Regenerative Therapy in Patients With a Revascularized Acute Anterior Myocardial Infarction and Depressed Ventricular Function

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Introduction and objectives. It is difficult to distinguish the effects early revascularization and regenerative therapy have on left ventricular function in patients with acute myocardial infarction (AMI). This study was an investigation into 3 groups of patients who had a revascularized anterior wall AMI and depressed left ventricular function (i.e., ejection fraction <45%). The aim was to compare changes in left ventricular function between patients who received regenerative therapy and those who did not.

Methods. Patients were randomly assigned to receive either an intracoronary infusion of autologous mononuclear bone marrow cells (Group I; n=10) or systemic administration of granulocyte colony-stimulating factor (G-CSF) (Group II; n=10), or to a control group (Group III; n=10). In Group I, intracoronary infusion was carried out 7(2) days after AMI. Group-II patients received a 10-day course of subcutaneous G-CSF injections, 10 μg/kg per day starting 5 days after AMI. Ventricular function was assessed at baseline and 3-month follow-up.

Results. A 20% increase in mean ejection fraction was observed in Group I, compared with increases of 4% (*P*<.01) and 6% (*P*<.05) in Groups II and III, respectively.

Conclusions. Intracoronary infusion of mononuclear bone marrow cells in patients with AMI and poor ventricular function was associated with better short-term functional recovery than previously reported. However, mobilization of stem cells by G-CSF did not have a significant influence on functional recovery.

Key words: *Stem cells. Granulocyte colony-stimulating factor. Acute myocardial infarction.*

Tratamiento regenerativo en pacientes con infarto agudo anterior revascularizado y función ventricular deprimida

Introducción y objetivos. La influencia de la revascularización precoz y del tratamiento regenerativo sobre la función ventricular en pacientes con infarto agudo de miocardio (IAM) resulta difícil de separar. En el presente estudio se analizan 3 grupos de pacientes con infarto de miocardio anterior revascularizado con función ventricular deprimida (fracción de eyección [FE] < 45%). Nuestro propósito fue comparar los cambios en la función ventricular entre los pacientes con y sin tratamiento regenerativo.

Métodos. Los pacientes fueron asignados aleatoriamente para recibir una infusión intracoronaria de células mononucleares autólogas de la médula ósea (grupo I; n = 10), la administración sistémica de factor estimulante de colonias granulocíticas (G-CSF) (grupo II; n = 10), o como grupo control (grupo III; n = 10). En los pacientes del grupo I la infusión intracoronaria de células fue efectuada 7 ± 2 días tras el IAM. Los pacientes del grupo II recibieron inyección subcutánea de G-CSF (10 μg/kg/día) durante 10 días consecutivos, comenzando 5 días después del IAM. Se efectuaron estudios de función ventricular basalmente y a los 3 meses de seguimiento.

Resultados. Se observó un incremento neto medio del 20% en FE en el grupo I, frente a un 4% (p < 0,01) y un 6% (p < 0,05) en los grupos II y III, respectivamente.

Conclusiones. La infusión intracoronaria de células mononucleares de la médula ósea en pacientes con IAM y función ventricular deprimida se asocia con una recuperación funcional a corto plazo de mayor proporción que la publicada previamente. Sin embargo, la movilización de células madre con G-CSF no tuvo una influencia significativa en la recuperación funcional.

Palabras clave: *Células madre. Factor estimulante de colonias granulocíticas. Infarto agudo de miocardio.*

INTRODUCTION

The favorable influence of early revascularization on left ventricular function in patients with acute myocardial infarction (AMI) has been widely studied.[1-7] Regenerative treatment may provide substantial additional benefit to patients with revascularized AMI.

ABBREVIATIONS

AMI: acute myocardial infarction
BMC: bone marrow cell
CFR: coronary flow reserve
CK-MB: creatine kinase MB fraction
EF: ejection fraction
G-CSF: granulocyte colony-stimulating factor
LAD: left anterior descending coronary artery
LV: left ventricle
MNBMC: mononuclear bone marrow cell

However, it is still difficult to differentiate and characterize both types of benefit in clinical studies. Several regenerative strategies are presently under evaluation. Different studies[8-16] have shown that intracoronary infusion of bone marrow cells (BMC) in patients with AMI results in favorable remodelling of the left ventricle (LV) and improvement in ejection fraction (EF). Additionally, experimental evidence in animal models[17] and different feasibility studies in humans[18-23] have suggested that cytokine-mobilized BMCs home to the infarcted heart and promote cardiac repair. Controversy about the efficacy of granulocyte-colony stimulating factor (G-CSF) still persists[18,24] and further information is required as cytokine treatment would be a highly desirable non-invasive method for myocardial regeneration. As a result, we compared these 2 different regenerative strategies against a control group of patients. Our aim was to assess whether autologous intracoronary BMC transplantation and G-CSF mediated stem-cell mobilization have comparable capabilities in restoring ventricular function after AMI. For this comparison, we selected patients with anterior wall AMI and poor baseline ventricular function, to better analyse functional recovery.

