Cellular Cardiomyoplasty Using Different Stem Cells in a Single Center

Fumin Zhang[*,1], Zhijian Yiang[1], Yijiang Chen[1], Wenzhu Ma[1], Xiang Gao[2], Chuanfu Li[3], Tuanzhu Ha[3] and Race L. Kao[*,3]

[1]*Department of Cardiology, Jiangsu Province Hospital, The First Affiliated Hospital of Nanjing Medical University, Nanjing, 210029, China*

[2]*Model Animal Research Center, Nanjing University, Nanjing, 210093, China*

[3]*Department of Surgery, East Tennessee State University, Johnson City, TN, 37614, USA*

Abstract: Cellular cardiomyoplasty is a procedure that uses live cells to replace, repair or enhance the biological function of a damaged or failing heart. Several types of autologous stem cells from adult human have been used for cellular cardiomyoplasty with encouraging outcomes. However, a direct comparison of different stem cells in a single clinical center has not been reported. We harvested stem cells from skeletal muscle (satellite cells) or bone marrow from different patients and utilized for cellular cardiomyoplasty. Eighteen male patients, suffering acute myocardial infarction were evenly assigned to coronary artery bypass graft and autologous satellite cell implantation or percutaneous coronary intervention and autologous bone marrow cell intra coronary infusion. Cardiac functions were assessed before and at six months after the treatment. All patients who survived the procedures without obvious arrhythmia, had an uneventful recovery, and were discharged from the hospital. One patient from each group was not subjected to coronary artery bypass graft or percutaneous coronary intervention. Significant improvement in NYHA classification and left ventricular ejection fraction were observed to a similar degree for both treatments at six month later. Comparable improvements in local contractility, blood perfusion, tissue viability, and wall thickness at infarct area for all patients who received either type of autologous stem cells were also found. Cellular cardiomyoplasty using either autologous satellite cells or autologous bone marrow cells has equal beneficial outcomes at six months follow-up. A longer follow-up time and large scale randomized controlled trail will be needed to determine the advantages of different stem cells.

Keywords: Cellular cardiomyoplasty, satellite cell, mononuclear cell, clinical trial.

1. INTRODUCTION

Adult stem cells are undifferentiated cells residing in differentiated tissues capable of self-renewal and proliferation to produce differentiated cells. Adult stem cells can yield the specialized cell types of the tissue from which it originated and are capable of developing into cell types that are characteristics of other tissues (plasticity). Self renewal and plasticity of adult stem cells have been well established in recent years [1-3]. Cell therapy has emerged as a strategy for the treatment of many human diseases [4-6]. The aim of cellular cardiomyoplasty is to replace, repair, or enhance the biological function of damaged myocardium or failing heart [7-9].

Since we initiated cellular cardiomyoplasty in 1989, our successful outcomes [10-13] have been confirmed by other investigators [14]. Clinical cellular cardiomyoplasty was first performed by Menasche's group in June of 2000 [15]. Since then, a number of small scale uncontrolled clinical trials have been reported by different investigative groups. Skeletal muscle satellite cells (myoblasts) have the advantages of autologous availability, can be proliferated *in vitro* to vast quantity, without tumorigenicity, more committed to myogenic differentiation, and are highly resistant to ischemia/hypoxia environment which allows good survival and engraftment after transplantation. Early clinical applications offered highly encouraging results from others and our observations that were covered by recent reviews [13, 16, 17]. Although the survival, feasibility, safety, and encouraging outcomes have been observed in long-term follow-up studies [15,18]; the definitive long-term efficacy requires large scale, placebo-controlled, double-blind randomized trials such as MAGIC study [19].

Adult bone marrow contains multiple cell populations of differentiated cells and undifferentiated cells (stem cells) such as hematopoietic stem cells (HSC), mesenchymal stem cells (MSC) and endothelial progenitor cells. A mixed population of bone marrow cells rather than purified stem cells is commonly used for cellular cardiomyoplasty [20-22]. The safety and ease of obtaining the cells, the observation of new muscle tissue formation, the improvement of contractile function, the enhancement of local perfusion, and the prevention of remodeling and deterioration of the injured heart have been documented in studies using experimental animals and reviewed recently [13, 23-25].

Despite a lack of clear understanding for the beneficial mechanisms, Phase 1 clinical studies have shown the feasi-

*Address correspondence to these authors at the (RLK) Department of Surgery, J. H. Quillen College of Medicine, East Tennessee State University, P.O. Box 70575, Johnson City, TN 37614-1708, USA; Tel: 423-439-8803; Fax: 423-439-8750; E-mail: kao@etsu.edu and (FZ) Department of Cardiology, Jiangsu Province Hospital, 300 Guan Zhou Road, Nanjing 210029, China; Tel: 86-25-3718836, Ext. 6664;
E-mail: fmzhang8@vip.sina.com

1876-5335/08 2008 Bentham Open

bility and safety of the procedure with encouraging functional improvements that are summarized in recent publications [13, 24, 26-28]. Treatment-control trials (TOPCARE-AMI, IACT, BOOST, MAGIC Cell-3-DES, ASTAMI) have been reported, but the number of patients involved are relatively small [29-34]. The randomized, placebo-controlled trials with small to medium size samples have also been reported recently [35-38]. So far, improvement in left ventricular ejection fraction, less heart enlargement, significantly improved blood flow reserve, lower rate of mortality, heart attacks, and hospitalization due to heart failure all indicate the efficacy of cellular cardiomyoplasty using bone marrow cells. The beneficial mechanisms and the possible factors regulating the differentiation of bone marrow stem cells into heart muscle cells are under current investigation by many groups.

Several types of autologous stem cells from adult human have been used for cellular cardiomyoplasty with beneficial outcomes. Other than the elegant study of comparing bone marrow versus circulating progenitor cells in a controlled crossover study [39], a direct comparison of different stem cells in a single clinical center has not been reported. This report summarized the pilot study using autologous stem cells harvested from skeletal muscle or bone marrow from different patients and compared their outcomes at six month after cellular cardiomyoplasty.

