial inflammation and C-reactive protein (CRP).

Stem cells and the statins

HMG-CoA reductase inhibitors (statins) are a powerful class of drugs with both lipid-lowering and pleiotropic effects on vascular function. Recent studies have suggested that statins may also have effects on stem cell number and function. Vasa and colleagues found that treatment with atorvastatin 40 mg per day for 4 weeks significantly increased the number of circulating EPCs and their migratory activity when compared to controls.[12] Moreover, recent data suggest that statins induce EPC proliferation and decrease EPC apoptosis, likely through activation of the PI3K-Akt survival signaling pathway. In addition, experimental studies have demonstrated statin-induced angiogenesis in normocholesterolemic animals, and accelerated re-endothelialization after balloon injury.[8]

Clinical trials

The beneficial effects of stem cells in animal models have recently been extended to patients with acute and chronic coronary syndromes. In a nonrandomized phase I study by Strauer *et al*, 20 male patients with acute ST-elevation MI (STEMI) were treated with primary percutaneous coronary intervention (PCI) + stenting +/- intracoronary transplantation of bone marrow cells in the IRA.[13] There was 100% glycoprotein IIb-IIIa inhibitor, ASA, clopidogrel, statin, beta-blocker and ACE inhibitor use, and patients were excluded if they had shock, severe comorbidity, or late presentation (>72 h). Seven days after initial presentation, the stem cell group underwent bone marrow aspiration, gradient centrifugation, and overnight cell culture, followed by intracoronary infusion in the IRA using a balloon angioplasty technique. After 3 months, patients in the stem cell group had a significant improvement in LV wall motion, with decreased infarct size and LV remodeling relative to baseline values and control patients. This was associated with significantly increased perfusion on single photon emission computed tomography (SPECT) imaging and a trend towards improvement in ejection fraction (EF). Importantly, no adverse effects of stem cell therapy were reported.

In the similar, nonrandomized Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) study by Assmus and colleagues, 20 patients with acute STEMI received primary PCI + stenting, +/- either intracoronary transplantation of bone marrow mononuclear cells (same day) or circulating EPCs (after 3-day cell culture). Cell transplantation occurred at 4.3±1.5 days post-MI. After 4 months of follow-up, patients in the stem cell group showed significant improvements in EF, reduced LV remodeling, and improvement in perfusion on positron emission tomography (PET) imaging. Again, no adverse effects were documented.[14]

Perin and colleagues recently reported a prospective, nonrandomized, phase I study of intramyocardial injection of stem cells in 21 patients with chronic ischemic cardiomyopathy, EF <40%, and reversible defects on SPECT imaging who were not candidates for PCI or coronary artery bypass grafting (CABG).[15]

Bone marrow was aspirated 4 hours prior to injection; stem cells were isolated by gradient centrifugation and then injected into the ischemic territory using NOGA catheter mapping. At 4-month follow-up, cell therapy patients demonstrated significant improvements in coronary calcium score and New York Heart Association (NYHA) class, LV remodeling, perfusion imaging, and EF. No adverse effects were reported with this technique.

The first randomized controlled trial of stem cell therapy, the BOOST trial, was recently presented at the American Heart Association 2003 meeting. Wollert and colleagues randomized 60 patients with acute STEMI to primary PCI +/- intracoronary stem cell infusion using a balloon angioplasty technique. Bone marrow was aspirated, centrifuged, and infused on the same day, 4-8 days post-MI. At 6 months, there was a significant improvement in the primary outcome of LVEF in the stem cell group (6.7%, p=0.028) compared to the baseline and control groups.[16] Similar benefit was seen in both men and women, regardless of lesion location. Interestingly, late presenters seemed to derive the greatest benefit from stem cell therapy. No adverse events were reported and post-MI Holter monitoring and 6-month follow-up electrophysiological studies did not document an increased incidence of arrhythmias in the cell therapy group.

A similar, smaller, randomized study of 40 patients with STEMI treated with primary PCI +/- intracoronary stem cell infusion was reported at the same meeting by Brehmet *et al*. A significant reduction in infarct size, accompanied by significantly increased perfusion on SPECT imaging was found in patients receiving cell therapy.

Safety

Early indications from clinical trials suggest that stem cell therapy may be a powerful new therapeutic strategy to minimize LV remodeling and promote myocardial regeneration in both acute and chronic coronary syndromes. However, several safety issues must be addressed before stem cells can be incorporated into clinical practice. Firstly, large randomized trials with sufficient follow-up and close monitoring for adverse events must be conducted to document both long-term efficacy and safety. Given the heterogeneous nature of bone marrow stem cells currently used in clinical trials, there is a theoretical risk of abnormal scar formation that might serve as a substrate for arrhythmias. Other possible adverse effects include uncontrolled cell growth within the heart and remote tumour angiogenesis. In addition, the safety of bone marrow aspiration in the post-MI period needs further study. Alternatively, the use of circulating peripheral stem cells may provide a safer approach. Ongoing studies will delineate the true risks associated with stem cell therapy.