METHODS

Study Design

This study was designed to compare 3 groups of patients with revascularized anterior wall AMI. Patients were randomly assigned to a control group or to regenerative treatment, either by intracoronary cell transfer or by systemic administration of G-CSF. The study was approved by all institutional and ethics committees. Since January 2005, we prospectively studied 30 patients with anterior wall AMI who met all the inclusion criteria and signed informed consent to be involved in this study. They were assigned to intracoronary BMC transplantation (Group I, n=10), subcutaneous administration of G-CSF (Group II,

n=10), or the control group with conventional treatment (no new actions) (Group III, n=10). Inclusion criteria were as follows: *a)* patients with anterior wall AMI arriving at the hospital within 12 hours of the onset of symptoms; *b)* poor LV-function (angiographic EF <45%); *c)* early reperfusion of the left anterior descending artery, by intravenous administration of fibrinolytics or by rescue percutaneous coronary intervention; and *d)* in all instances, full stent revascularization of the left anterior descending artery supplying the anterior wall was mandatory to enter the study. Exclusion criteria included: *a)* age >80 years; *b)* mechanical complications of AMI or cardiogenic shock; *c)* hematologic disorders; and d) concomitant malignant or pre-malignant systemic disease. Once fulfilling these criteria after the first cardiac catheterization, patients who agreed to enter the study were randomly assig

ned to either group. The main purpose of the study was to compare the serial LV function and coronary flow reserve (CFR) studies performed at baseline and at 3-month follow-up cardiac catheterization, in all groups. Functional recovery at 3-month evaluation was analyzed by changes in EF and other angiographic indices of LV-performance. The changes in post-extrasystolic potentiation of the LV were also analyzed for each group of patients.

Patient Groups

All 30 patients were initially managed at the intensive care unit according to their clinical and hemodynamic conditions. Early intravenous fibrinolytics were always administered upon hospital arrival. Full stent revascularization was also performed in all patients, either at acute early catheterization (as a rescue procedure) or at catheterization performed 3 to 5 days later in patients with successful fibrinolytic therapy. Once study eligibility was established, the patient signed an informed consent form to enter the study. Then, randomization by telephone was performed. The extent of AMI was evaluated by the peak CK-elevation and the summation in mm of Q waves (ΣQ) on the electrocardiogram performed at discharge. All patients were sent home under early afterload-reducing therapy and antithrombotic regimen. Functional class (NYHA) was evaluated in all patients at 3-month follow-up.

Group I (n=10)

All 10 patients underwent a second cardiac catheterization 5 to 12 days after AMI (7 [2] days) where intracoronary infusion of MNBMCs into the infarcted area was performed. On the morning of cardiac catheterization, up to a volume of 80-100 mL of marrow was obtained under local anesthesia by aspiration from the iliac crest. MNBMCs were isolated by density gradient

centrifugation over Ficoll-Hypaque technique in a sterile, semiautomated device COBE 2991. After three washes, MNBMCs were filtered and resuspended in 10 mL of 0.9% sodium chloride supplemented with preservative-free 0.1% heparin. Aliquots were obtained for cell count and cytofluorometric analysis of the CD34+ cell content and their subsets.

Cells were directly transferred into the infarcted zone by the use of a coaxial balloon catheter, which was placed within the site of the stented segment. Balloon size was selected according to vessel size in order to achieve complete occlusion of the vessel and to stop flow during cell injection. So, backflow of cells was prevented and distal stagnant flow facilitated cell exposure. The cell suspension was injected through the distal tip of the balloon over 2 to 4 minutes.

Group II (n=10)

After successful stent revascularization was achieved, patients received a 10-day course of r-hu-G-CSF (Neupogen, Amgen, Thousand Oaks, CA) subcutaneously, starting on day 5 post-AMI at a dose of 5 µg/kg every 12 hours. Samples of peripheral blood were obtained on days 0, 3, 5 and 10 for determination of total white blood cell (WBC) count, total number of circulating CD34+ cells and their subsets by immunophenotyping. To quantify the CD34+ cells and subsets of progenitors present in peripheral blood we used three-color-inmunofluorescence cytometry in accordance with ISHAGE guidelines.[25]

Group III (n=10)

Patients assigned to the control group underwent early reperfusion and ulterior stent revascularization of the left anterior descending artery 3 to 5 days post- AMI. LV-function studies were performed at this time and were considered the baseline condition for evaluating the extent of LV-damage.

