Transcoronary Infusion of Bone Marrow Derived Multipotent Stem Cells to Preserve Left Ventricular Geometry and Function After Myocardial Infarction

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ABSTRACT

Objectives: Myocardial damage after myocardial infarction (MI) was deemed irreversible after late reperfusion. Administration of multipotent stem cells (MSC) into such infarct may regenerate the myocardium and capillary network. The objectives were to study the feasibility and safety of MSC infusion and its effect on infarct size and left ventricular (LV) function.

Methods: We conducted a pilot study in patients who survived ST-elevation MI with late reperfusion therapy and remained hemodynamically stable. Bone marrow derived MSC was infused into a patent infarct-related coronary artery during brief low pressure (2 atm) balloon inflation. A 3-T gadolinium-based MRI was performed at baseline and 8 weeks later to evaluate infarct area and LV function.

Results: We enrolled 10 patients, age 63.8 ± 2.8 years $5.2 \pm 4.12 \times 10^6$ MSC were infused via coronary artery 24.8 ± 16 days after infarction. The procedures were successful in all patients without any in-hospital event. Infarct size by MRI decreased by 5.84% ($P = .018$) over 8 weeks. Mean baseline left ventricular ejection fraction (LVEF) was $44.1\% \pm 9\%$ and was $46.3\% \pm 9\%$ at 8 weeks ($P = .34$). A trend of smaller LV end-systolic volume with 65.02 ± 18.2 ml vs 63.04 ± 21.89 ml ($P = .09$) with no change of LV end-diastolic volume observed.

Conclusion: MSC infusion into coronary circulation was feasible and safe after myocardial infarction. Infarct size was reduced with preservation of LV geometry.

Myocardial damage after myocardial infarction (MI) was deemed an irreversible process. Timely reperfusion treatment either by thrombolytic therapy or percutaneous coronary intervention (PCI) limits the infarct size. Even after early coronary reperfusion, salvage of the myocardium may be incomplete and loss of myocardium may initiate the remodeling process.¹ Unfortunately many patients with acute myocardial infarction will not present themselves within such a time window. Late reperfusion of an occluded coronary artery usually does not reduce the infarct size though it may have other potential effects such as minimizing remodeling. In animal models of myocardial infarction, infusion of multipotent stem cells (MSC) was feasible with evidence suggesting new islets of myocardium by histology.² Infusion of human multipotent stem cells into the already infarcted area may replace myocardium.^{3,4,5} We set out to prove the hypothesis that infusion of such cells is feasible and safe in patients who have had an acute myocardial infarction with large infarct size and did not have reperfusion therapy in a timely fashion. Such cell infusion

therapy may improve the left ventricular (LV) remodeling process.

Methods

Patients

We selected patients who survived ST-elevation MI with late or no reperfusion therapy (>12 h after onset of chest pain), whose transmural infarction area was large judging from appearance of the Q-wave and visible wall motion abnormality by echocardiogram or LV angiography. However, they had to be hemodynamic and electrically stable at least 5 days after the index infarction. Their infarct-related arteries had to be well patent without residual stenosis ($<30\%$) and with good distal flow ($>$ TIMI grade 2 flow).

We excluded patients with persistent occlusion of the infarct-related artery, patients who had contraindication to bone marrow aspiration, patients with systolic blood pressure <90 mm Hg, heart rate >110 bpm, signs of pulmonary congestion, patients who had electrical instability, for example, episodes of symptomatic arrhythmia, and patients who could not provide a consent form.

Procedures

After documenting the patency of the infarct-related artery, the patient was admitted to the hospital. Bone marrow was harvested under local anesthesia using at least 2 sites of puncture area at the posterior iliac crest by a staff hematologist. Bone marrow was then processed by Ficoll density gradient centrifugation. The final cell preparations were washed twice with 0.5% human albumin and phosphate solution 30 to 60 minutes after harvest. The cells were then incubated at room temperature. Total cell count was done by manual hemocytometer. Cells were characterized by flow cytometry with direct conjugated antibodies against hematopoietic progenitor cells (CD34+). The cell preparation was stored in the blood bank (<24 hr) and was rewashed and rewarmed before intracoronary infusion.