2. PATIENTS AND METHODOLOGY

2.1. Patient Selection

The protocol and procedure of this clinical evaluation were approved by the Institutional Review Board for human study and Jiangsu Province Health Ministry, China. The procedures and methods were in compliance to the Helsinki Declaration of 1975. Written informed consent was obtained from all patients. Eighteen male patients with coronary heart diseases who suffered from acute myocardial infarction were recruited and evenly assigned to the two treatment groups. The average age of the patients was 64 years (27 - 79 years) who had a history of left ventricular myocardial infarction and in majority of patients with a depressed left ventricular ejection fraction (LVEF). The heart function of the selected patients was classified as II-IV of New York Heart Association functional classification (NYHA). Two-D echocardiography, technetium-99m methoxyisobutylisonitrile (^{99m}Tc-MIBI) and ^{18}F-deoxyglucose were employed to examine the cardiac function, myocardial perfusion, and viable cardiomyocytes, respectively, before and at six month after cell transplantation as reported in our earlier publication [40].

For the patients who received bone marrow derived stem cells, they were suffering from a recent myocardial infarction and percutaneous coronary intervention (PCI) was performed with balloon angioplasty and stent deployment for the selected vessels. Written informed consent was obtained from every patient with cardiac functional evaluations and cell administration scheduled three weeks after PCI procedure. All the patients who were treated with satellite cells, they all suffered from a recent myocardial infarction with coronary angiogram and cardiac functional evaluation performed. After obtaining written informed consent, muscle biopsy was procured from each patient and coronary artery bypass graft (CABG) procedure and satellite cell implantation were scheduled at three weeks later. Holter monitor was used to monitor electrocardiogram during hospitalization and at six months follow-up.

2.2. Isolation and Culture of Satellite Cells

Biopsy specimens (2-5 g) were taken from the *vastus lateralis* muscle under local anesthesia from each patient on an outpatient basis. The biopsy specimens were placed in Hanks' balanced salt solution without Ca^{++} and Mg^{++} but containing 1% antibiotics (5,000 IU/ml penicillin and 5,000 μg/ml streptomycin), and immediately transported to the laboratory for isolation of skeletal muscle satellite cells as previously described [10-13]. Briefly, the biopsy sample was minced (~1 mm^3) and dissociated using digestive enzymes (1% collagenase and 0.2% hyaluronidase; Sigma, St. Louis, MO) in Medium 199 at 37°C. The cell suspension was centrifuged at 650 x g for 10 min and washed with Medium 199 (Sigma) plus 10% fetal bovine serum (FBS) and 1% antibiotics. Skeletal muscle satellite cells were cultured in Medium 199 containing 20% FBS and 1% antibiotics at 37°C under humidified atmosphere of 95% air with 5% CO_2. The media were changed every 3 to 4 days and cell density was maintained below 75% ~ 80% confluences throughout the culture in order to avoid the formation of myotubes.

During the day for CABG and cell implantation procedures, the cultured cells free of microbial contamination were recovered. The satellite cells were thoroughly washed before being suspended in 4 ml of serum free medium 199 and injected (15 to 40 sites) into the peri-infarct and infracted areas. A small fraction of the cell suspension was used for cell counting, purity, viability, and myogenic capability determinations. The cell number was determined using an automated hemocytometer. The purity (>85%) of the satellite cells was documented with anti-CD56 monoclonal antibody using fluorescence-activated cell sorting (FACS) and the viability (>95%) using trypan blue exclusion. To examine the ability of satellite cells to form multinucleated myotubes *in vitro*, the cells were placed in 24 well tissue cultured plates with 2 x 10^4 cells per well and the medium was changed to fusion medium containing reduced serum (2% FBS). Two weeks later, the cultures were observed for the formation of myotubes. All the satellite cell preparations from patients were observed to form multinucleated myotubes indicating the myogenic characteristic of cell preparations.

2.3. Isolation and Culture of BMSCs and BMMCs

Twenty to forty ml of bone marrow was aspirated from the posterior iliac crest of each patient under local anesthesia into syringe with heparin (5,000 IU). Venous blood of 200 ml was obtained with heparin to allow separation of plasma and cells by centrifugation. The cells were cultured in Iscove's modified Dulbecco medium (IMDM) containing 20% autologous plasma and 1% antibiotics. 72 hours later, the adherent cells were recovered, cultured in the same medium, and subcultured every 3 to 4 days to maintain cell density below 80% confluence as bone marrow mesenchymal stem cells (BMSCs). The non-adherent cells were subjected to Ficoll density-gradient centrifugation [20] before being extensively washed and cultured in IMDM culture medium with fresh medium added every 3 to 4 days as bone marrow mononuclear cells (BMMCs). A small fraction of the cell

suspension was used for cell counting, purity, and viability determinations. Better than 90% of purity (with anti-CD133 monoclonal antibody for BMMCs using FACS) and >95% viability (trypan blue exclusion) without microbial contamination were observed for all cell preparations. The cells were washed with IMDM containing 20% autologous plasma and combined before being finally suspended in 9 ml of the same medium for intra coronary administration. The cell suspensions (3 ml) were infused slowly over three minutes into the culprit coronary artery through the central lumen of an over-the-wire balloon catheter with balloon inflated at low pressure to temporary stop the antegrade blood flow. Between each infusion, three minutes were allowed with balloon deflated to restore normal blood perfusion.

2.4. Statistical Analysis

All values were presented as means ± SEM. Two-sample t-tests were performed for comparisons between groups. Paired t-tests were used to compare the data for before and after cellular cardiomyoplasty. SAS-PC was used for the analysis.

3. RESULTS

Table **1** summarized the basic clinical characteristics of the patients that were not different between the two groups. There was one patient in each group (S4 and M5) who did not receive the concomitant vascular procedure. Patient S4 had respiratory and urethra infection that delayed his CABG procedure for three months, after opening the chest, his left anterior descending coronary artery (LAD) and right coronary artery (RCA) were completely occluded and coronary bypass procedure could not be performed. Patient M5 only had 50% stenosis of his LAD and balloon angioplasty or stent deployment was not required. All patients survived the procedures without obvious arrhythmia, had an uneventful recovery, and were discharged from the hospital.

