Direct intramyocardial percutaneous delivery of autologous bone marrow in patients with refractory myocardial angina

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Background Intramyocardial injection of autologous bone marrow (ABM) may induce angiogenesis. We tested the safety and feasibility of catheter-based direct percutaneous intramyocardial delivery of ABM in patients with refractory angina pectoris.

Methods Ten patients (9 men, 67 ± 8 years) with refractory angina (Canadian Cardiovascular Society class III-IV) and documented myocardial ischemia were enrolled. After left ventricular electromechanical mapping, freshly aspirated and filtered ABM was percutaneously injected into target myocardial ischemic areas. Clinical symptoms (as assessed according to the Canadian Cardiovascular Society class), quality of life, and myocardial perfusion were evaluated before the procedure and through the follow-up.

Results In all patients, ABM was successfully injected into the target regions. No periprocedural complications occurred. At 12 months, no major cardiac events (death, acute myocardial infarction, stroke, and malignant ventricular arrhythmias) occurred. Severity of angina improved of ≥2 classes in 3 patients. Quality of life showed a significant improvement in all patients. Myocardial perfusion in the target regions improved in 4 of 8 patients.

Conclusions Direct percutaneous intramyocardial delivery of ABM appears feasible and safe. Further evaluation is warranted to test its clinical efficacy. (Am Heart J 2006;151:674-80.)

Therapeutic options in patients with refractory angina pectoris are limited.[1,2] Therapeutic angiogenesis is a new experimental strategy for inducing neovascularization by use of factors that stimulate blood vessel formation.[3,4] Direct intramyocardial injection of pluripotent endothelial cell precursors may represent the ideal method to activate arteriogenesis in ischemic regions. Bone marrow contains many kinds of immature cells, which could differentiate into hematopoietic cells, and endothelial progenitor cells.[5] A subset of $CD34^+$ cells derived from bone marrow has the capacity to differentiate into endothelial cells in vitro and to be introduced into the inner surface of small vessels in vivo.[5,6] Recent experimental studies demonstrated that autologous bone marrow (ABM) cell implantation enhances angiogenesis in ischemic heart or skeletal muscle models.[7-12]

The purpose of this study was to assess the safety and feasibility of direct intramyocardial injection of ABM in patients with refractory angina pectoris.

Methods

Study design

Patients enrolled in this registry fulfilled the following criteria: (1) severe angina (Canadian Cardiovascular Society [CCS] functional class III or IV) despite conventional maximal drug treatment, (2) noncandidate to the conventional revascularization strategies, and (3) clear evidence of reversible myocardial ischemia. All patients signed an informed consent form. The local ethics committee approved the study protocol and gave permission to treat a maximum of 10 patients in this pilot study to test the safety and feasibility of direct intramyocardial percutaneous ABM cell injection.

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Table I. Clinical characteristics of patients

Patient	Age (y)	Sex	CCS class	CASS	Diabetes mellitus	Pre-AMI	Pre-CABG	Pre-PCI	EF (%)
1	71	M	IV	3	No	Yes	Yes	Yes	47
2	71	M	IV	3	No	Yes	Yes	Yes	55
3	57	M	III	2	No	Yes	No	Yes	63
4	75	M	IV	3	Yes	Yes	Yes	Yes	62
5	60	M	III	3	No	Yes	No	Yes	60
6	53	M	III	3	No	Yes	Yes	Yes	48
7	75	M	III	3	Yes	No	Yes	Yes	50
8	66	M	IV	3	No	Yes	Yes	Yes	50
9	72	M	IV	3	No	Yes	Yes	Yes	33
10	69	F	IV	3	Yes	Yes	Yes	Yes	64

AMI, acute myocardial infarction; *CABG*, coronary artery bypass graft; *CASS*, extension of the coronary artery disease according to the Coronary Artery Surgery Study; *EF*, LV ejection fraction; *PCI*, percutaneous coronary intervention.

