Initial clinical outcomes of intracoronary infusion of autologous progenitor cells in patients with acute myocardial infarction

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Abstract

BACKGROUND: Myocardial infarction (MI) is an irreversible cardiomyocytes injury which begins after 15-20 minutes of coronary artery occlusion. The extent of infarction is modulated by a number of factors including collateral blood supplies, medications, and ischemic preconditioning. Although angioplasty and thrombolytic agents can relieve the cause of the infarction, the time from the occlusion onset to reperfusion determines the degree of irreversible myocardial injury. Experimental studies suggested that stem cells and progenitor cells derived from bone marrow can be used in the repair of cardiac tissue after acute MI. This study was designed to investigate the feasibility, safety and initial clinical outcome of intracoronary infusion of autologous progenitor cells in patients with acute MI.

METHODS: Patients with a history of anterior MI and a left ventricular ejection fraction (LVEF) less than 35 % who were candidates for coronary angioplasty were randomly allocated in a 1:1 ratio to either control or bone marrow cell groups (each including 16 patients). Thallium scan and 17-segment echocardiography analysis for regional wall motion abnormality were performed before and 1 and 6 months after intracoronary infusion of bone marrow cells. The same tests were also conducted for the control group at identical time intervals. Quantitative variables were compared by independent t-test and paired t-test. Statistical significance was assumed at a value of $P < 0.05$.

RESULTS: LVEF in the case and control groups increased to 39.37 ± 2.47% and 31.00 ± 1.87%, respectively ($P = 0.069$ and 0.1, respectively). Wall motion abnormality index (WMAI) decreased insignificantly in both groups. Perfusion defect scores (PDSs) decreased significantly in the case group.

CONCLUSION: In this study, autologous mesenchymal stem cell transplantation by intracoronary catheter during angioplasty in patients with a history of severe LV dysfunction caused mild increases in LVEF.

Keywords: Myocardial Infarction Left Ventricular Failure, Stem Cell.

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Introduction

Myocardial infarction (MI) is, by nature, an irreversible myocardial injury.[1] Regional systolic function and regional metabolism decrease within a few heart beats of a sudden decrease in myocardial perfusion.[2] In some patients, impaired diastolic relaxation may precede global systolic abnormalities. Irreversible cardiomyocytes injury begins after 15-20 minutes of coronary artery occlusion.[3] The subendocardial myocardium has high metabolic needs and thus is most vulnerable to ischemia.[4] The extent of the infarction depends on the duration and severity of the perfusion defect.[5] However, the extent of infarction is also modulated by a number of factors including collateral blood supplies, medications, and ischemic preconditioning.[6] Beyond contraction and fibrosis of myocardial scar, progressive ventricular remodeling of non-ischemic myocardium can further reduce cardiac function in the weeks to month after the initial event.[7]

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Many of the therapies available to clinicians todaycan significantly improve the prognosis of patients with acute myocardial infarction.[8] Although angioplasty and thrombolytic agents can relieve the cause of the infarction, the time from onset of occlusion to reperfusion determines the degree of irreversible myocardial injury.[9] No clinical medication or procedure has been proved efficient in replacing myocardial scar with functioning contractile tissue. Therefore, stem cells and progenitor cells derived from bone marrow have been proposed to be used in repairing the cardiac tissue after acute myocardial infarction.[10,11] Experimental studies suggested that bone marrow-derived or blood-derived progenitor cells may contribute to the regeneration of infracted myocardium[12] and enhance neovascularization of ischemic myocardium.[12,13] Furthermore, studies have revealed that the subset of cells in the bone marrow can be mobilized to peripheral circulation and can localize the endothelial flow surface of the vascular prosthesis.[13] In addition, intracoronary infusion or intramyocardial injection of adult progenitor cells in animal models resulted in sustained improvement of cardiac function after experimentally induced myocardial infarction.[11,14] Therefore, this study aimed to investigate the feasibility, safety and initial clinical outcome of intracoronary infusion of autologous progenitor cells in patients with acute MI.