Therapeutic strategies

There are a number of technical issues to be resolved in the rapidly evolving field of stem cell transplantation.[3,4] The optimal cell population remains to be determined, although success has been achieved with EPCs, MSCs, as well as whole bone marrow mononuclear cells. Direct comparisons and further refinements in cell isolation and culture will help identify the ideal stem cells for clinical use. Several routes of delivery exist, each with distinct advantages and limitations. The intravenous route is simple and noninvasive, but the number of cells reaching ischemic myocardium may be limited by uptake in the lung. Intracoronary delivery is relatively simple and could be widely implemented in catheterization labs, however, it depends on a patent and functional vasculature to reach target myocardium. Direct intramyocardial injection circumvents this issue; however, this technique is labour intensive and expensive.

Future directions

The combination of vascular growth factor gene therapy and stem cells may augment neovascularization of ischemic myocardium by providing an endothelial substrate that is better able to respond to angiogenic signals. Another approach

involves the generation of transgenic stem cells that express vascular growth and survival factors such as VEGF or endothelial nitric oxide synthase (eNOS) that could directly induce local vasculogenesis as well as angiogenesis via a paracrine effect. Finally, the recent identification of multipotent cardiac stem cells residing in human myocardium raises the exciting therapeutic possibility of inducing the heart to heal itself from within.[17,18]

Conclusions

Bone marrow-derived stem cells offer a novel approach to treating both acute and chronic cardiovascular disease, and ongoing clinical trials will determine their optimal use in MI, heart failure, stroke, and peripheral vascular disease. Although the exact cell population, route of delivery, and long-term safety must be determined before stem cell therapy can enter into clinical practice, these issues do not appear insurmountable. Ultimately, the combination of stem cell therapy with early revascularization and aggressive medical therapy may make "dying of a broken heart" a thing of the past.

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Abstracts of Interest

Randomized Controlled Clinical Trial of Intracoronary Autologous Bone Marrow Cell Transfer Post Myocardial Infarction

WOLLERT KC, MEYER GP, LOTZ J, ET AL.
HANNOVER, GERMANY

Experimental data and small, open, uncontrolled clinical feasibility studies have suggested that transplantation of autologous bone marrow cells (BMC) to the ischemic area may enhance LV function after myocardial infarction (MI). However, data from prospectively designed, controlled clinical trials are lacking. We performed a randomized, controlled trial in patients after acute ST-elevation MI and successful primary or rescue percutaneous coronary intervention (PCI). The change in left ventricular ejection fraction (LVEF), as determined by magnetic resonance imaging (MRI; assessed by two investigators blinded for treatment assignment), 5 to 6 months after MI as compared to baseline was defined as the primary end point. Patients with hypokinesia or akinesia of 2/3 of the LV anterior, septal, lateral, or inferior wall (as determined by angiography immediately after PCI) were eligible for the trial. After providing informed consent, patients were randomized to the control (CON, n=30) and BMC (n=30) groups and underwent MRI to determine baseline LVEF. In the BMC group, 128±33 mL of bone

marrow was then obtained. Nucleated BMC were enriched by 4% gelatin-polysuccinate sedimentation and were transplanted (4 to 8 days post MI) into the infarct-related coronary artery through the central lumen of an over-the-wire balloon catheter (25±9 x108 nucleated BMC, 9.5±6.3 x10^6 CD34pos cells). After 5 to 6 months, MRI was repeated in all patients. There were no significant differences between the CON and BMC groups with regard to age, infarct localization, time to PCI (median 8.0 vs 9.8 h), maximum creatine kinase levels, and baseline LVEF (51.3±9.3 vs 50.0±10.0%). After 5 to 6 months, LVEF in the CON and BMC groups had improved by 0.7±8.1% and 6.7±6.5%, respectively (P<0.01, all data are shown as mean±SD). There was no evidence for proarrhythmic effects in the BMC group, as determined by repeated Holter monitoring and an electrophysiological study 5 to 6 months after BMC transplantation. The results from this randomized, controlled trial indicate that intracoronary transplantation of autologous BMC is safe and enhances LV function in patients post MI and successful PCI.

Circulation 2003;108:2723.

Adult Cardiac Stem Cells are Multipotent and Support Myocardial Regeneration

BELTRAMI AP, BARLUCCHI L, TORELLA D, ET AL. VALHALLA, USA.

The notion of the adult heart as a terminally differentiated organ without self-renewal potential has been undermined by the existence of a subpopulation of replicating myocytes in normal and pathological states. The origin and significance of these cells has remained obscure for lack of a proper biological context. We report the existence of Lin(-) c-kit(POS) cells with the properties of cardiac stem cells. They are self-renewing, clonogenic, and multipotent, giving rise to myocytes, smooth muscle, and endothelial cells. When injected into an ischemic heart, these cells or their clonal progeny reconstitute well-differentiated myocardium, formed by blood-carrying new vessels and myocytes with the characteristics of young cells, encompassing approximately 70% of the ventricle. Thus, the adult heart, like the brain, is mainly composed of terminally differentiated cells, but is not a terminally differentiated organ because it contains stem cells supporting its regeneration. The existence of these cells opens new opportunities for myocardial repair.

Cell 2003;114:763-776.

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