Angina and quality of life assessment

Severity of angina was defined according to the CCS functional classification.[13] A ≥2 classes' improvement in the CCS functional class from baseline through the follow-up was considered clinically relevant. Quality of life was assessed according to the Seattle Angina Questionnaire (SAQ).[14] A change of ≥10 points in SAQ scores from baseline through the follow-up was considered clinically relevant.[14]

Angiographic and echocardiographic studies

Coronary angiography was performed preprocedurally (within 7 days) and at 30 days and 6 months after the procedure. Collateral flow was defined according to the Rentrop score.[15] Angiograms were reviewed in random sequence by 2 blinded interventional cardiologists. Left ventricular (LV) ejection fraction was assessed by 2-dimensional echocardiography according to the area-length method. Echocardiography was performed preprocedurally (within 7 days) and at 30 days and 6 and 12 months after the procedure.

Myocardial perfusion study

Patients underwent myocardial perfusion imaging using single photon emission computed tomography (SPECT) or positron emission tomography (PET). Myocardial perfusion examinations were performed preprocedurally (within 7 days) and at 30 days and 6 and 12 months after the procedure. The SPECT was performed by using technetium-99m sestamibi. Standard exercise (Bruce protocol) or dipyridamole (0.56 mg/kg in 4 minutes) was used to induce ischemia. The PET studies were performed using an ECAT 931 PET scanner (Siemens/CTI, Knoxville, TN). Nitrogen-13 ammonia (dose 10 MBq/kg) was injected at rest and after infusion of dipyridamole (0.56 mg/kg in 4 minutes).[16] For qualitative interpretation, images were read by 2 experienced blinded observers.

Bone marrow aspiration and analysis

Bone marrow was aspirated from the iliac crest at approximately 1 hour before the procedure using preservative-free heparinized glass syringes (20 U heparin/1 mL fresh ABM). The aspirated bone marrow was immediately macrofiltered using 300-μm and 200-μm stainless steel filters sequentially. An experienced hematologist performed the procedure under sterile conditions. A bone marrow smear was immediately evaluated to confirm a normal morphology of the bone marrow that would be used for intramyocardial injection. The laboratory analysis of ABM included colony-forming unit (CFU) assay and flow cytometry immunophenotyping. The CFU assays were performed as follows: cells were tested either for CFU granulocyte-macrophage in complete methylcellulose culture medium (Methocult, Stem Cell, Vancouver, Canada) or for CFU fibroblast after plating adherent cells on 100-mm Petri dishes and were then cultured for 14 days in Ham's F12 medium added with FCS 20% (Poyesis, Trieste, Italy). For flow cytometry immunophenotyping, the cells were labeled with the following monoclonal antibodies purchased from Becton Dickinson (Franklin Lakes, NJ) and used according to manufacturer's instructions: phycoerythrin-CD34 and phycoerythrin/cianine-5-CD45. For an isotype control, nonspecific mouse antibodies were used. A minimum of 100.000 cells/sample was analyzed with a 6-parameter dual-laser flow cytometer (FACScalibur, Becton Dickinson).