Materials and Methods

This study was done from June 2002 –January 2004. Patients were eligible if they had a history of anterior MI, by history, electrocardiography, and cardiac enzymes during the past month. All included patients had a left ventricular ejection fraction (LVEF) less than 35% and were candidates for coronary angioplasty. Patients with multivessel coronary artery disease, pulmonary edema, radiogenic shock, advanced renal or hepatic dysfunction, or cancer were excluded. Informed consents were obtained from all patients. Moreover, the study protocol was approved by the local Ethics Committee of Isfahan University of Medical Sciences. Although, 55 patients were initially admitted, only 20 had the inclusion criteria and were randomly allocated in a 1:1 ratio to the either control or case (bone marrow cell) groups. After angioplasty and stent deployment, coronary artery was occluded at the origin of the stent and progenitor cells were injected in 3 steps of 3 minutes duration.

Harvest and transfer of bone marrow cells

Bone marrow aspirates were obtained under local anesthesia with a standard Jamshidi needle with heparin (50 U/ml) from posterior iliac crests. Bone marrow-derived mononuclear cells (BMCs) were isolated by layering on a Ficoll-Paque gradient. Cell populations included hematopoietic progenitor cells. A hemocytometer was used to estimate the number of nucleated cells in the final preparation of bone marrow cells. Nucleated cell viability was assessed by trypan blue exclusion. Nucleated cells were cultured in an M199 medium, 10% human serum supplemented with 50 ng/ml vascular endothelial growth factor (VEGF), 1 ng/ml basic fibroblast growth factor (bFGF), and 2 ng/ml insulin-like growth factor-1 (IGF-1). The cells were incubated overnight at 37ºC in a fully humidified atmosphere with 5% CO_2. Then, cells were washed twice, resuspended in 5 ml human serum and infused into the infarct artery during the angioplasty procedure.

Selective coronary and left ventricular (LV) angiography were carried out according to the standard Seldinger method.[15]

SPECT thallium scan

Perfusion defects in 17 segments were assessed by scintigraphy using bull's-eye analysis[15] for both the control and case groups before and six months after the procedure.

Echocardiography

The 17-segment model for regional wall motion analysis of LV and LVEF was evaluated before, and 1 and 6 months after intracoronary infusion of bone marrow cells. It was also assessed at the same intervals in the control group.

Statistical analysis

Data was expressed as mean ± SEM. Initially, the Kolmogorov-Smirnov test was used to determine the normality. Then categorical variables were compared with the use of one way ANOVA. Statistical significance was assumed at a level of $P < 0.05$. All statistical analyses were performed using $SPSS_{15}$.

Results

On average, 128 ± 5.5ml of bone marrow was aspirated from the posterior iliac crest during the local anesthesia. The average preparation of bone marrow cells contained $24.6 \times 108 \pm 8.4 \times 108$ nucleated cells with approximately 99% viability.

Baseline characteristics of the patients are shown in table 1. Treatment with aspirin, Captopril, and Lovastatin was initiated during the hospitalization for MI and continued until the follow-up examination at the 6th month. Ticlopidine was used by both groups for 1 month after angioplasty. There were no deaths or reoccurrence of MI. None of the patients had any malignant arrhythmia during the follow-up period, had any clinical findings suggestive of exacerbation of heart failure or re-stenosis of the stent lesion in the infarct

artery. In addition, there were no increases in white blood cell (WBC) counts in any of the patients 24 hours after the intracoronary transfer of bone morrow cells.

LVEF was 33.37 ± 2.8% and 29.00 ± 1.87% respectively in the bone marrow treated and control groups before treatment. There was no significant difference between these values. In the bone marrow group, the LVEF increased to 39.37 ± 2.47% in 6 months which was significantly different (P = 0.069) from the values before treatment. An insignificant increase was also observed in the level of LVEF (31.00 ± 1.87%) in the control group. Although the difference in the LVEF values between the bone marrow and control groups before treatment was not significant, it was significant after 6 months.