At six months after cellular cardiomyoplasty, the NYHA was significantly improved ($P<0.01$) in both groups and similar improvement was found either using satellite cells or bone marrow cells (Table **2**). Patient S7 has uncorrected severe aortic regurgitation and was the only patient who did not show any improvement at follow-up examination. Al-

Table 1. Patients' Clinical Data and the Results of Coronary Angiogram

Patients for Satellite Cell Implantation				Patients for Bone Marrow Cell Infusion			
Case	**Age**	**Acute MI**	**Coronary Angiogram**	**Case**	**Age**	**Acute MI**	**Coronary Angiogram**
S1	74	Anterior wall	100% stenosis at LAD & LCX; 30% stenosis at middle RCA	M1	38	Anterior wall	Diffuse stenosis at middle and distal LAD
S2	67	Anterior wall	30% stenosis at LCM; LAD occluded; 95% stenosis at proximal LCX &RCA	M2	75	Anterior wall	90% stenosis at proximal LAD; 80% at middle LAD
S3	55	Anterior wall	90% stenosis at middle LCM; 90% stenosis at proximal LAD; 70% stenosis at distal LAD, proximal, middle LCX; Diffuse stenosis at RCA	M3	71	Anterior & inferior walls	100% stenosis at proximal LAD; 100% at proximal RCA
S4[#]	74	Extensive anterior wall with aneurysm	60% stenosis at LCM; 99% stenosis at LAD; Diffuse stenosis at RCA;	M4	78	Inferior wall	85% stenosis at proximal RCA; 100% at aproximal LCX; 90% stenosis at Diagonal Two stenoses at RCA
S5	62	Inferior wall	90% stenosis at proximal LAD; 85% stenosis at middle LAD; 80% stenosis at distal LCX; 80% stenosis at proximal RCA	M5[#]	27	Anterior wall	50% stenosis at proximal LAD
S6	73	Lateral wall	60-90% diffusion stenosis at LAD; occlusion at proximal LCX; 70% & 95% stenoses at proximal and middle RCA, respectively	M6	60	Extensive anterior wall	100% stenosis at proximal LAD; 70% stenosis at middle & distal LCX
S7	72	Anterior wall	100%stenosis at proximal LAD; 80% stenosis at Proximal LCX; 50% stenosis at Proximal RCA	M7	49	Extensive anterior wall	100% stenosis at proximal LAD
S8	68	Anterior wall with aneurysm	70% stenosis at proximal LAD; occlusion at distal LAD; 60% stenosis at proximal LCX; 85% stenosis at proximal RCA	M8	79	Anterior wall	100% stenosis at proximal LAD; 80% at middle LAD
S9	67	Inferior wall	90%stenosis at proximal LAD 95% stenosis at middle LCX 99% stenosis at middle RCA	M9	56	Extensive anterior wall	100% stenosis at proximal LAD

[#] = No coronary artery bypass graft (S4) or no percutaneous coronary intervention (M5). LAD = left anterior descending coronary artery. LCM = left main coronary artery. LCX = left circumflex coronary artery. RCA = right coronary artery.

Table 2. Functional Outcomes with Type, Number and Site of Cell Administration

Patients for Satellite Cell					Patients for Bone Marrow Cell						
CASE	NYHA Before	NYHA After	Satellite Cells (in 10^6)	Site	CASE	NYHA Before	NYHA After	Cell Number (in 10^6)			Site
								BMSC	BMMC	SUM	
S1	II	I	1.4	LV apex	M1	II	I	1.1	0	1.1	LAD
S2	III	I	3.0	LV apex	M2	II	I	0.9	6.1	7.0	LAD
S3	III	I	3.0	LV apex	M3	III-IV	II	2.6	0	2.6	LCX
S4	III-IV	II	7.0	LV apex and peri-aneurysm	M4	III	I	1.3	3.4	4.7	RCA
S5	II	I	1.2	Inferior wall	M5	II	I	1.2	3.8	5.0	LAD
S6	II	I	1.3	Lateral wall	M6	III	I	1.1	2.4	3.5	LAD
S7#	III	III	1.7	LV apex	M7	III	I	2.7	2.8	5.5	LAD
S8	II	I	1.2	LV apex and peri-aneurysm	M8	II	I	3.5	2.3	5.8	LAD
S9	II	I	1.6	Inferior wall	M9	III	I	1.0	1.6	2.6	LAD
Mean ± SEM	2.5 ± 0.2	1.3* ± 0.2	2.4 ± 0.3		Mean ± SEM	2.6 ± 0.2	1.1* ± 0.1	1.7 ± 0.3	2.5 ± 0.6	4.2 ± 0.6	

NYHA= New York Heart Association Classification (for III-IV 3.5 was used for calculation); BMMC=Bone Marrow Mononuclear Cell; BMSC=Bone Marrow Mesenchymal Stem Cell; SUM=BMMC + BMSC; LV=Left Ventricular; LAD=Left Anterior Descending Coronary Artery; LCX=Left Circumflex Coronary Artery; RCA=Right Coronary Artery. # = patient has uncorrected severe aortic regurgitation. * = Significant different from "Before" group with P<0.01.

though the total cell number given in the bone marrow cell group was higher than the satellite cell group, the difference was not statistical difference (P = 0.056).

Significant improvement in ejection fraction was observed for both groups of patients at six months after cell therapy (Table **3**). Improvement in LVEF was found in every patient with the exception of patient S7 who suffered uncorrected severe aortic regurgitation. The increase in LVEF was higher in bone marrow cell group but the difference between the different cell groups was not statistical difference (P = 0.09). No significant changes were observed for left ventricular diastolic diameter (LVDD). The results from 2-D echo, ^{99m}Tc-MIBI, ^{18}F-deoxyglucose evaluations showing similar improvement (Fig. **1**) in local contractility, blood perfusion, tissue viability, and wall thickness at infarct area for all patients received either type of autologous stem cells by using the same methods as reported by us in our earlier publication [40]. Occasional arrhythmias were detected by Holter monitoring, however arrhythmias resolved by themselves and treatment was not necessary.