Left ventricular electromechanical mapping and percutaneous myocardial injection

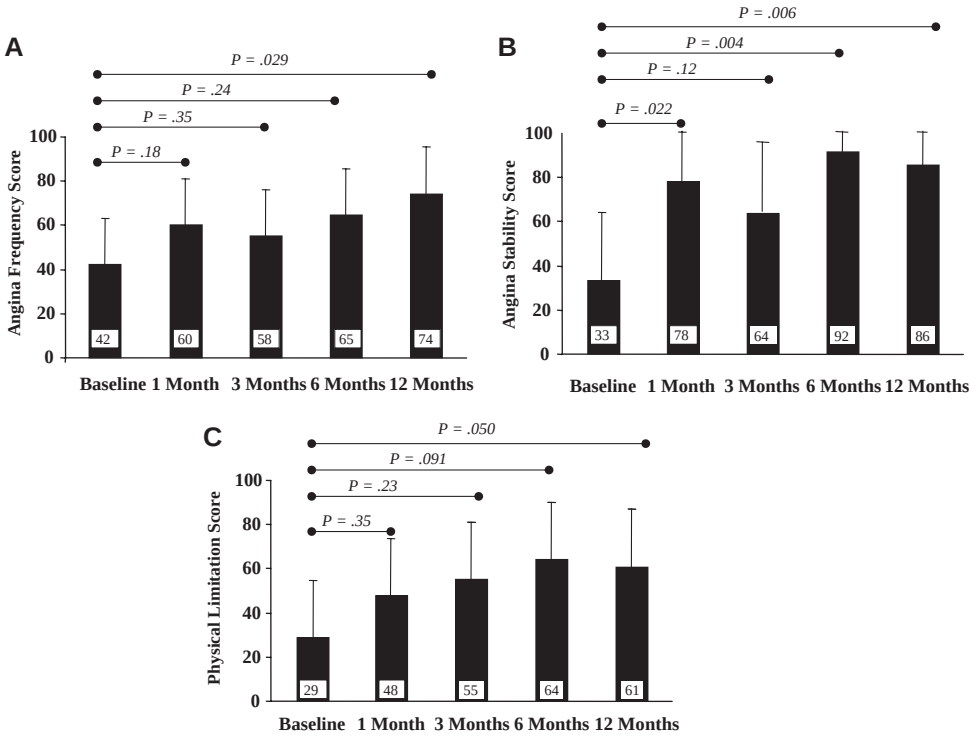
Left ventricular electromechanical mapping was performed with the NOGA system (Biosense Webster, Johnson & Johnson Corp, Diamond Bar, CA) according to a described method.[17,18] Briefly, the mapping catheter (Biosense Webster), a 7-F fused-tip catheter with a miniature passive magnetic filed sensor embedded within its distal tip that determines the position and rotation of the distal catheter segment, was introduced via a femoral arterial puncture and advanced to the left ventricle. Local functional analysis (wall motion) was based on linear local shortening (LLS). Unipolar and bipolar endocardial potentials were recorded from the tip electrode, and measurements based on these local intracardiac signal amplitudes formulated a guide to myocardial viability. The combination of these 2 data sets permitted assessment of electromechanical function that identifies foci of myocardial ischemia. According to the endocardial potential (normal >11 mV, low 5-11 mV) and the LLS during a cardiac cycle, 3 combinations may occur: (1) normal kinesis and voltage (normal myocardium), (2) hypokinesis and normal voltage (ischemic or stunned or hibernated myocardium), and (3) ipokinesis and low voltage (necrotic or scarred

Table II. Bone marrow aspiration and characteristics

Patient	Aspirated ABM (mL)	TNCs ($\times 10^6$)	CFU-GM ($\times 10^4$)	CD34+/CD45+ (% of total cells)	CD34+/CD45– (% of total cells)
1	25	1250	0.37	2.27	0.02
2	20	390	0.25	1.95	0.02
3	18	311.5	1.70	2.21	0.02
4	30	535.8	0.40	4.47	0.06
5	30	815	1.01	0.40	1.2
6	30	1047	1.42	2.00	1
7	60	832	1.00	3.90	0
8	60	897	0.95	4.10	0
9	30	454	0.52	1.48	0.05
10	30	382.5	1.18	1.50	0.02

CFU-GM, colony-forming unit granulocyte-macrophage; *TNC*, total nucleated cells.