Diagnostic and Therapeutic Cardiac Catheterizations

A diagnostic left heart catheterization, including left ventriculography and coronary angiography, was undertaken in all 30 patients. Stent revascularization of the left anterior descending artery was performed in all patients 0 to 5 days post-AMI. We always used a Cypher® stent (Johnson&Johnson, Cordis, Miami, Florida) for treatment of the culprit lesion. In 10 patients, additional stent implantation was needed at remote sites in a non-related artery. In the cath lab patients received 2 mg/kg of intravenous unfractionated heparin, continuing after the procedure with low-molecular-weight heparin (Fragmin®) 10 000 IU anti-Xa/day, ticlopidine 500 mg/day and aspirin 150 mg/day, at least, for 4 weeks.

A 3-month follow-up cardiac catheterization was performed in all 30 patients, where functional studies were repeated under conditions similar to baseline studies.

LV-Function Studies

In every condition of the study we performed at least one 30º right anterior oblique LV-angiogram. In all groups, the LV-angiogram performed 3 to 12 days after AMI was considered a baseline study while the late angiogram was performed at 3-month follow-up. During each ventriculogram, attempts were made to obtain a sinus and a post-extrasystolic beat for analysis, in order to study contractile reserve behaviours. Post-extrasystolic beats were obtained by inducing premature beats with the catheter, once a well opacified cardiac cycle with a normal sinus beat had been filmed. In all instances, the R-R´ interval of the induced premature beat and the post-extrasystolic pause were recorded and measured. There were no significant differences among groups in such intervals (Table 1).

Measurements and calculations were made off line in our own core lab, where end-diastolic and end-systolic silhouettes were drawn using the CASS system by 2 expert angiographers who were unaware of the patient group or study conditions. LV-volumes and EF were derived and regional wall motion was analyzed. The method by Sheehan[1] was used for the asynergy study, dividing the superimposed silhouettes in 100 radii of wall shortening, from end-diastole to end-systole. The abnormal contracting segment (ACS) was defined as the percentage of radii showing akinesia or dyskinesia. The areas of the anterior wall affected by the infarct included the ACS plus the surrounding hypokinetic radii. Thus, the functional behaviour of the infarcted myocardium after treatment was observed serially enabling possible recovery to be evaluated. The serial evolution of the contractile reserve was evaluated by the post-extrasystolic potentiation.

Measurements of Velocities and CFR

The FloMap® system (Cardiometrics; Mountain View; California) was used. Baseline CFR-studies were performed immediately after stent revascularization. A 0.014” intracoronary Doppler guide wire was positioned distal to the stent and flow velocities were recorded continuously. Average peak velocity was obtained at baseline and after an intracoronary bolus of 60 µg of Adenosine. CFR was calculated as the ratio between maximal flow velocity during the peak effect of the adenosine injection and basal flow velocity. At 3-month follow-up, a similar Doppler study was repeated in all patients following the same methodology.

TABLE 1. **Clinical and Angiographic Baseline Data***

	Group I (Cell-Infusion)	Group II (G-CSF)	Group III (Control)	*P*
Age, years	52 (12)	54 (9)	55 (11)	NS
Gender, male	8	9	7	NS
Recanalization time, h	4 (2)	5 (3)	4 (3)	NS
Peak CK, IU/dL	4455 (1573)	4936 (3091)	3063 (1672)	NS
Peak CK-MB, IU/dL	464 (264)	579 (472)	248 (197)	NS
∑Q-wave	26 (16)	34 (19)	25 (9)	NS
LV-ejection fraction, %	37 (5)	39 (5)	38 (6)	NS
LVEDP, mm Hg	30 (8)	29 (10)	25 (7)	NS
ACS, %	32 (12)	36 (11)	28 (14)	NS
No. of affected radii, %	40 (16)	47 (14)	36 (11)	NS
Post-PVC potentiation, %	15 (8)	11 (7)	13 (8)	NS
Coronary flow reserve	1.5 (0.3)	1.4 (0.3)	1.7 (0.4)	NS
R-R interval, ms	983 (172)	890 (161)	935 (179)	NS
R-R' interval, ms	465 (111)	600 (166)	580 (288)	NS
R'-R interval, ms	1152 (280)	1155 (227)	940 (314)	NS

*ACS indicates abnormal contracting segment; CK, creatine-kinase; LV, left ventricle; LVEDP, left ventricular end-diastolic pressure; PVC. premature ventricular contraction.