The values of wall motion abnormality index (WMAI) in the bone marrow treated and control groups (36.88 ± 2.5% and 35.60 ± 4.8%, respectively) were not significantly different before treatment. The WMAI decreased to 36.00 ± 3.86% and 34.40 ± 4.46% respectively in the bone marrow treated and control groups after 6 months. The differences were not significant in either group. The difference between WMAI in the bone marrow and control groups after treatment was not significant.

Perfusion defect scores (PDSs) were 31.00 ± 3.04 and 35.00 ± 4.09 in the bone marrow treated and control groups before treatment. There was no significant difference between these values. Although after 6 months, the PDSs decreased to 21.88 ± 4.27 and 31.00 ± 4.50 in the bone marrow treated and control groups, respectively, the difference was only significant in the first group (P ≤ 0.05). The difference in the PDSs between the bone marrow and control groups was significant after 6 months (P ≤ 0.05).

Functional class index (FCI) was assessed by a cardiologist according to New York Heart Association classification. FCI was 2.38 ± 0.26 and 2.2 ± 0.20 in the bone marrow treated and control groups before treatment. There was a significant difference between these values. In the bone marrow group, the FCI decreased to 1.13 ± 0.12 after 6 months which was significantly different from the value before treatment. Similarly, the FCI significantly decreased to 1.06 ± 0.24 in the control group. The difference in FCI between the bone marrow and control groups was significant after 6 months. The results are shown in table 2.

Table 1. Basic characteristics of the two studied groups

	Control Group (n = 16)	**Case Group (n = 16)**
Age (yrs)	45.20 ± 3.16	48 ± 2.48
Sex (Male%)	90%	66%
Diabetes mellitus	25%	33%
Hyperlipidemia	40%	33%
Hypertension	0%	33%
Current cigarette use	40%	32%

Table 2. Evaluated parameters in the two studied groups

	Control group		**P**	**Bone marrow cell treated group**		**P**
	Before	**After 6 months**		**Before**	**After 6 months**	
LVEF* %	29±1.87	31±1.87	≤ 0.05	33.37±2.80	39.37±2.47	0.069
FCI** (NYHA)	2.20 ± 0.20	1.06±0.24	0.005	2.38±0.26	1.13±0.12	< 0.001
WMAI***%	35.60 ± 4.8	34.4 ± 4.46	0.388	36.88 ± 2.50	36.00±3.86	0.757
Defect score	35.00±4.09	31.00±4.50	0.052	31.00±3.04	21.88±4.27	0.065

* Left ventricular ejection fraction
** Functional class index
*** Wall motion abnormality index

Discussion

Rapid reperfusion of the infarct-related coronary artery is of great importance in salvaging ischemic myocardium and limiting the infarct size in patients with acute MI. When done expeditiously and expertly, percutaneous transluminal coronary angioplasty with stent implantation is the method of choice to re-establish coronary flow.[16] Unfortunately, myocardial necrosis starts rapidly after coronary occlusion, usually before reperfusion can be achieved.[17]

The loss of viable myocardium initiates a process of adverse LV remodeling, leading to chamber dilatation and contractile dysfunction in many patients.[18] In this context, much interest has been paid to experimental studies showing that cardiac transfer of unfractionated bone marrow cells, or stem cells, and progenitor cells derived from bone marrow can enhance functional recovery after acute MI.[10]

Previous clinical investigations indicated that infusion of autologous bone marrow cells into the infarct-related coronary artery would be feasible after acute MI.[19,20] Our randomized controlled clinical trial addressed the effects of autologous bone marrow cell therapy on LV function recovery after acute ST segment elevation MI. On average, 128 ml of bone marrow were aspirated from 10 patients that resulted in the recovery of around 24.6 million cells per infusion. Clinical follow-up of patients revealed that the method was safe and no side effects such as fever, acute thrombosis, reoccurrence of MI or proarrhythmia were observed.