Figure 1

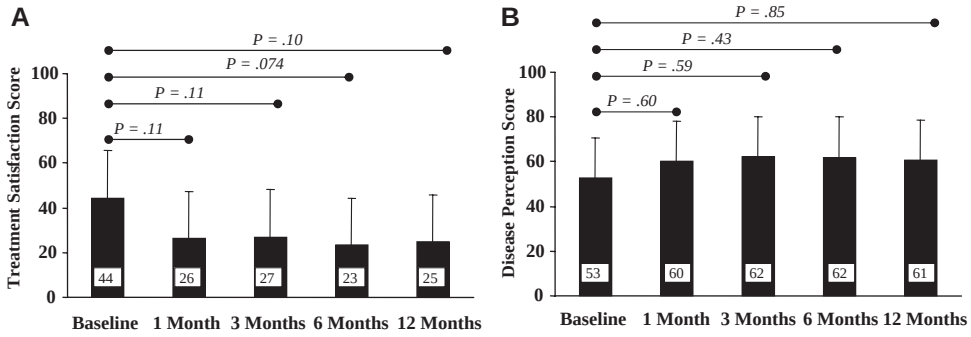


Angina frequency (**A**), angina stability (**B**), and physical limitation (**C**) scores according to the SAQ at baseline and at 12 months.

myocardium).[17,18] To quantify the degree of myocardial perfusion demonstrated visually by the mapping images, the long axis of the heart was divided into 4 regions: anterior, inferoposterior, septal, and lateral. Mean values for LLS and unipolar and bipolar endocardial potentials were calculated for ischemic myocardium (area of electromechanical uncoupling on NOGA mapping). In addition, for comparative analysis with nuclear imaging, LLS and voltage values from ischemic regions were compared with the corresponding perfusion values on the resting SPECT and PET studies.

Percutaneous injection was performed using a MYOSTAR injection catheter (Biosense Webster).[18] This catheter is a modified 7-F mapping catheter. The distal tip of the catheter incorporates a 27-G needle that can be protruded 3 to 5 mm. The catheter was placed in a retrograde fashion via the femoral sheath to the aortic valve. The injection catheter (incorporating an electromechanical mapping tip sensor) was manipulated to acquire stable points (local shortening value <3 mm) within the target region–based ventricular contractions as evidence of needle penetration into the myocardium. The location and the

Figure 2



Treatment satisfaction (**A**) and disease perception (**B**) scores according to the SAQ at baseline and at 12 months.

Table III. Bone marrow injection characteristics and clinical outcome

Patient	Injected ABM (mL)	No. of injections and injection sites	TNCs ($\times 10^6$)	Injected CD34+/CD45+ ($\times 10^6$)	Target LV region	CCS class	Quality of life (SAQ)				
							Angina stability	Angina frequency	Physical limitation	Satisfaction	Disease perception
1	7	14	350	7.95	Anterolateral	U	U	U	U	U	U
2*	10	10	195	3.80	Anteroseptal	U	U	U	U	U	U
3	10	10	173	3.82	Lateral	I	I	I	I	U	I
4	9	9	160.7	7.18	Lateral	U	I	U	I	U	U
5	5	10	173.5	4.50	Anterolateral	U	I	I	I	U	U
6	10	10	217.2	4.34	Inferolateral	U	I	I	I	U	I
7	7	14	322.6	6.29	Inferior	I	I	I	I	U	I
8	7	10	156	4.50	Inferolateral	I	I	I	I	U	I
9	10	10	151	2.23	Anteroseptal	U	U	U	U	U	U
10	10	10	127	1.90	Anterolateral	U	U	U	U	U	U

I, improved through the follow-up (ie, ≥2 classes for CCS and ≥10 points for SAQ scores); *U*, unchanged through the follow-up.
*This patient experienced acute heart failure 7 days after the procedure because of acute atrial fibrillation.

number of injections were selected according to the site and extension of the ischemic area as evaluated by noninvasive studies (scintigraphy perfusion imaging) as well as by the NOGA mapping performed before the injection procedure. The number of injections and the final number of cells injected were not prespecified but left at the operator's discretion according to the extension of the target area. Each injection of at least 10^7 nucleated cells was delivered in a volume of 0.5 to 1 mL, depending on cell concentration: when the solution was highly concentrated (ie, $\geq 40 \times 10^6$ cells), each injection was of 0.5 mL. The lumen was filled with 0.2 mL of sterile saline before entry into the circulation and was again flushed with 0.2 mL of sterile saline after each injection. After completion of the injection, the needle was retracted and the catheter was removed to another endocardial site. The density of injection sites depended on each subject's LV endomyocardial anatomy and the ability to achieve a stable position on the contractions. Care was taken to avoid the mitral valve apparatus, the LV apex, and regions of myocardium with known previous infarction and thinning (determined by the preprocedure echocardiogram or unipolar voltage potentials <7.4 mV). Care was also taken to avoid significant (>1 mV) ST elevation shown on the intracardiac electrogram during catheter positioning before intramyocardial injection.