Statistical Analysis

All data are presented as mean (SD). Student pair *t* test was used to compare means within groups, while analysis of variance and Scheffé test swere used to compare means between groups. Pearson correlation coefficients (*r*) were calculated to assess linear associations. A power calculation (1-β) of the Anova was performed for the comparison of gain in EF. A *P* value less than .05 was considered significant.

RESULTS

Clinical, Angiographic, and Laboratory Findings

Table 1 shows the main clinical and angiographic baseline data in all 3 groups. There were no significant differences among groups in recanalization time, peak enzyme elevation, ∑Q-waves or baseline ventricular function. All patients survived AMI. Twelve needed inotropic drugs during the acute phase. None had complications secondary to any cardiac catheterization performed during the hospital phase or at follow-up. In group I patients, the total number of mononuclear cells infused was 9 (3)×10^8. Of these, 17 (13)×10^6 were CD34+. Patients from group II evidenced mobilization by significant elevation of circulating WBC and CD34+ cells during G-CSF administration. Peak levels of circulating progenitors were observed at day 5 after the onset of G-CSF administration. Table 2 summarizes these findings. At 3-month evaluation, 24 patients were in functional class I; 4 patients were in functional class II and 2 in functional class III. No restenosis was observed at cardiac catheterization. Minimal lumen

diameter at the treated segment was 2.6 (0.7) and late loss was 0.3 (0.4) mm. No differences in functional class or luminal measurements were observed among groups.

LV Volumes and Function

Table 3 shows the changes observed in LV-volume and function parameters in all 3 groups. There were significant reductions in LV-volumes at follow-up in group I patients. Patients from groups II and III did not have similar LV-volume reduction at follow-up. The EF increased significantly at follow-up in group I, showing no significant changes in groups II and III. The extent of recovery in EF at follow-up did not differ between groups II and III. In addition, the percentage of ACS and of the affected radii were significantly reduced at follow-up in group I but showed no significant changes in groups II and III patients.

Degree of Functional Recovery

Table 4 shows the net gain observed in functional parameters in all 3 groups. Group I patients always showed a favorable gain in EF ranging from +11% to +34%. Patients from group II had a gain in EF ranging from –22% to +18%. The gain in EF in patients from the control group III ranged from –6% to +21%. The gain in EF, ACS and number of affected radii were significantly higher in patients treated with intracoronary cell infusion. There was a significant correlation (*r*=0.81; *P*<.001) between the gain in EF and the reduction in the number of affected radii in the anterior wall suggesting that improvement in global function was mainly obtained

TABLE 2. Infused and Mobilized Cells*

	Group I Infused Cells	Group II Circulating Cells
MNCs×10^8	9 (3)	–
CD34+ cells×10^6	17 (13)	–
WBC×10^6 /mL, day 5 of treatment	–	48 (16)
WBC×10^6 /mL, day 10 of treatment	–	54 (16)
Granulocytes×10^6 /mL, day 5 of treatment	–	43 (15)
Granulocytes×10^6 /mL, day 10 of treatment	–	42 (16)
CD34+ cells×10^3/mL, day 5 of treatment	–	88 (79)
CD34+ cells×10^3/mL, day 10 of treatment	–	29 (25)

*MNCs indicates mononuclear cells; WBC, white blood cell count.

by recovery of the infarcted anterior wall. Interestingly, group I showed a significant improvement, not only in sinus EF but also in post-extrasystolic EF, which was not observed in the remaining groups (Figure). This may suggest an additional functional improvement in contractile reserve in patients receiving intracoronary cell transfer. There was an inverse and significant correlation (r=–0.45; P=.02) between the peak creatine-kinase and the gain in EF, suggesting a worse functional

recovery in patients with greater damage. The baseline post-extrasystolic potentiation correlated (r=0.39; P=.04) with the gain in sinus EF at follow-up. In addition, there was a significant correlation (r=0.80; P<.001) between the 3-month post-extrasystolic EF and the net gain in sinus EF. CFR did not correlate with LV-function parameters and the gain in CFR did not correlate with the gain in EF in any group.