Cell infusion was done in the cardiac catheterization suite. The infarct-related artery was approached in a similar fashion to the standard PCI procedure. Over the wire balloon catheter was passed into the artery via standard guiding catheter and guide wire. The balloon was inflated with low pressure (<2 atm) at the proximal part of the target coronary vessel usually at the stented segment. The guide wire was removed for the facilitation of cell infusion. The solution containing MSC was infused via the lumen of the balloon catheter for cycles of 3 minute duration while the balloon was inflated. The balloon was allowed to be deflated from time to time when chest pain or arrhythmia became unbearable to the patient. The infusion process was repeated after 2 minutes rest in the same manner until all the solution was totally infused. Intracoronary nitroglycerin was used liberally during the infusion period. Coronary angiogram was repeated to ascertain the vessel patency after finishing the cell infusion.

The patients' vital signs and ECG were monitored continuously during the cell infusion process. They were observed in the recovery area until they were transferred to the coronary care unit (CCU). The sheath removal and access site hemostasis were done manually, usually 6 hours after procedure completion. They were discharged home 24 to 48 hours after CCU observation. They were given instruction on lifestyle adjustment, as well as level of optimal activity they could do after discharge from the hospital.

A cardiac MRI (3-T system; Philips, Best, Netherlands) was performed 3 to 5 days before cell infusion therapy and was repeated 8 weeks afterward. All images were acquired using a phased-array cardiac surface coil during breath-holds (maximum, 12 sec) and were ECG triggered. Global and regional LV functions were assessed with breath-hold cine-MRI (fast gradient-echo sequence) in the short and horizontal long axis views. In short axis view, the left ventricle was completely encompassed by contiguous 8 mm thick slices. After 15 to 20 minutes, intravenous administration of Gd-DTPA (0.2 mmol/kg),

"late enhancement" imaging was performed. All MRI images analysis and interpretation were analyzed off-line by an MRI cardiologist. The reading was done blindly on various settings without knowing the sequence of images. The specific information of interests were LV end-diastolic and end-systolic volumes (LVEDV and LVESV), LV ejection fraction (LVEF), and infarct volume. The measurements were done with proprietary software.

Patients were given an appointment prior to discharge. The patient was provided with a phone number for any emergency contact. Clinic visit and follow-up MRI were prearranged. Patients were being followed in our cardiology clinic for at least 12 months. The study was approved by the hospital ethics committee in November 2005. Inform consents were obtained with patients' signature or fingerprint.

Endpoints

The primary endpoints of the study were feasibility and safety of intracoronary administration of bone marrow derived MSC into the infarct area of the myocardium. We expected that enough cells ($>1 \times 10^6$) could be obtained with acceptable discomfort and infusion of such cells via the coronary balloon catheter should not cause any hemodynamic or electrical instability. The secondary endpoints were various index of the left ventricle, that is, LV volume, LV mass, LV function, and infarct size. We expected all LV measurement to show less remodeling or not to change significantly.

Statistic Analysis

This was a pilot project of 10 patients. MRI index of LV measurements were presented as continuous variables and a nonparametric Wilcoxon test was used to compare these parameters. *P* values of <.05 were considered significant.

Results

From December 2005 to January 2007, 10 patients who survived acute, relatively large myocardial infarction (determined by Q-wave and visible wall motion abnormality by echocardiogram or LV angiography) were enrolled. A total of 5 patients were referred to us from an outside hospital after having their infarct-related artery patency established by PCI at the referring hospital. Their age ranged from 51 to 78 years with a mean of 64 years of age (Table 1). Seven patients were male; 8 out of 10 were anterior wall in location; 50% received some form of reperfusion during the initial index infarct presentation. The time from the onset of chest pain and their reperfusion therapy was >12 hours in all cases and it took average of 272 minutes for the patient to receive reperfusion therapy after admission. All of them eventually underwent PCI for the infarct-related artery. A total of 9 out of 10 patients had stent implantation for the

Figure 1. Static picture of cine-MRI of patient with large anterior wall MI in short and long axis views. Left ventricular ejection fraction was measured to be 29% before multipotent stem cell infusion (A) and to be 40.8% at 8 weeks after (B).

infarct-related artery. The average time for PCI was 35 hours after the index infarction.