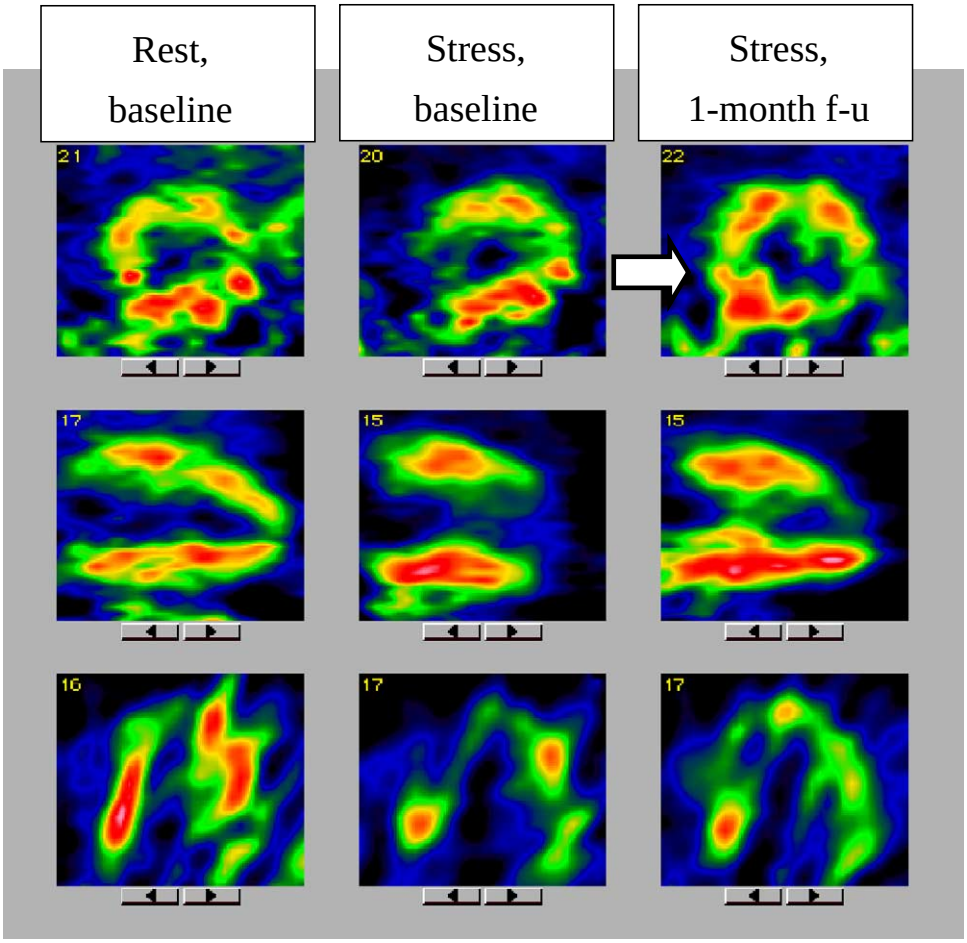
Study end point

The end point of this pilot study was to establish the safety and feasibility of Biosense-guided ABM transplantations, evaluated as incidence of periprocedural and postprocedural major adverse cardiac events. A major adverse cardiac event was defined as a combined end point of death, acute myocardial infarction, revascularization procedures (percutaneous or surgical) for procedure-related complications, LV perforation, sustained ventricular arrhythmia, and stroke.

Statistical analysis

Continuous variables are given as mean ± 1 SD. The 5 items of the SAQ at baseline and through the follow-up (1, 3, 6, and 12 months) were analyzed with repeated-measures analysis of variance with time as the fixed factor and each patient as the random factor. Comparison versus baseline values was performed using the Dunnett *t* test. Probability values <.05 were considered significant. Data were analyzed with SPSS (SPSS, Chicago, IL) for Windows 10.0.