DISCUSSION

Reperfusion Therapy

Recovery of ventricular function occurs in a significant proportion of patients after AMI and modern reperfusion strategies have been associated with increased recovery of function and enhanced survival. However, no recovery or even worsening may also be observed in another proportion of patients. Factors influencing such different outcomes after reperfusion therapy in AMI have been widely studied.[2,4,6,7] Time delay to reperfusion and the adequacy of coronary reflow are of paramount importance.[2,6] A lower peak level of creatine kinase, an estimate of the extent of necrosis, has also been identified as a strong independent predictor of subsequent LV-function recovery.[4,6] As a result, many factors and conditions may influence the degree of functional recovery after AMI in any given patient.

The evaluation of functional recovery also needs consideration. Improvement of wall motion in the infarct

TABLE 3. Parameters of Left Ventricular Volumes and Function*

	Group I (Cell-Infusion)		Group II (G-CSF)		Group III (Control)	
	Baseline	3-Month	Baseline	3-Month	Baseline	3-Month
LVEDVI, mL/m^2	142 (35)	134 (29)	141 (21)	182 (46)	140 (30)	159 (43)
LVESVI, mL/m^2	89 (27)	61 (19)	87 (15)	110 (50)	83 (25)	87 (35)
EF, %	37 (5)	58 (9)	37 (5)	42 (14)	39 (6)	45 (8)
ACS, %	33 (12)	8 (10)	39 (7)	25 (16)	27 (13)	16 (9)
Affected radii, %	47 (6)	24 (17)	52 (6)	44 (20)	46 (11)	36 (20)

*ACS indicates abnormal contracting segment. EF, ejection fraction; LVEDVI, left ventricular end-diastolic volume index; LVESVI, left ventricular end-systolic volume index.

TABLE 4. Net Gain in Functional Recovery*

	Group I (Cell-Infusion)	Group II (G-CSF)	Group III (Control)	*P* (Anova)
Gain in EF	20 (8)	4 (13)†	6 (10)‡	.003
Gain in ACS	−20 (15)	−12 (11)	−6 (3)‡	.05
Gain in affected radii	−26 (11)	−9 (16)‡	−12 (12)	.05
Gain in CFR	0.9 (0.7)	1.1 (0.5)	0.4 (0.7)	NS
Gain in ΣQ wave	−12 (10)	−13 (10)	−6 (5)	NS

*ACS indicates abnormal contracting segment; CFR, coronary flow reserve; EF, ejection fraction.
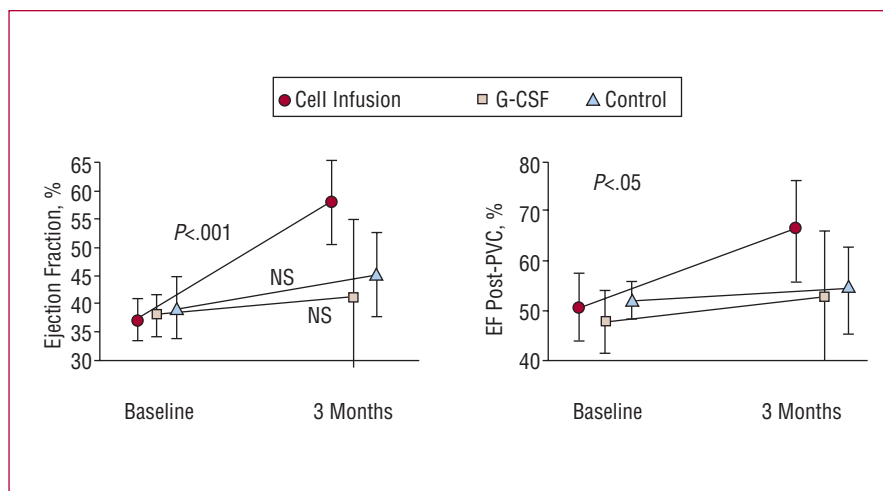†P<.01 versus group I.
‡P<.05 versus group I.

Figure. Graphic representation of the sinus and post-extrasystolic evolution of EF in all 3 groups. EF indicates ejection fraction; G-CSF, granulocyte-colony stimulating factor; PVC, premature ventricular contraction.

region is delayed and follows a time course that may vary in the early phase. Minor worsening may even be observed early after reperfusion and seems to be associated with marked regression in compensatory hyperkinesia in the non-infarcted areas.[4,5] Three days after AMI has been considered the best time to measure the final functional status early after myocardial damage.[5] In a multi-center trial,[7] most functional improvements occurred by day 14 after AMI. At 90 days, full recovery was observed in 22% of patients and over 50% of patients had a greater than 5% improvement in ejection fraction, whereas 16% of patients had a decrease in ejection fraction of over 5%. Thus, we actually know that reperfusion and revascularization in AMI, combined with modern pharmacological strategies, provide a wide degree of functional recovery ranging from a clear worsening to full recovery. This must be taken into account when evaluating the possible benefits of a new adjuvant therapy for AMI in clinical studies.