4. DISCUSSION

Cellular cardiomyoplasty is aiming at regenerating damaged myocardium, restoring lost perfusion and regaining ventricular function. After myocardial injury, the permanent loss of cardiomyocytes and subsequence remodeling often initiates the development of heart failure and unfavorable clinical manifestations. Since we initiated cellular cardiomyoplasty in 1989, promising results from experimental animals and clinical trials clearly support the safety, feasibility, and certain efficacy of this new therapy. However, the mechanisms of benefit, the type of progenitor cells, the time and route of cell administration, as well as the dose of cells given are unanswered questions. This is a pilot clinical trial performed at a single center to compare the skeletal muscle satellite cells versus bone marrow stem cells for the possibility of designing a randomized, controlled, double-blinded study.

Using experimental animals the skeletal muscle satellite cells are better than bone marrow derived cells [41-42] or have similar beneficial effect for both cells [43]. From a recent review [44], after evaluating all experimental and clinical publications using skeletal myoblasts versus bone marrow stem cells, the authors concluded that further studies with longer-term basis will be needed to make any decision. Other than comparing bone marrow versus circulating progenitor cells in a controlled crossover study [39], a direct comparison of different stem cells in a single clinical center has not been reported. This study is unique in the following ways: 1). the study was performed in a single clinical center, 2) all the patients received standard clinical care after myocardial infarction (either CABG or PCI), and 3) cellular cardiomyoplasty was performed at 3 weeks after myocardial infarction for both groups.

When a study was performed in a single clinical center, the care and evaluations to each patient (2-D echo, ^{99m}Tc-MIBI, ^{18}F-deoxyglucose) were more uniform and directly comparable before and after treatment. Similar improvement in local contractility, blood perfusion, tissue viability, and wall thickness at infarct area was observed for all patients received either type of autologous stem cells by using the methods we published before [40]. A longer-term comparison will be necessary based on the BOOST trial [31] that observed significant improvement at 6 month but no difference at 18 months for the cell versus control group. This may suggest that the satellite cells should perform better than the bone marrow derived stem cells due to the beneficial effects remained for 4 years or longer [15,18]. However, long-term (1 year) beneficial outcomes using bone marrow derived stem cells were observed by the TOPCARE-AMI [29] and REPAIR-AMI trials [38] that were different from BOOST trial [31] ASTAMI trial [35] and the other report [37]. One

Table 3. Left Ventricular Diastolic Diameter and Left Ventricular Ejection Fraction Data Before and After Cellular Cardiomyoplasty

Patients for Satellite Cell Implantation						
Case	LVDD (mm)			LVEF (%)		
	Before	After	Change	Before	After	Change
S1	52	48	-4	35	46	+11.0
S2	47	42	-5	40	45.4	+5.4
S3	57	48	-9	40	41	+1.0
S4	60	53	-7	37	48	+11.0
S5	54	53	-1	58	60	+2.0
S6	58	49	-9	45	52	+7.0
S7[#]	58	64	+6	40	35	-5.0
S8	57	55	-2	50	65	+15.0
S9	55	54	-1	40	42	+2.0
Mean ± SEM	55.3 ± 1.3	51.8 ± 2.0	-3.6 ± 1.6	42.8 ± 2.4	48.3* ± 3.1	+5.5 ± 2.1

Patients for Bone Marrow Cell Infusion						
Case	LVDD (mm)			LVEF (%)		
	Before	After	Change	Before	After	Change
M1	42	46	+4	65.6	69.6	+4.0
M2	53	53	0	62.4	67.3	+4.9
M3	76	76	0	22.1	35.9	+13.8
M4	54	58	+4	42.0	51.0	+9.0
M5	64	61	-3	37.8	55.8	+18.0
M6	54	50	-4	58.8	65.3	+6.5
M7	63	57	-6	35.0	46.0	+11.0
M8	58	52	-6	42.0	56.0	+14.0
M9	60	52	-8	45.0	55.0	+10.0
Mean ± SEM	58.2 ± 3.1	56.1 ± 2.9	-2.1 ± 1.3	45.6 ± 4.7	55.8* ± 3.6	+10.1 ± 1.5

LVDD = Left Ventricular Diastolic Diameter in mm; **LVEF** = Left Ventricular Ejection Fraction in %. # = patient has uncorrected severe aortic regurgitation. * = Significant different from "Before" group with $P<0.05$.

major difference between these studies was attributed to the solutions used with mononuclear cells [45]. TOPCARE-AMI and REPAIR-AMI trials used culture medium with autologous serum while the other trials used saline or saline with serum or plasma [31, 35, 37]. Store mononuclear cells in saline plus plasma resulted in functional impairment of the cells [45]. For our study, culture medium with 20% autologous plasma was used to suspend the bone marrow cells.

Although bone marrow mesenchymal stem cells, bone marrow mononuclear cells, and circulating progenitor cells represent different population of cells, recent meta-analyses combined them under the term adult or autologous bone marrow-derived cells [46-48]. Representing 500–1,000 patients, bone marrow cell-treated groups had significantly improved left ventricular ejection fraction, reduced infarct scar size, and decreased left ventricular end-systolic volume as compared to controls. Lower rates of recurrent myocardial infarction, death, re-hospitalization for coronary heart disease, and repeat revascularization are additional favorable outcomes of cell therapy. Greater cell numbers seem to be associated with more improvement, further supporting the notion that the delivered cells are producing the beneficial effects.