Figure 3



Nitrogen-13 ammonia PET at rest, during stress at baseline, and at 1-month follow-up after transmyocardial delivery of ABM. Note the increased myocardial perfusion at the anterior septum wall during stress at the 1-month evaluation (arrow).

Results

Clinical characteristics of the patients

Ten patients were treated from October 2001 to March 2002 (Table I). All patients had refractory severe angina caused by a severe coronary artery disease nonsuitable to conventional treatment. Two patients had spinal cord stimulators. All patients were taking nitrate and antiplatelet agents; all but one patient were also taking β-blockers and calcium antagonists.

Autologous bone marrow aspiration

Autologous bone marrow (36 ± 16 mL) was aspirated from the iliac crest 1 hour before the procedure. The principal characteristics of the ABM (with normal morphology in all instances) are summarized in Table II.

Intramyocardial ABM injection

Direct percutaneous intramyocardial delivery of ABM was successful in all cases. The mean amount of ABM injected was 8.5 ± 1.8 mL (range 5-10 mL) (Table II). A total of 113 (mean 11 ± 2, median 10) percutaneous catheter-based myocardial injections was performed. The cell population consisted a mean of 2.38% ± 1.25% CD34+/CD45+ cells. We injected a mean of 4.6 ± 1.5 × 10^6 CD34+/CD45+ cells per patient. In all but one case (patient 10), ≥2 × 10^6 CD34+/CD45+ cells were injected in the target area. No intraprocedural complications (eg, ventricular tachycardia or fibrillation and cardiac perforation) occurred.

Inhospital outcome

The inhospital clinical course was uneventful in all cases. Patients were discharged from the hospital 48

hours after the procedure. No case of pericardial effusion was detected by echocardiography performed soon after the procedure and before discharge. Continuous electrocardiographic monitoring was maintained for 24 hours after injection and disclosed no sustained arrhythmias. No evidence of postprocedure acute myocardial infarction or recurrent ischemia in any patient was found. The creatine kinase-MB was not elevated above normal limits in any patient after injection.

Clinical outcome

One patient experienced acute heart failure 7 days after the procedure because of acute atrial fibrillation. No cases of death, acute myocardial infarction, and malignant ventricular arrhythmias occurred. Severity of angina, as assessed according to the CCS classification, remarkably (≥2 classes) improved in 3 patients (30%). Changes in quality of life (as assessed by the SAQ) are summarized in Figures 1 and 2 and in Table III. We found a significant improvement in the physical limitation, angina frequency, and angina stability scores, whereas the disease perception and the treatment satisfactory scores were quite unchanged. Left ventricular ejection fraction was 53% ± 10% at baseline and 57% ± 16% 12 months after the procedure ($P = .44$). Complete myocardial perfusion imaging data at baseline and follow-up were available in 8 patients. The severity and extension of the ischemic target area decreased in 4 of the 8 patients (Figure 3). Of these 4 patients, 3 had significant improvements in CCS class.

Discussion

This pilot study demonstrated in a preliminary format that direct percutaneous myocardial injection of ABM in patients with refractory angina pectoris is feasible and safe. The absence of any acute adverse event with this catheter system confirms previous observations[5,19-21] and provides encouraging data supporting that direct intramyocardial percutaneous delivery appears to be a safe and feasible vehicle for myocardial angiogenesis. Tse et al[19] treated 8 patients with stable refractory angina with direct percutaneous intramyocardial delivery of ABM cells. No major intraprocedural complications occurred. After 3 months, the authors observed a significant reduction of anginal episodes, improvement in target wall motion, and a reduction in the percentage mass of hypoperfused myocardium. Perin et al[20] reported a prospective, nonrandomized, open-label study where 14 patients underwent transendocardial percutaneous ABM injection (15 injections of 0.2 mL). No major periprocedural complications occurred. No sustained arrhythmias were associated with the injection procedures, nor did any significant arrhythmia occur while the patients were hospitalized. There were no sustained ventricular arrhythmias found on 24-hour Holter monitoring at baseline or when repeated after the injection procedure and no significant differences in the number of percentage of premature ventricular contractions. No postprocedural pericardial effusions were seen on 2-dimensional Doppler echocardiograms. At 4 months, there was a significant reduction in total reversible defect and improvement in global LV function within the treatment group and between the treatment and control groups. No complications were reported. The same results have been reported by Fuchs et al,[21] who performed catheter-based ABM injection in 10 patients with refractory angina. No serious adverse effects were observed; in particular, there were no arrhythmias, evidence of infection, myocardial inflammation, or increased scar formation.