Compared to control group, the LVEF in the bone marrow cell treated group increased significantly 6 months after the infusion of autologous bone marrow cells into the infarct-related coronary artery. However, the difference in LVEF between the two groups was not significant before the treatment. Thus, the difference observed might not have been caused by the stent but was rather a result of bone marrow treatment. Strauer et al. reported insignificant increments of LVEF in both groups.[19] Lunde et al. showed that intracoronary injection of mononuclear stem cells caused an increase of 7.6 ± 10.4% in mean LVEF (baseline value: 46.3 ± 9.5%) in both groups. In contrast to our results, they did not observe a significant difference between two the groups.[21] Schachinger et al. indicated that at four months follow-up, the absolute improvement in the global LVEF was significantly greater in the case (bone marrow cell) group than in 3.0 ± 6.5%; $P < 0.01$). They stated that patients with a baseline LVEF at or below the median value of 48.9% enjoyed the most benefits,[22] which is in agreement with our results.

The enhanced LVEF in the treated group compared to the control group probably due to the bone marrow treatment emphasizes the need for additional therapeutic strategies to promote functional recovery in patients with acute MI. Serial LVEF measurements in patients with reperfused myocardium after acute MI have revealed no significant improvements in LVEF (from the baseline at the 5th-7th days to the follow-up at the 3rd-6th months).[23,24] These results are consistent with insignificant improvements of LVEF in the control group. The LVEF improvement has been suggested to be a result of improved regional systolic wall motion in the infarct border zone and not due to the improved LV remodeling at the 6th month.[25]

In contrast to our results, Strauer et al. found that compared to the standard therapy group, intracoronary injection of monoclonal stem cells decreased regional wall motion abnormality index in the cell therapy group after three months.[19] This inconsistency could be related to the different patients selected by the two studies. Strauer et al. also reported insignificant LVEF increments in both groups since they studied MI patients with a mean LVEF of 57 ± 8% but in our study the mean LVEF was around 30%.[19]

Therefore, patients in this study have substantial functional impairment.

The 19.2% rise in LVEF in the treated group versus the 3.7% increase in the control group emphasizes the need for additional therapeutic strategies to enhance functional recovery in patients with acute MI. The obtained increase was also higher than LEVF improvement (up to 4%) that can be achieved when coronary potency is reestablished within 4 hours of symptoms onset.[23]

The 19.2% increase in this study is greater that LVEF improvements reported by other studies.[25] This difference could be due to selection of patients with very low LVEF or due to the added growth factor to cells during the overnight culture.

Our results are consistent with the reports suggesting cell transfer to be more beneficial than established strategies to promote functional recovery after acute MI, such as percutaneous coronary interventions, PCI with stent implantation, and post infarction pharmacotherapy.[14,26]

This study was not designed to assess the underlying mechanisms of treatment with bone marrow cells that promote functional recovery after acute MI. Apparently, trans differentiations of bone marrow derived hematopoietic stem cells to cardiac myocytes cannot account for their beneficial effects. Instead, recent papers have highlighted the potential of bone marrow cells to promote paracrine effects in

the ischemic tissues (e.g. secretion of angiogenic factors), and suggest that paracrine signaling, rather than cell incorporation, promotes functional recovery.[23,24] It is also possible that adding bFGF, IFG-I and VEGF promoted the differentiation of CD 34+ cells in the medium to endothelial cell colonies and led to aforementioned improvements. The presence of VEGF has been reported to be critical for endothelial differentiation in vitro, even though bFGF and IGF-1 enhanced endothelial colony formation.[27] Furthermore, the usage of colony-stimulating factor was avoided since high rates of in-stent restenosis have been reported after intracoronary transfer of granulocyte and mobilized peripheral blood mononuclear cells.[28] This effect may be mediated by enhancing neutrophil recruitment at sites of tissue injury.[27]

It is of interest to note that nucleated bone marrow cells are significantly smaller than expanded mesenchymal stromal cells ex vivo,[28] which may explain why we and others[19] did not observe infarctions after intracoronary transfer of bone marrow cells.

Finally, the results of our study indicated that intracoronary bone marrow stem cell transplantation is safe and feasible. Overall, the results are encouraging and support the idea that autologous bone marrow cells can be used to enhance LV functional recovery in patients after acute MI and decrease the incidence of heart failure.

Conflict of Interests

Authors have no conflict of interests.

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