Mesenchymal Stem Cells and Their Potential as Therapeutics in Ischemic Heart Disease

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There has been a rapid progress in research to reveal the molecular mechanism underlying therapeutic effect of mesenchymal stem cells (MSCs) on cardiovascular diseases. To maximize the beneficial potency of MSCs, various factors such as growth factors and cytokines are applied both directly and indirectly. In addition to modification of stem cell itself, safe and feasible stem cell sources including umbilical cord blood and adipose tissue are actively developed. In conclusion, modified MSCs from various adult tissue were showed beneficial and therapeutic effects on cardiovascular diseases.

Keywords: Mesenchymal stem cell; Myocardial infarction; Modification

Introduction

Despite the rapid advances in medical therapy and coronary revascularization procedures, coronary artery disease (CAD) remains the major cause of mortality in many countries.1 In patients with severe CAD, persistent myocardial ischemia in myocardium resulted in progressive loss of cardiomyocytes with development of heart failure. Recent experimental studies have demonstrated adult stem cells can improve cardiac function, and these findings have prompted the development of different cellular transplantation approaches for heart diseases refractory to conventional therapy after myocardial infarction.2 Although experimental and clinical trials have shown potential benefit of cell therapy for myocardial regeneration, the long-term safety, the optimal timing and cell delivery method remains unclear.2–9

Stem Cell Therapy for Myocardial Infarction

Mesenchymal stem cells (MSCs) represent a stem cell population present in adult tissues that can be isolated, expanded in culture, and characterized in vitro and in vivo.10 MSCs differentiate readily into chondrocytes, adipocytes, osteocytes, and they can support hematopoietic stem cells or embryonic stem cells in culture.11 Evidence suggests MSCs can also express phenotypic characteristics of endothelial, neural, smooth muscle, skeletal myoblasts, and cardiac myocyte cells.12 When introduced into the infarcted heart, MSCs prevent deleterious remodeling and improve recovery, although further understanding of MSC differentiation in the cardiac scar tissue is still needed. MSCs have been injected directly into the infarct myocardium, or they have been administered in intra-coronary or intrave-
ous route. Examination of the interaction of allogeneic MSCs with cells of the immune system indicates little rejection by T cells. Persistence of allogeneic MSCs in vivo suggests their potential “off the shelf” therapeutic use for multiple recipients. Research continues to support the desirable traits of MSCs for development of cellular therapeutics for many tissues, including the cardiovascular system.

**Modification of Stem Cells**

One of the reasons for marginal improvement after cellular transplantation could be a significant cell death rate of implanted cells after grafting into injured hearts. Therefore, a strategy to overcome the poor survival rate of implanted cells is critical for improving the efficiency of stem cell therapy. Activation of Akt, a serine-threonine kinase, in cardiomyocytes has been shown to protect against apoptosis after ischemia/reperfusion injury.

We performed the study to examine whether MSCs transduced with Akt enhance cardiac repair after transplantation into the ischemic porcine heart. MSCs isolated from porcine bone marrow and transduced with myr-Akt were transplanted into porcine hearts after experimental myocardial infarction (MI) using intracoronary injection [Group I, vehicle; Group II, MSCs; Group III, Akt-MSCs]. Myocardial single photon emission tomography was performed to assess myocardial function and the infarcted area. Pigs were also sacrificed for immunohistochemical characterization and histologic analysis. In addition, in vitro assays were performed to examine the resistance of Akt-MSCs to H₂O₂ stimulation. Transplantation of MSCs into the ischemic porcine myocardium (Group II) increased the left ventricular ejection fraction (ΔLV EF; −6.3±15.1% versus 5.8±11.3%, p<0.001) and in Δarea of MI (6.8±5.6% versus −17.0±7.6%, p<0.001).

Akt-MSCs were more resistant to apoptosis, and the levels of extracellular signal-regulated protein kinase activation and vascular endothelial growth factor were higher in H₂O₂-stimulated Akt-MSCs. Cellular transplantation of Akt-MSCs further enhances the repair of injured myocardium compared to MSC transplantation alone by increasing the number of viable MSCs after cellular transplantation.