G-CSF Mobilization

Mobilization of stem cells by G-CSF has recently attracted attention. However, the beneficial effects of G-CSF-induced stem-cell mobilization have been questioned. Different clinical studies have evaluated the feasibility and safety of recombinant G-CSF treatment in patients with AMI.[18-23] A clinical trial observed an increased rate of in-stent coronary restenosis.[18] However, most of the clinical studies suggest that G-CSF may provide LV-functional benefits and has no influence on restenosis rate. Recently, Zohlnhofer et al[24] have demonstrated that stem-cell mobilization by G-CSF in patients with AMI and successful mechanical reperfusion has no influence on infarct size, LV-function or coronary restenosis. Our results confirm this line of evidence showing again that patients receiving G-CSF and stent

revascularization did not differ from controls. Nevertheless, many questions still arise on the role of cytokine treatment in AMI. Individual responses to G-CSF in the number of circulating progenitors during treatment may vary widely among patients. In a previous feasibility study we observed a direct and significant correlation between circulating levels of mobilized progenitors obtained on day 5 after the start of treatment with G-CSF and the gain in EF at follow-up, suggesting a better functional recovery with higher levels of circulating progenitors.[22] At present, it seems that G-CSF treatment in revascularized AMI provides a wide degree of functional recovery, but does not differ from control groups. Several reasons may explain why G-CSF treatment may fail to promote significant myocardial regeneration. One biological explanation may lie in the loss of properties of mobilized progenitor cells. The exposure of stem cells to cytokines such as G-CSF, both in vivo for mobilization purposes and in vitro in expansion cultures, has been shown to induce changes in the expression of adhesion molecules.[26] The loss of their adhesive properties during circulation could impair the homing potential at myocardial infarction.

Intracoronary Infusion of BMC

Different studies in humans have demonstrated that intracoronary injection of autologous BMCs may have a beneficial effect on post-infarction remodeling and perfusion. Significant improvements in ventricular function may range from a 2% to 9% increase in EF.[8-16] The biological mechanism underlying such beneficial effects remain unknown. Our results clearly show that patients with adjuvant intracoronary cell-infusion had a remarkable improvement in LV-function (a mean 20% increase in EF) that significantly differed from a mean 4% and 6% increase in groups II and III patients, respectively. In addition, at follow-up patients

TABLE 5. **Number of Injected Cells and Functional Recovery in Previous Studies***

Reference	Injected Cells×10^8	Injected CD34+ Cells×10^6	Baseline EF, %	Gain in EF,%
Strauer et al[8]	0.28	0.59 (0.78)	51 (14)	2
Asmus et al[9]	–	7.35 (7.3)	52 (9)	8
Britten et al[10]	2.38 (0.79)	5.5 (2.8)	44 (10)	5
Fernández Avilés et al[12]	0.78 (0.41)	–	51 (7)	6
Schächinger et al[13]	2.13 (0.75)	2.6 (2.5)	50 (10)	8
Wollert et al[11]	24.6 (9.4)	9.5 (6.3)	50 (10)	7
Janssens et al[14]	1.72 (0.72)	2.8 (1.7)	49 (7)	3
Lunde et al[15]	0.68†	0.7†	42 (11)	8
Schächinger et al[16]	2.36 (1.74)	3.6 (3.6)‡	48 (9)	5.5
Our study	9 (3)	17 (13)	37 (5)	20

*EF indicates ejection fraction.
†Median.
‡CD34+, CD133+, CD45+.

with intracoronary cell-infusion showed a significant increase in maximal contractile capacity (post-extrasystolic EF), which was not observed in groups II and III. Post-extrasystolic potentiation provides an effective stimulus for contractile reserve of acutely dysfunctional ischemic myocardium.[27] The analysis of this capacity might help to discriminate between the functional recovery observed in certain patients with revascularized AMI and the additional recovery afforded by an effective regenerative treatment. Loss or similar responsiveness at follow-up may represent the progression to non-viability. However, improvements in contractile reserve at follow-up represent further benefit.