Bone marrow aspiration was done successfully in all 10 patients at an average of 24 days after index infarction. No patients required a second aspiration procedure. An average of 5.21×10^6 cells were finally obtained and sent to the cardiac catheterization laboratory for infusion within 24 hours. There was no bleeding or hematoma at the bone marrow aspiration site. All patients received standard regimen of antiplatelet medications (aspirin and clopidogrel) and anticoagulation medications (heparin or low molecular weight heparin) during the cell infusion session. It took an average of 17 minutes to infuse the cell suspension into the infarct area via the patent infarct-related artery. A total of 7 patients had chest pain during cell infusion, 4 patients developed ST-deviation (either ST-depression or ST-elevation), and 6 out of 10 did have a brief episode of premature ventricular contraction (PVC) and short run ventricular tachycardia (VT) during the infusion period. All ECG changes were transient and posted no

hemodynamic consequences. All patients remained stable in the CCU and in the medical ward. They were all discharged within 72 hours after the procedure. They received the conventional medication for post myocardial infarction. First MRI was done an average of 22 days after index infarction and the second MRI was done an average of 64 days after the first MRI.

All patients were, on average, clinically stable at 8 weeks follow-up; 9 out of 10 were in Canadian Classification 1. One patient was readmitted because of chest pain, but repeat angiography did not show any new lesion or restenosis and he was discharged the next day. Of interest, 2 patients developed eosinophil elevation noted on their complete blood count and 1 patient developed exfoliative dermatitis. All blood cell count and skin derangements resolved spontaneously on subsequent follow-up. Unfortunately at the time of this writing, 1 patient died with sudden cardiac death at 6 months after the cell infusion procedure. The sudden death may relate to the underlying disease or to the procedure.

Table 1. Demographic Data of Ten Patients who Underwent MSC Infusion After Acute MI

Age	63.8 y (51–78)
Male:Female	7:3
Anterior:Inferior MI	8:2
Number of patients receiving thrombolysis	5/10
Timing of thrombolysis after admission	272 min
Number of patients receiving PCI with stent	9 / 10
Timing of PCI after MI	34.9 hr
Hemodynamics at time of MSC infusion	
HR	76 bpm
SBP	117 mm Hg
CHF	0/10
Angiography at time of MSC infusion	
Patent IRA	10/10
Abnormal wall motion	10/10
Angiographic LVEF	41.1%±9.99%
Bone Marrow Procedure	
Timing of bone marrow procedure	24.4 days
Enough bone marrow obtained	10/10
Number of cells	5.2±4.12 million cells
Duration of cell infusion	17.3±9 min
Number of patients with chest pain during infusion	7/10
Number of patients with ST-T change during infusion	4/10
Number of patients with PVC or short run VT	6/10
MRI	
Timing of first MRI after index infarction	22 d
Timing of second MRI after index infarction	64 d

LVESV on average was smaller at 8 weeks (63.04±21.89 ml) compared to before cell infusion (65.02±18.2 ml). There was no change in LVEDV (114.65±18.3 ml before vs 114.56±25.42 ml at week 8). LVEF thus improved slightly from 44.13% ± 9.0% to 46.39% ± 9.3%. Infarct size (scar volume) got smaller over the course of 8 weeks after cell infusion. Scar volume (as % of LV) at baseline varied from 12.92 to 48.29 with average of 27.67±12.11. Scar volume at week 8 varied from 10.97 to 33.0 with average of

Table 2. MRI Parameters Before and After MSC Infusion

MRI Parameters	Baseline	8 Weeks	P Value
Mean infarct size (%LV), mean	27.67±12	21.83±7	.018
Infarct size, range	12.92–48.29	10.97–33.0	
Absolute change of infarct size		–5.84%	
LVEF, mean	44.13±9.0	46.39±9.3	.34
LVEF, range	29.0–58.4	32.6–63.2	
Absolute change of LVEF		+2.26%	
LV end-diastolic volume mean	114.65±18.3	114.56±25.42	.98
LV end-systolic volume mean	65.02±18.2	63.04±21.89	.69

21.83±7.2. The change in infarct area by absolute of 5.84% was statistically significant ($P = .018$) but the increase in ejection fraction of 2.26% was not statistically significant ($P = .34$).