Granulocyte colony stimulating factor (G-CSF) enhances bone marrow stem cell mobilization during acute myocardial infarction. There is increasing evidence that stem cell mobilization to the heart and their differentiation into cardiac cells is a naturally occurring process. The effects of G-CSF on myocardial infarction were very controversial, and the conflicting results were reported. Several studies reported that G-CSF therapy did not improve left ventricular recovery in patients with acute myocardial infarction after successful mechanical reperfusion.

Kang et al. raised the concerns about the safety of G-CSF because of the potential for an increase in the rate of in-stent restenosis (ISR). Our recent study revealed that G-CSF administration enhanced the ISR significantly which suppressed by sirolimus-eluting stent in porcine myocardial infarction model. We implanted Bare stents and drug eluting stent (DES) in coronary arteries (bare stents, Group I; bare stents with G-CSF, Group II; DES, Group III; DES with G-CSF, Group IV, n=10 in each group). G-CSF (10 μg/kg/day) was injected for 7 days after stent implantation. G-CSF in bare stents enhanced ISR, and DES could be a good strategy to prevent the G-CSF-stimulated proliferation and migration of smooth muscle cells, which could be responsible for neointimal hyperplasia.
New Stem Cell Sources

In case of adult tissue stem cells, both number and function of a stem cell was depressed in a senile patient at severe coronary risk factors. Therefore, marrow-derived stem cell, obtained from such patients, may not work well, as was shown in the experimental animal. Based on this, we tested cardiomyogenic potential of the human umbilical cord blood (UCB) derived MSCs, which is a candidate for new stem cell source, since it can be obtained from younger population. Other sources of MSCs from adipose tissue (AD), umbilical cord matrix, and menstrual blood etc. are under investigation. Umbilical cord blood and adipose tissue have traditionally been discarded as a by-product of the birth process and liposuction. Umbilical cord blood stem cell transplants are less prone to rejection probably because the cells have not yet developed the features that can be recognized and attacked by the recipient's immune system.\(^\text{40,41}\) Both the versatility and availability of these stem cells make them potent resources for transplant therapies.\(^\text{42-45}\) Our colleagues compared gene expression patterns and the data analysis is processed (Fig. 1). These data could provide the basis for investigation of specific characteristics involved in the cell therapy of UCB-MSCs to cardiovascular diseases.

We elucidated whether human UCB and AD derived MSCs survive and engrat in experimentally induced ischemic rat myocardium. MSCs were successfully isolated and characterized from human UCB and AD. Myocardial infarction was induced by ligation of left anterior descending coronary artery (LAD) for 30 minutes followed by release in rats. Cells (1-2\(\times\)10\(^6\)) or media only (control) were injected in 3 designated points around the infarcted area and immunosuppressive agent using cyclosporine was treated during follow-up time. Before and 4 weeks after injection, the echocardiography was performed. Tracking of human cells were performed in situ hybridization for human X and Y chromosome and bioluminescence imaging. And histological studies were performed by H&E staining, masson trichrome staining, and immunohistochemistry.

In situ hybridization and bioluminescence imaging revealed transplanted human-derived MSCs were engrafted in the myocardium.

Myocardial fibrosis was significantly decreased in UCB and AD derived MSC-treated infarcted myocardium compared with control one (Fig. 2). Scar tissue from cell-treated animals was significantly populated with cardiomyocyte like cells indicated by alpha-smooth

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**Fig. 1.** Different cDNA microarray patterns of gene expression between UCB- and BM-MSCs were displayed as a graph.
Fig. 3. Fractional shortening and ejection fraction were assessed by echocardiographical analysis 2 weeks MSCs injection. In both UCB-MSCs and AD-MSCs injected rats, FS and EF were improved than vehicle injected rats, *p<0.05 vs. Vehicle.

Fig. 4. The number of MSCs on infarcted myocardium was increased by TNF-α pretreatment. Noggin, an antagonist of bone morphogenic protein, attenuated the engraftment of TNF-α-treated MSCs. *p<0.05 vs. MSC group, †p<0.05 vs. TNF-α MSC group

In summary, MSCs from different sources were provided for development of stem cell therapeutics, and their potential use in the cardiovascular system is currently under investigation in the laboratory and clinical settings.

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