Cellular cardiomyoplasty procedures [49-50] all suffer from very low cell retention and massive cells death after treatment irrespective of the type of stem cells studied [51-54]. Although the satellite cell (>85% purity) and bone marrow cell (>90% purity) preparations employed in our study still contain a mixed population of cells, the cell purity is significantly higher than most of the published reports. Higher purity of stem cells has the potential of higher retention and survival rates without the complications of non-stem cells for the treatment. Muscle samples of 5 to 10 g will be the recommended size for satellite cell isolation. Smaller

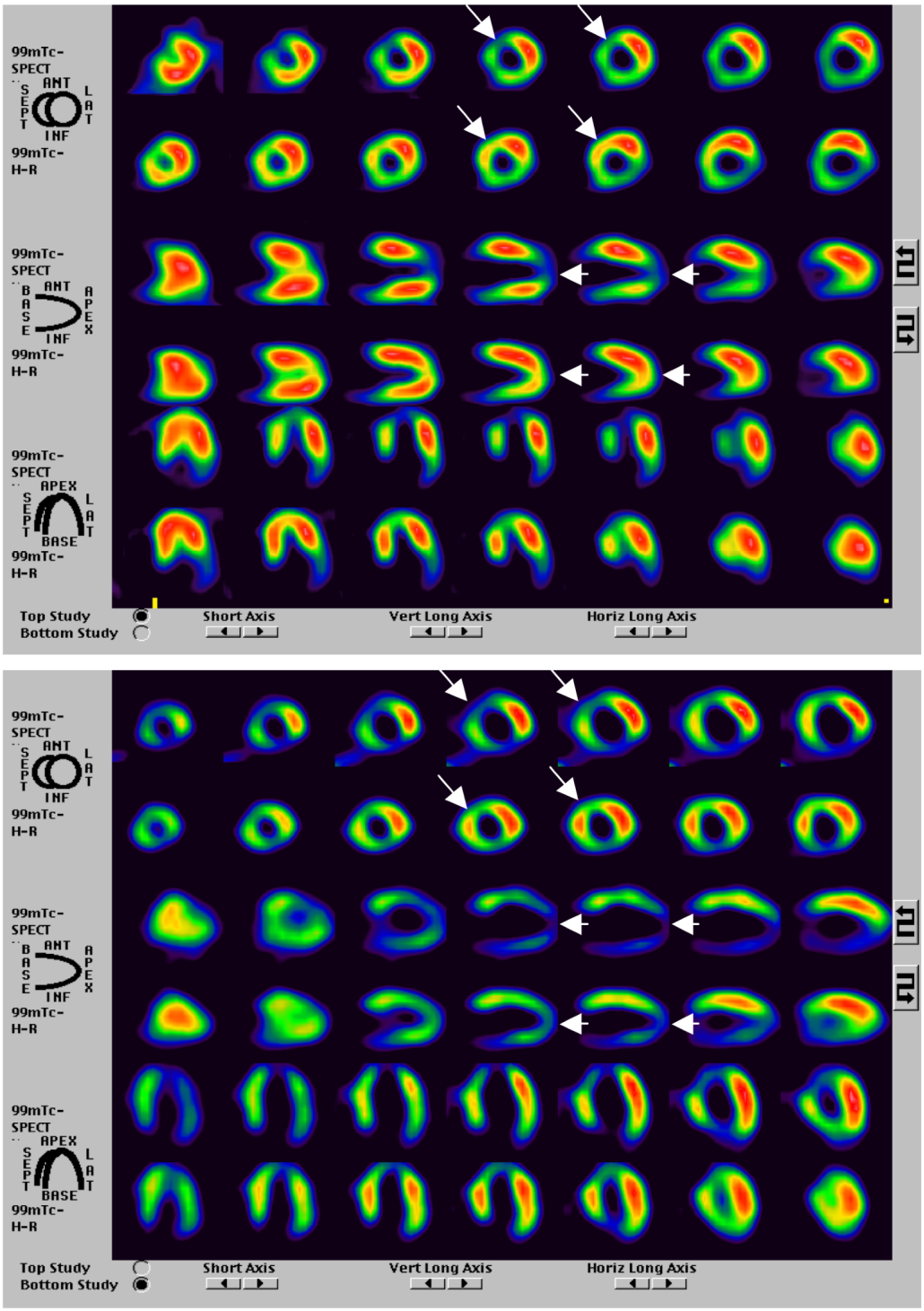

Fig. (1). Perfusion images using technetium-99m methoxyisobutylisonitrile (^{99m}Tc-MIBI). The ^{99m}Tc-MIBI perfusion images of the short-axis (top), vertical long-axis (middle) and horizontal long-axis (bottom) from a representative patient of satellite cell implantation (upper panel) and a representative patient of bone marrow cell infusion (lower panel). The upper lines were obtained before cell therapy and the lower lines were results at six month after cell therapy. The arrows pointed to the sites where perfusion was significantly improved due to cell administration.

samples make the isolation procedure difficult and require longer culture time to have sufficient satellite cells. This study only compared the 6 months follow-up results and longer duration follow-up will be necessary especially judged from the lesson learned by the BOOST trial that observed significant improvement at 6 months but no difference at 18 months for the cell versus control group.

From the original design, cellular cardiomyoplasty is intended for patients who still have reasonable cardiac reserve. This is to allow the patients to survive the procedure and also allow the implanted cells to generate sufficient myocytes (remained to be proven) to improve the cardiac function. Although some studies have applied the procedure to patients of severe left ventricular dysfunction [18,19] with

encouraging outcomes, selecting patients that still have sufficient cardiac reserves (LVEF = 40~50%) not only resulted in better improvement but also offered higher success rate as reported by the other clinical trials [55, 56]. It is well known that 2D echocardiography is observer and interpreter dependent. Therefore, the same operator and same interpreter were used for this study to minimize the variation. This further supported that the study conducted in a single center for different stem cells or implantation procedures will have higher reliability. For the future study, concomitant MRI or other procedure will be better than sole 2D echocardiography for ventricular functional evaluation. Arrhythmia has not been a problem for our clinical experience as indicated before [40] and further supported by other clinical trials [55,56]. From the MAGIC trial [19], no difference between the control and cell treated groups on time to first arrhythmia and no death can be attributed to arrhythmic event clearly indicated that arrhythmia is not a concern for cellular cardiomyoplasty even with satellite cells. With two procedures that both have the potential to improve cardiac function as reported in this study; it will be very hard to delineate which one and how much of each procedure contributes to the improved ventricular function. However, with the patients who received only cell therapy without revascularization procedure S4 (LVEF 37 to 48%; NYHA III-IV to II) and M5 (LVEF 37.8 to 55.8%; NYHA II to I) indicated that cell therapy can be the major contributor to the improved ventricular function. Unfortunately, the numbers of patients are too small for the current pilot study and randomized, controlled, double-blinded study [19] or using cell therapy as the sole treatment will be needed to clearly answer this question.