As in these previous studies, we found a somewhat clinical benefit after ABM cell injection. Severity of angina remarkably (≥2 classes) improved in 3 of 10 patients. At 12 months, we found a significant improvement in the physical limitation, angina frequency, and angina stability scores. The fact that the treatment satisfaction score did not improve and paradoxically deteriorated with time may signify a disproportion between the expectations that a patient had and the actual benefit subjectively perceived. Furthermore, as in other studies involving percutaneous intramyocardial procedures, we cannot dismiss the strong placebo effect associated with this procedure.

ABM and myocardial angiogenesis

Bone marrow is a natural source of multiple factors involved in angiogenesis.[5,7,8,11,12,22] During tissue ischemia, endothelial progenitor cells mobilize from bone marrow and home where neovascularization is needed, differentiating into mature endothelial cells.[5] Endothelial progenitor cells have also been shown to differentiate into cardiomyocytes.[23,24] Autologous bone marrow cell transplantation was shown to enhance angiogenesis and improve cardiac function in experimental heart models.[5,12,25-27] Preliminary experience in various clinical settings seem to support this beneficial effect.[27-30] Selective intracoronary transplantation of ABM in patients during immediate postinfarction seems to positively affect remodeling processes.[27-29]

We injected a mean of $4.6 \pm 1.5 \times 10^6$ CD34+/CD45+ cells per patient: in all but one case, $\geq 2 \times 10^6$ CD34+/CD45+ cells were injected in the target area. It is not clear what the amount of cells to be injected in the target area is to potentiate angiogenesis. Tse et al[19] did not clearly report these data in their study. Fuchs et al[12] performed 12 injections of 0.2 mL each (total 2.4 mL). A total of $32.6 \pm 27.5 \times 10^6$ mL nucleated cells was injected. The CD34+/CD45+ fraction was 2.6% ± 1.6%, of which 47.9% ± 15.1% coexpressed CD45. From these data, we can extrapolate that approximately 4.0×10^6 CD34+/CD45+ cells were injected in each patient. Perin et al[20]

performed 15 injections of 0.2 mL each (total 3.0 mL). In each patient was injected $25.5 \pm 6.3 \times 10^6$ nucleated cells and approximately 6.0×10^6 CD34+/CD45+ cells.

Study limitations

The number of patients is only sufficient to derive preliminary data about the safety and feasibility of ABM direct intramyocardial percutaneous injection in patients with refractory angina. No information is available about cell survival and differentiation along the cardiac myocyte or endothelial lineage after intramyocardial needle injection. The clinical improvements and the improvements in target area perfusion may suggest the need for a large double-blind randomized study. Large studies will need to take into consideration possible risks of tumorigenicity caused by homing of new cells at sites of active angiogenesis.