The mean 20% increase in EF observed in our patients from group I contrasts with the lower values reported in other studies,[8-16] where direct cell infusion was similarly applied to patients with AMI (Table 5). Two reasons might account for these differences. On one hand, the total number of CD34+ cells directly infused in this study is 2 to 5 times higher than that previously reported. A higher concentration of progenitors delivered locally to the infarcted myocardium might play a role in providing better functional responses at follow-up. In experimental studies with rats, Schuster et al[28] observed that increasing the number of angioblasts trafficking to the infarct zone resulted in dose-dependent neovascularization, with development of progressively larger-sized capillaries. Hofmann et al[29] have recently monitored myocardial homing and biodistribution of BMCs after therapeutic injection in post-AMI patients, demonstrating that a small fraction of intracoronary tranplanted cells did actually home to the myocardium while no cells were detected after intravenous infusion of labeled BMCs. Thus, the number and type of infused cells, the method of preparation as well as the way of delivery could play an important role. An experienced hematologist may try to obtain the largest amount of mononuclear cells from bone marrow because the number

of transferred cells may influence the final result. On the other hand, in most studies on cell therapy evaluating changes in ventricular function after AMI, the mean baseline EF was near to normal values (mean, 50%). In patients with small infarcts and, consequently, almost normal EF, the functional benefit of a given treatment may be more difficult to evaluate if contractile reserve is not investigated. The comparative study of large infarcts with poor ventricular function may better test potential functional restoration. REPAIR-AMI trial[16] has also observed better functional responses in patients with AMI and poor LV-function.

CONCLUSIONS

The main limitation of our study is the small number of patients, but it is powered enough to detect a 14% difference in EF gain between groups (power=91.6%). The strict selection of patients was designed to analyze large infarcts on the same territory supplied by the same artery. The study of a uniform model of AMI may limit the number of observations but might improve their accuracy. Additionally, the small size of the sample does not warrant the efficacy of the randomization nor the homogeneous distribution among groups of baseline parameters that could also influence changes in ventricular function.

We conclude that intracoronary transfer of BMC in patients with revascularized anterior wall AMI and poor LV-function promotes a significant short-term functional improvement of the infarcted area of higher proportions than previously reported. This functional recovery also seems to exert a higher contractile reserve, as evaluated by the post-extrasystolic potentiation. On the contrary, stem cell mobilization by G-CSF in patients with anterior wall AMI and successful mechanical reperfusion had no significant influence on the degree of functional recovery as compared with a control group of patients.

REFERENCES

1. Sheehan FH, Mathey DG, Schofer J, Krebber HJ, Dodge HT. Effect of interventions in salvaging left ventricular function in acute myocardial infarction: a study of intracoronary streptokinase. Am J Cardiol. 1983;52:431-8.

2. Sheehan FH. Determinants of improved left ventricular function after thrombolytic therapy in acute myocardial infarction. J Am Coll Cardiol. 1987;9:937-44.

3. Sheehan FH, Doerr R, Schmidt WG, Bolson EL, Uebis R, von Essen R, et al. Early recovery of left ventrivular function after thrombolythic therapy for acute myocardial infarction: an important determinant of survival. J Am Coll Cardiol. 1988;12: 289-300.

4. Suárez de Lezo J, Pan M, Romero M, Segura J, Sancho M. Determinants of functional recovery after thrombolytic therapy. Rev Esp Cardiol. 1988;41:426-36.

5. Schmidt WG, Sheehan FH, von Essen R, Uebis R, Effert S. Evolution of left ventrivular function after intracoronary thrombolysis for acute myocardial infarction. Am J Cardiol. 1989;63:497-502.

6. Lancellotti P, Albert A, Berthe C, Pierard LA. Full recovery of contraction late after myocardial infarction: determinants and early predictors. Heart. 2001;85:521-6.

7. Solomon SD, Glynn RJ, Greaves S, Ajani U, Rouleau JL, Menapace F, et al. Recovery of ventricular function after myocardial infarction in the reperfusion era: the healing and the early afterload reduction therapy study. Ann Intern Med. 2001; 134:451-8.

8. Strauer BE, Brehm M, Zeus T, Kostering M, Hernandez A, Sorg RV, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cells transplantation in humans. Circulation. 2002;106:1913-8.

9. Assmus B, Schächinger V, Teupe C, Britten M, Lehmann R, Döbert N, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation. 2002;106:3009-17.

10. Britten MB, Abolmaali ND, Assmus B, Lehmann R, Honold J, Schmitt J, et al. Infarct remodeling after intracoroanary progenitor cell treatment in patients with acute myocardial infarction (TOPCARE-AMI): mechanistic insights from serial contrast-enhanced magnetic resonance imaging. Circulation. 2003;108: 2212-8.