Although the optimal time after myocardial infarction for cellular cardiomyoplasty has not been established in any clinical study [13, 26, 49, 50], performing the treatment at a similar time after disease onset as in our study will allow more direct comparison between different stem cells employed. After acute myocardial infraction (MI), the healing process can be divided into 4 phases: cardiomyocyte death, acute inflammation, tissue granulation, and remodeling or repair [57]. It is well known that MI is an inflammatory disease, and there is compelling evidence that the innate immune response plays an important role in myocardial ischemia-reperfusion injury and coronary heart disease [57-61]. Ischemia-reperfusion significantly increases tumor necrosis factor, interleukins-1, -6, and -8, interferon, and intercellular adhesion molecule-1 gene expression in myocardium [61-63]. These pro-inflammatory and immunoregulatory cytokines appear to be directly involved in the progression of myocardial ischemia-reperfusion injury, myocardial dysfunction, ventricular remodeling, coronary heart disease, and cardiac hypertrophy [61-63].

Because innate immune and inflammatory responses are involved in myocardial ischemic injury, investigation of the innate immune modulation and anti-inflammatory activities of stem cells during cellular cardiomyoplasty may reveal the beneficial mechanisms. Adult tissues have been used to isolate stem cells that show plasticity, escape immune recognition, and inhibit immune responses. Adult stem cells can easily proliferate to vast numbers and be applied to inhibit immune responses by suppressing T and B cell proliferation, inducing T regulatory cells, modulating B cell and dendritic cell functions, and have been used to treat transplant rejection [64-65]. Stem cells do not induce lymphocyte proliferation and are not the targets for cytotoxic lymphocytes or natural killer cells. No adverse events during or after adult stem cell transplantation have been observed, and no ectopic tissue formation has been noted [64-66]. Other than paracrine or endocrine effects, anti-proliferative, anti-inflammatory, and immunomodulatory effects of stem cells to prevent infarct expansion and ventricular remodeling may be the major benefits [13, 64-68].

If anti-inflammatory and immunosuppressive effects are the primary beneficial mechanisms of cellular cardiomyoplasty, this may partially explain why different kinds of stem cells and different times of cell treatment (after acute MI or an old infarct) resulted in similar favorable outcomes [13, 67]. Stem cells can mediate immune suppression and anti-inflammatory effects through secreted soluble factors and contact-dependent mechanisms [65, 69]. More importantly, mesenchymal stem cells can exert anti-inflammatory effects even at the site of inflammation (similar to after MI) [69]. Since a significant fraction of the stem cells is lost by apoptosis after cellular cardiomyoplasty, and apoptotic cells can have long-lasting effects, a major benefit of cell therapy may derive from immune modulation and anti-inflammation effects. This is a pilot clinical trial performed at a single center to compare the skeletal muscle satellite cells versus mononuclear cells and mesenchymal stem cells derived from bone marrow for the possibility of designing a randomized, controlled, double-blinded study. The beneficial mechanisms of cellular cardiomyoplasty require additional studies.

ABBREVIATIONS

ASTAMI	=	Autologous stem cell transplantation in acute myocardial infarction
BOOST	=	BOne marrOw transfer to enhance ST-elevation infarct regeneration
BMMC	=	Bone marrow mononuclear cells
BMSC	=	Bone marrow mesenchymal stem cells
CABG	=	Coronary artery bypass graft
FACS	=	Fluorescence-activated cell sorting
FBS	=	Fetal bovine serum
HSC	=	Hematopoietic stem cells
IACT	=	Intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease
IMDM	=	Iscove's modified Dulbecco medium
LAD	=	Left anterior descending coronary artery
LVDD	=	Left ventricular diastolic diameter
LVEF	=	Left ventricular ejection fraction
MAGIC	=	Myoblast autologous grafting in ischemic cardiomyopathy

MAGIC Cell-3-DES	=	Myocardial regeneration and angiogenesis in myocardial infarction with g-csf and intra-coronary stem cell infusion-3-drug eluting stents
MI	=	Myocardial infarction
MSC	=	Mesenchymal stem cells
NYHA	=	New York heart association functional classification
PCI	=	Percutaneous coronary intervention
RCA	=	Right coronary artery
REPAIR-AMI	=	Reinfusion of enriched progenitor cells and infarct remodeling in acute myocardial infarction
^{99m}Tc-MIBI	=	Technetium-99m methoxy-isobutylisonitrile
TOPCARE-AMI	=	Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction

REFERENCES

[1] Wagers AJ, Weissman IL. Plasticity of adult stem cells. Cell 2004; 116: 639-48.

[2] Lakshmipathy U, Verfaillie C. Stem cell plasticity. Blood Rev 2005; 19: 29-38.

[3] Vieyra DS, Jackson KA, Goodell MA. Plasticity and tissue regenerative potential of bone marrow-derived cells. Stem Cell Rev 2005; 1: 65-9.

[4] Warburton D, Koh C, Chung HM, Cha KR, Kim KS. Meeting report: the first international collaborative symposium on stem cell research, Seoul, Korea. Stem Cells 2007; 25: 1337-8.

[5] Singec I, Jandial R, Crain A, Nikkhah G, Snyder EY. The leading edge of stem cell therapeutics. Annu Rev Med 2007; 58: 313-28.

[6] Burt RK, Loh Y, Pearce W, *et al*. Clinical applications of blood-derived and marrow-derived stem cells for nonmalignant diseases. JAMA 2008; 299: 925-36.

[7] Jawad H, Ali NN, Lyon AR, Chen QZ, Harding SE, Boccaccini AR. Myocardial tissue engineering: a review. J Tissue Eng Regen Med 2007; 1: 327-42.

[8] Wu KH, Mo XM, Liu YL, Zhang YS, Han ZC. Stem cells for tissue engineering of myocardial constructs. Ageing Res Rev 2007; 6: 289-301.

[9] Giraud MN, Armbruster C, Carrel T, Tevaearai HT. Current state of the art in myocardial tissue engineering. Tissue Eng 2007; 13: 1825-36.

[10] Chiu RC, Zibaitis A, Kao RL. Cellular cardiomyoplasty: myocardial regeneration with satellite cell implantation. Ann Thorac Surg 1995; 60: 12-8.