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References

1. Kim MC, Kini A, Sharma SK. Refractory angina pectoris. J Am Coll Cardiol 2002;39:923-34.
2. Mannheimer C, Camici P, Chester MR, et al. The problem of chronic refractory angina. Report from ESC Joint Study Group on the Treatment of Refractory Angina. Eur Heart J 2002;23:355-70.
3. Simons M, Bonow RO, Chronos NA, et al. Clinical trials in coronary angiogenesis: issues, problems, consensus. An expert panel summary. Circulation 2000;102:e73-e86.
4. Carmeliet P. Mechanism of angiogenesis and angiogenesis. Nat Med 2000;6:389-95.
5. Asahara T, Masuda H, Takahashi T, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 1999;85:221-8.
6. Kawamoto A, Gwon HC, Iwaguro H, et al. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. Circulation 2001;103:634-7.
7. Kobayashi T, Hamano K, Li TS, et al. Enhancement of angiogenesis by the implantation of self bone marrow cells in an ischemic heart model. J Surg Res 2000;89:189-95.
8. Tomita S, Li RK, Weilsel RD, et al. Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. J Thorac Cardiovasc Surg 2002;123:1132-40.
9. Kocher A, Schuster MD, Szabolcs MJ, et al. Neoangiogenesis of ischemic myocardium by human adult bone marrow–derived angioplasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med 2001;7:430-6.
10. Kamihata H, Matsubara H, Nishiue T, et al. Improvement of collateral perfusion and regional function by implantation of peripheral blood mononuclear cells into ischemic hibernating myocardium. Arterioscler Thromb Vasc Biol 2002;22:1804-10.
11. Shintani S, Murohara T, Ikeda H, et al. Augmentation of postnatal neovascularization with autologous bone marrow transplantation. Circulation 2001;103:897-903.
12. Fuchs S, Baffour R, Zhou YF, et al. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. J Am Coll Cardiol 2001;37:1726-32.
13. Campeau L. Grading of angina pectoris. Circulation 1976;54:522-3.
14. Spertus JA, Winder JA, Dewhurst TA, et al. Development and evaluation of the Seattle Angina Questionnaire: a new functional status measure for coronary artery disease. J Am Coll Cardiol 1995;25:333-41.
15. Rentrop KP, Thornton JC, Feit F, et al. Determinants and protective potential of coronary arterial collaterals as assessed by an angioplasty model. Am J Cardiol 1988;61:677-84.
16. Choi Y, Huang SC, Hawkins RA, et al. A simplified method for quantification of myocardial blood flow using nitrogen-13-ammonia and dynamic PET. J Nucl Med 1993;34:488-97.
17. Gepstein L, Hayam G, Ben-Haim SA. A novel method for non-fluoroscopic catheter based electroanatomical mapping of the heart: in vitro and in vivo accuracy results. Circulation 1997;95:1611-22.
18. Vale PR, Losordo DW, Tkebuchava T, et al. Catheter-based myocardial gene transfer utilizing nonfluoroscopic electromechanical left ventricular mapping. J Am Coll Cardiol 1999;34:246-54.
19. Tse HF, Kwong YL, Chan JKF, et al. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. Lancet 2003;361:47-9.
20. Perin EC, Dohmann HFR, Borojevic R, et al. Transendocardial, autologous bone marrow cells transplantation for severe, chronic ischemic heart failure. Circulation 2003;107:2294-302.
21. Fuchs S, Satler LF, Kornowski R, et al. Catheter-based autologous bone marrow myocardial injection in no-option patients with advanced coronary artery disease. J Am Coll Cardiol 2003;41:1721-4.
22. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 1997;276:71-4.
23. Condorelli G, Borello U, de Angelis L, et al. Cardiomyocytes induce endothelial cells to trans-differentiate into cardiac muscle: implication for myocardial regeneration. Proc Natl Acad Sci U S A 2001;98:10733-8.
24. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001;410:701-5.
25. Kamihata H, Matsubara H, Nishiue T, et al. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. Circulation 2001;104:1046-52.
26. Kawamoto A, Tkebuchava T, Yamaguchi JJ, et al. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. Circulation 2003;107:461-8.
27. Tateishi-Yuyama E, Matsubara H, Murohara T, et al. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomized controlled trial. Lancet 2002;360:427-35.
28. Assmus B, Schachinger V, Teupe C, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). Circulation 2002;106:3009-17.
29. Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 2002;106:1913-8.
30. Stamm C, Westphal B, Kleine HD, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet 2003;361:45-6.