Discussion

This study found that obtaining stem cells by bone marrow aspiration was practical and safe after myocardial infarction, which was in agreement with most clinical trials. There was no hematoma at any of the iliac crest. Enough marrow and stem cells were obtained. In a stable patient, infusion of such cells into the infarct zone via coronary route was also safe. Of interest, 70% of our patients had chest pain and ST changes during the infusion period, the causes of which were uncertain. One could speculate that there may be some viable myocardium that began to resume normality and the temporary balloon occlusion during the infusion period made it ischemic again. However most of the chest pain and ECG abnormalities were transient and did not pose any subsequent threat to the patient. All of them were discharged within 72 hours after the procedure.

The unique thing about this study was that the patients selected had a large infarct size. We used the new Q wave by ECG and with definite wall motion abnormality as the criteria without knowledge of infarct volume by MRI. Most of our patients had scar formation documented by late enhancement volume of 27% of LV on average (range 13%–48%) despite reperfusion therapy. Of all the patients who had reperfusion therapy during the acute phase of chest pain, 50% were late (>12 hours after the onset of symptom). Most of the patients in other clinical trials during acute MI had their reperfusion therapy done in a very timely fashion that is, within 4 hours after the onset of symptoms.^{6,7} Technically, all our patients got reperfusion by patent artery

Figure 2. Gadolinium enhanced MRI in short axis view before multipotent stem cell infusion (A) and 8 weeks after (B). The infarct zone shown in the left panel was seen as white in the anterior wall which become clearer in the right panel. This represented the reduction of infarct volume of the left ventricle from 43.7% to 25.6%.

from PCI. However in our study as well as others,^{8,9} the possibility that stunted myocardium might very well improve over time cannot be excluded although it is not likely the case in our study because of the time delay.

We observed the changes in LV geometry in a favorable direction. The shrink in scar size of 5% absolute reduction ($P = .018$) was not unexpected. Many mechanisms may be playing a part. The initial relatively fresh infarct is usually quite congested in term of cellular infiltrate and tissue edema. The uptake of gadolinium could be over read by such mechanism.¹⁰ The subsiding tissue congestion over time could very well be the mechanism.¹¹ However, the regeneration of new islets of myocardium can also be the cause of infarct size reduction. The infusion of MSC could have a dual effect on anti-inflammatory and tissue replenishing effects.

Smaller LVESV with stability of LVEDV was noted and suggested that remodeling of LV was favorable. This showed that cell infusion may not be harmful to the remodeling process. Despite reperfusion, the infarct zone usually becomes thinner and expands.¹² It is difficult to conceive that infusion of approximately 5 million stem cells will replace the whole myocardium lost in the acute occlusion process, but it showed that some stem cells might begin to have a regenerative effect. The shrinking of scar volume could be due to the subsiding tissue edema around the infarct tissue with the uptake of gadolinium was less at a subsequent time frame.

The technique of cell delivery and cell type used were also worth mentioning. We chose the intracoronary infusion via catheter rather than direct myocardial injection. The latter method was used when the patient had to undergo coronary bypass surgery. The reason for balloon occlusion was to create a homing effect of myocardial ischemia for the stem cell to stay around the infarct area. Intracoronary infusion

of the stem cell with temporary balloon occlusion resulted in the uptake of the cell by radioisotope study. Stem cells labeled with FDG 18 were noted after using such a method.¹³ The ease of procedure makes this method attractive for cell delivery. Results may have been different if we were to infuse the cells via collaterals to the infarct zone. We also believed we used the specific cell type of MSC (CD34+). We could have used just bone marrow mononuclear cells similar to previous clinical trials, but we felt that a more specific group of cells may function better.¹⁴

Other than being a small study, the study was not done in a randomized control fashion. A randomized controlled trial of similar methods in an early phase of MI, where all patients did receive PCI as the standard for reperfusion relatively early, did show a similar trend of infarct size reduction although it did not show any improvement in LV systolic function.¹⁵ This study generates the hypothesis that with the delayed or no reperfusion within a big infarct area, infusion of multipotent stem cells can improve the LV index.

Conclusions

Multipotent stem cell extraction from bone marrow aspiration given to patients who recently survived a large myocardial infarction appears feasible. Intracoronary infusion of such cells was safe in 10 cases. Repeat MRI at 60 days showed smaller infarct size, but no change in LV size and function. The lack of control precludes linking the functional or remodeling changes with cell infusion.

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