[11] Kao RL, Chua R-J, Eds. Cellular cardiomyoplasty: myocardial repair with cell implantation. Austin, TX: Lande 1997.

[12] Kao RL. Transplantation of satellite cells for myocardial regeneration. In: Kipshidae NN, Serruys PW, Eds. Handbook of cardiovascular cell transplantation. 1st ed. London, England: Martin Dunitz 2004; pp. 91-105.

[13] Kao RL, Ganote CE, Pennington DG, Browder IW. Myocardial regeneration, tissue engineering and therapy. In: Prakash S. Artificial cells, cell engineering and therapy. 1st ed. Cambridge, England: Woodhead Publishing Ltd. 2007; pp. 349-65.

[14] Taylor DA, Atkins BZ, Hungspreugs P, *et al*. Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. Nat Med 1998; 4: 929-33.

[15] Hagège AA, Marolleau JP, Vilquin JT, *et al*. Skeletal myoblast transplantation in ischemic heart failure: long-term follow-up of the first phase I cohort of patients. Circulation 2006; 114(Suppl I): 108-13.

[16] Prockop DJ, Olson SD. Clinical trials with adult stem/progenitor cells for tissue repair: let's not overlook some essential precautions. Blood 2007; 109: 3147-51.

[17] McMullen NM, Pasumarthi KB. Donor cell transplantation for myocardial disease: does it complement current pharmacological therapies? Can J Physiol Pharmacol 2007; 85: 1-15.

[18] Dib N, Michler RE, Pagani FD, *et al*. Safety and feasibility of autologous myoblast transplantation in patients with ischemic cardiomyopathy: four-year follow-up. Circulation 2005; 112: 1748-55.

[19] Menasché P, Alfieri O, Janssens S, *et al*. The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. Circulation 2008; 117: 1189-200.

[20] Tomita S, Li RK, Weisel RD, *et al*. Autologous transplantation of bone marrow cells improves damaged heart function. Circulation 1999;100(Suppl II): 247-56.

[21] Wang JS, Shum-Tim D, Galipeau J, *et al*. Marrow stromal cells for cellular cardiomyoplasty: feasibility and potential clinical advantages. J Thorac Cardiovasc Surg 2000; 120: 999-1005.

[22] Orlic D, Kajstura J, Chimenti S, *et al*. Bone marrow cells regenerate infarcted myocardium. Nature 2001; 410: 701-5.

[23] Christoforou N, Gearhart JD. Stem cells and their potential in cell-based cardiac therapies. Prog Cardiovasc Dis 2007; 49: 396-413.

[24] Leri A, Kajstura J, Anversa P, *et al*. Myocardial regeneration and stem cell repair. Curr Probl Cardiol 2008; 33: 91-153.

[25] Luo J, Chiu RCJ, Tim-Shum D. Bone marrow stromal cells as "universal donor cells" for myocardial regeneration therapy. In: Prakash S, Ed. Artificial cells, cell engineering and therapy. Cambridge, England: Woodhead Publishing Ltd. 2007; pp. 333-48.

[26] Jolicoeur EM, Granger CB, Fakunding JL, *et al*. Bringing cardiovascular cell-based therapy to clinical application: perspectives based on a National Heart, Lung, and Blood institute Cell Therapy Working Group meeting. Am Heart J 2007; 153: 732-42.

[27] Schuleri KH, Boyle AJ, Hare JM. Mesenchymal stem cells for cardiac regenerative therapy. Handb Exp Pharmacol 2007; 180: 195-218.

[28] Segers VF, Lee RT. Stem-cell therapy for cardiac disease. Nature 2008; 451: 937-42.

[29] Schächinger V, Assmus B, Britten MB, *et al*. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. J Am Coll Cardiol 2004; 44: 1690-9.

[30] Strauer BE, Brehm M, Zeus T, *et al*. Regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease: the IACT Study. J Am Coll Cardiol 2005; 46: 1651-8.

[31] Meyer GP, Wollert KC, Lotz J, *et al*. Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months' follow-up data from the randomized, controlled BOOST (BOne marrOw transfer to enhance ST-elevation infarct regeneration) trial. Circulation 2006; 113: 1287-94.

[32] Kang HJ, Lee HY, Na SH, *et al*. Differential effect of intracoronary infusion of mobilized peripheral blood stem cells by granulocyte colony-stimulating factor on left ventricular function and remodeling in patients with acute myocardial infarction versus old myocardial infarction: the MAGIC Cell-3-DES randomized, controlled trial. Circulation 2006; 114(Suppl I): 145-51.

[33] Lunde K, Solheim S, Aakhus S, *et al*. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. N Engl J Med 2006; 355: 1199-209.

[34] Meluzín J, Mayer J, Groch L, *et al*. Autologous transplantation of mononuclear bone marrow cells in patients with acute myocardial infarction: the effect of the dose of transplanted cells on myocardial function. Am Heart J 2006; 152: 975.e9-15.

[35] Lunde K, Solheim S, Aakhus S, *et al*. Exercise capacity and quality of life after intracoronary injection of autologous mononuclear bone marrow cells in acute myocardial infarction: results from the Autologous Stem cell Transplantation in Acute Myocardial Infarction (ASTAMI) randomized controlled trial. Am Heart J 2007; 154: 710.e1-8.

[36] Erbs S, Linke A, Adams V, *et al*. Transplantation of blood-derived progenitor cells after recanalization of chronic coronary artery occlusion: first randomized and placebo-controlled study. Circ Res 2005; 97: 756-62.

[37] Janssens S, Dubois C, Bogaert J, *et al*. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation

myocardial infarction: double-blind, randomised controlled trial. Lancet 2006; 367: 113-21.

[38] Schächinger V, Erbs S, Elsässer A, *et al*. Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial. Eur Heart J 2006; 27: 2775-83.

[39] Assmus B, Honold J, Schächinger V, *et al*. Transcoronary transplantation of progenitor cells after myocardial infarction. N Engl J Med 2006; 355: 1222-32.