11. Wollert KC, Meyer GP, Lotz J, Lichtenberg SR, Lippolt P, Breidenbach C, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomized controlled clinical trial. Lancet. 2004;364:141-8.

12. Fernandez-Aviles F, San Roman JA, Garcia-Frade J, Fernández ME, Peñarrubia MJ, de la Fuente L, et al. Experimental and clinical regenerative capability of human bone marrow cells after myocardial infarction. Circ Res. 2004;95:742-8.

13. Schachinger V, Assmus B, Britten MB, Honold J, Lehmann R, Teupe C, 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.

14. Janssens S, Dubois C, Bogaert J, Theunissen K, Deroose C, Desmet W, 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.

15. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. N Engl J Med. 2006;355:1199-209.

16. Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. N Engl J Med. 2006;355:1210-21.

17. Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, et al. Mobilized bone marrow cells repair the infarcted heart improving function and survival. Proc Natl Acad Sci USA. 2001;98:10344-9.

18. Kang HJ, Kim HS, Zhang SY, Park KW, Cho HJ, Koo BK, et al. Effects of intracoronary infusion of peripheral blood stem-cells mobilized with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomized clinical trial. Lancet. 2004;363:751-6.

19. Ince H, Petzsch M, Kleine HD, Eckard H, Rehders T, Burska D, et al. Prevention of left ventricular remodeling with G-CSF after acute myocardial infarction: Final one-year results of the FIRSTLINE-AMI trial (Front-integrated revascularization and stem cell liberation in evolving acute myocardial infarction by granulocyte colony-stimulating factor). Circulation. 2005;112:73-80.

20. Ince H, Petzsch M, Kleine HD, Schmidt H, Rehders T, Körber T, et al. Preservation from left ventricular remodeling by front-integrated revascularization and stem cell liberation in evolving acute myocardial infarction using granulocyte-colony stimulating factor (FIRSTLINE-AMI). Circulation. 2005;112:3097-106.

21 Valgimigli M, Rigolin GM, Cittanti C, Malagutti P, Curello S, Percoco G, et al. Use of granulocyte-colony stimulating factor during acute myocardial infarction to enhance bone marrow stem cell mobilization in humans: clinical and angiographic safety profile. Eur Heart J. 2005;26:1838-45.

22. Suárez de Lezo J, Torres A, Herrera I, Pan M, Romero M, Pavlovic DJ, et al. Effects of stem-cell mobilization with recombinant human granulocyte colony stimulating factor in patients with percutaneously revascularized acute anterior myocardial infarction. Rev Esp Cardiol. 2005;58:253-61.

23. Kuethe F, Figulla HR, Herzau M, Voth M, Fritzenwanger M, Opfermann T, et al. Treatment with granulocyte colony-stimulating factor for mobilization of bone marrow cells in patients with acute myocardial infarction. Am Heart J. 2005;150:115.e1-7.

24. Zohlnhofer D, Ott I, Mehilli J, Schomig K, Michalk F, Ibrahim T, et al. Stem cell mobilization by granulocyte colony-stimulating factor in patients with acute myocardial infarction: a randomized controlled trial. JAMA. 2006;295:1003-10.

25. Keeney M, Chin-Yee I, Weir K, Popma J, Nayar R, Sutherland DR. Single platform flow cytometry absolute CD34+ cell counts based in the ISHAGE guidelines International Society of Hematotherapy and Graft Ingineering. Cytometry. 1998;34:61-70.

26. Herrera C, Sánchez J, Torres A, Pascual A, Rueda A, Alvarez MA. Pattern of expression of CXCR4 and adhesion molecules by human CD34+ cells from different sources: role in homing efficiency in NOD/SCID mice. Haematologica. 2004;89:1037-45.

27. Dyke SH, Cohn B, Gorlin R, Sonnemblick EH. Detection of residual myocardial function in coronary artery disease using post-extrasistolyc potentiation. Circulation. 1974;50:694-9.

28. Schuster MD, Kocher A, Seki T, Martens TP, Xiang G, Homma S, et al. Myocardial neovascularization by bone marrow angioblasts results in cardiomyocyte regeneration. Am J Physiol Heart Circ Physiol. 2004;287:525-32.

29. Hofmann M, Wollert KC, Meyer GP, Menke A, Arseniev L, Hertenstein B, et al. Monitoring of bone marrow cell homing into the infarcted human myocardium. Circulation. 2005;111: 2198-202.