[40] Zhang F, Yang Z, Chen Y, *et al*. Clinical cellular cardiomyoplasty: technical considerations. J Card Surg 2003; 18: 268-73.

[41] Agbulut O, Vandervelde S, Al Attar N, *et al*. Comparison of human skeletal myoblasts and bone marrow-derived CD133+ progenitors for the repair of infarcted myocardium. J Am Coll Cardiol 2004; 44: 458-63.

[42] Guarita-Souza LC, Carvalho KA, Rebelatto C, *et al*. Cell transplantation: differential effects of myoblasts and mesenchymal stem cells. Int J Cardiol 2006; 111: 423-9.

[43] Thompson RB, Emani SM, Davis BH, *et al*. Comparison of intracardiac cell transplantation: autologous skeletal myoblasts versus bone marrow cells. Circulation 2003; 108(Suppl II): 264-71.

[44] Ye L, Haider HKH, Sim EK. Adult stem cells for cardiac repair: a choice between skeletal myoblasts and bone marrow stem cells. Exp Biol Med (Maywood) 2006; 231: 8-19.

[45] Seeger FH, Tonn T, Krzossok N, *et al*. Cell isolation procedures matter: a comparison of different isolation protocols of bone marrow mononuclear cells used for cell therapy in patients with acute myocardial infarction. Eur Heart J 2007; 28: 766-72.

[46] Hristov M, Heussen N, Schober A, *et al*. Intracoronary infusion of autologous bone marrow cells and left ventricular function after acute myocardial infarction: a meta-analysis. J Cell Mol Med 2006; 10: 727-33.

[47] Lipinski MJ, Biondi-Zoccai GG, Abbate A, *et al*. Impact of intracoronary cell therapy on left ventricular function in the setting of acute myocardial infarction: a collaborative systematic review and meta-analysis of controlled clinical trials. J Am Coll Cardiol 2007; 50: 1761-7.

[48] Abdel-Latif A, Bolli R, Tleyjeh IM, *et al*. Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. Arch Intern Med 2007; 167: 989-97.

[49] Bartunek J, Dimmeler S, Drexler H, *et al*. The consensus of the task force of the European Society of Cardiology concerning the clinical investigation of the use of autologous adult stem cells for repair of the heart. Eur Heart J 2006; 27: 1338-40.

[50] Dimmeler S, Burchfield J, Zeiher AM. Cell-based therapy of myocardial infarction. Arterioscler Thromb Vasc Biol 2008; 28: 208-16.

[51] Müller-Ehmsen J, Krausgrill B, Burst V, *et al*. Effective engraftment but poor mid-term persistence of mononuclear and mesenchymal bone marrow cells in acute and chronic rat myocardial infarction. J Mol Cell Cardiol 2006; 41: 876-84.

[52] Al Kindi A, Ge Y, Shum-Tim D, *et al*. Cellular cardiomyoplasty: routes of cell delivery and retention. Front Biosci 2008; 13: 2421-34.

[53] Anderl JN, Robey TE, Stayton PS, *et al*. Retention and biodistribution of microspheres injected into ischemic myocardium. J Biomed Mater Res A 2008 [Epub ahead of print].

[54] Laflamme MA, Murry CE. Regenerating the heart. Nat Biotechnol 2005; 23: 845-56.

[55] Gavira JJ, Herreros J, Perez A, *et al*. Autologous skeletal myoblast transplantation in patients with nonacute myocardial infarction: 1-year follow-up. J Thorac Cardiovasc Surg 2006; 131: 799-804.

[56] Siminiak T, Fiszer D, Jerzykowska O, *et al*. Percutaneous transcoronary-venous transplantation of autologous skeletal myoblasts in the treatment of post-infarction myocardial contractility impairment: the POZNAN trial. Eur Heart J 2005: 26: 1188-95.

[57] Frangogiannis NG. The mechanistic basis of infarct healing. Antioxid Redox Signal 2006; 8: 1907-39.

[58] Frantz S, Bauersachs J, Kelly RA. Innate immunity and the heart. Curr Pharm Des 2005; 11: 1279-90.

[59] Linde A, Mosier D, Blecha F, *et al*. Innate immunity and inflammation—New frontiers in comparative cardiovascular pathology. Cardiovasc Res 2007; 73: 26-36.

[60] Baumgarten G, Kim SC, Stapel H, *et al*. Myocardial injury modulates the innate immune system and changes myocardial sensitivity. Basic Res Cardiol 2006; 101: 427-35.

[61] Kapadia S, Dibbs Z, Kurrelmeyer K, *et al*. The role of cytokines in the failing human heart. Cardiol Clin 1998; 16: 645-56.

[62] Kelly RA, Smith TW. Cytokines and cardiac contractile function. Circulation 1997; 95: 778-81.

[63] Ono K, Matsumori A, Shioi T, Furukawa Y, Sasayama S. Cytokine gene expression after myocardial infarction in rat hearts: possible implication in left ventricular remodeling. Circulation 1998; 98: 148-56.

[64] Uccelli A, Pistoia V, Moretta L. Mesenchymal stem cells: a new strategy for immunosuppression? Trends Immunol 2007; 28: 219-26.

[65] Stagg J. Immune regulation by mesenchymal stem cells: two sides to the coin. Tissue Antigens 2007; 69: 1-9.

[66] Solheim S, Seljeflot I, Lunde K, *et al*. Inflammatory responses after intracoronary injection of autologous mononuclear bone marrow cells in patients with acute myocardial infarction. Am Heart J 2008; 155: 55.e1-9.

[67] Thum T, Bauersachs J, Poole-Wilson PA, *et al*. The dying stem cell hypothesis: immune modulation as a novel mechanism for progenitor cell therapy in cardiac muscle. J Am Coll Cardiol 2005; 46: 1799-802.

[68] Tomchuck SL, Zwezdaryk KJ, Coffelt SB, *et al*. Toll-like receptors on human mesenchymal stem cells drive their migration and immunomodulating responses. Stem Cells 2008; 26: 99-107.

[69] Rasmusson I. Immune modulation by mesenchymal stem cells. Exp Cell Res 2006; 312: 2169-79.

Received: October 5, 2008 Revised: October 27, 2008 Accepted: October 30, 2008