Scintigraphic Assessment of the Effects of Bone Marrow–Derived Mononuclear Cell Transplantation Combined with Off-Pump Coronary Artery Bypass Surgery in Patients with Ischemic Heart Disease

Hiroyuki Yaoita, MD[1]; Shinya Takase, MD[2]; Yukio Maruyama, MD[1]; Yoshiyuki Sato, MD[2]; Hirono Satokawa, MD[2]; Nobuo Hoshi, MD[3]; Nobutaka Ono, MD[4]; Tsuguo Igari, MD[5]; Hitoshi Ohto, MD[5]; and Hitoshi Yokoyama, MD[2]

[1]First Department of Internal Medicine, Fukushima Medical University, Fukushima, Japan; [2]Department of Cardiovascular Surgery, Fukushima Medical University, Fukushima, Japan; [3]Second Department of Pathology, Fukushima Medical University, Fukushima, Japan; [4]Shirakawa Kousei General Hospital, Shirakawa, Japan; and [5]Division of Blood Transfusion and Transplantation Immunology, Fukushima Medical University, Fukushima, Japan

Myocardial SPECT may be useful for assessment of the therapeutic effects and the mechanisms of cardiac regeneration medicine. We aimed to assess first the feasibility and the short-term safety of autologous bone marrow–derived mononuclear cell transplantation (BMCT) into the ischemic myocardium in patients who undergo off-pump coronary artery bypass surgery (OPCAB). In addition, we aimed to assess our hypothesis that the BMCT may help ameliorate myocardial perfusion in patients with ischemic heart disease (IHD) using myocardial perfusion scintigraphy. **Methods:** We performed BMCT in 10 patients with IHD during OPCAB. Cells for BMCT were collected by intraoperative bone marrow aspiration or by preoperative cellular apheresis after pretreatment with granulocyte colony-stimulating factor. After OPCAB was performed in all graftable ischemic areas, a total of $3.4 \pm 1.2 \times 10^9$ mononuclear cells, including $5.2 \pm 1.6 \times 10^6$ CD34-positive (CD34+) cells, were injected into ungraftable ischemic myocardial areas. Dipyridamole-stress and resting ^{99m}Tc myocardial SPECT was performed before and 1 mo after the procedures. **Results:** BMCT was performed safely in all patients. Compared with before treatment, myocardial ^{99m}Tc tracer uptake on the dipyridamole-stress image increased similarly in BMCT- and OPCAB-treated areas, whereas tracer accumulation at rest did not change in all myocardial areas. The improvement of myocardial perfusion was not correlated with the total number of mononuclear cells transplanted. However, it was positively correlated with the number of transplanted CD34+ cells: ^{99m}Tc tracer uptake after/before BMCT (ratio) = $1.091 \times (\text{CD34+ cell number } [\times 10^6])^{0.074}$ ($r^2 = 0.48$, $P < 0.05$), although new development of coronary vessels was not documented cineangiographically. Myocardial histopathology in 2 of 3 autopsy cases revealed coronary angiogenesis in the areas corresponding to the sites of BMCT. **Conclusion:** The present study demonstrates the feasibility of BMCT combined with OPCAB. This therapy improves myocardial perfusion possibly via CD34-related development of coronary microvessels.

Key Words: regeneration; SPECT; coronary artery bypass surgery; bone marrow

J Nucl Med 2005; 46:1610–1617

Regeneration of myocardial components by bone marrow–derived cells has been suggested to have a therapeutic potential in ischemic heart disease (IHD) (*1–10*). Among several attempts to deliver bone marrow–derived stem cells or progenitor cells to myocardium, bone marrow–derived mononuclear cell transplantation (BMCT) into ischemic myocardium has been suggested to be effective for patients who undergo coronary artery bypass grafting (CABG) (*3,9*). In these pioneer works, BMCT was combined with on-pump CABG. On the other hand, reports on BMCT combined with off-pump CABG (OPCAB) are rare (*8*), and the patient number enrolled is small. OPCAB has several advantages for patients who plan to receive CABG (more stable hemodynamics during CABG and fewer complications and better prognosis than on-pump CABG). Therefore, the feasibility of BMCT combined with OPCAB remains to be determined.

Although myocardial perfusion or function improves after BMCT in patients with IHD (*2,3,9*), it remains to be determined what kinds of cells participate in the therapeutic effects of BMCT. The usefulness of CD34 positivity—one of the hematopoietic stem cell markers—as a tool for human stem cell therapy, including their dose–response relation, remains to be determined. In addition, there is little his-

Received Apr. 27, 2005; revision accepted Jun. 27, 2005.
For correspondence or reprints contact: Hitoshi Yokoyama, MD, Department of Cardiovascular Surgery, Fukushima Medical University, Hikarigaoka 1, Fukushima, 960-1295 Japan.
E-mail: hitoshiy@fmu.ac.jp

topathologic information on humans with respect to the mechanisms of the therapeutic effects of BMCT.

In the present study, we hypothesized that BMCT into ungraftable ischemic myocardial area may be feasible in patients with IHD who undergo OPCAB and may improve myocardial perfusion at least partially via a CD34 positivity-dependent mechanism. To assess this hypothesis, we performed autologous BMCT in combination with OPCAB in patients from whom consent was obtained. Myocardial perfusion and viability were assessed by dipyridamole-stress and resting ^{99m}Tc myocardial perfusion SPECT. Our results may contribute to development of BMCT in IHD by confirming its feasibility and providing information on at least some of the mechanisms involved.

MATERIALS AND METHODS

Patients

Ten patients were included in this study, which was performed between April 2002 and March 2005. The indications for autologous myocardial BMCT were the presence of myocardial ischemia that is not suitable for percutaneous coronary intervention or CABG. Accordingly, the candidates for this study had both graftable and ungraftable ischemic myocardial areas. The exclusion criteria for myocardial BMCT were the history or presence of malignant neoplasms, active diabetic retinopathy, age >80 y or <10 y, active inflammatory disease, or myocardial infarction within 3 mo after its onset. The diagnosis of IHD and the indications for OPCAB were determined by ^{99m}Tc myocardial perfusion SPECT and coronary cineangiography. The follow-up cineangiogram was obtained 4 wk after surgery. The patient characteristics are summarized in Table 1. The endpoint of the present study is mortality by any cause.

The investigation conforms with the principles outlined in the Declaration of Helsinki (*11*) as well as Title 45, U.S. Code of Federal Regulations, part 46, Protection of Human Subjects (revised November 13, 2001; effective December 13, 2001), and with the Guideline for Human Study by the Ethics Committee of Fukushima Medical University. Approval was given by the Ethics Committee. The consent form stated that the patient who needs surgical treatment of IHD may choose either treatment—that is, OPCAB or OPCAB combined with BMCT. All patients chose OPCAB combined with BMCT, and written consent was obtained from all patients before the study.

Bone Marrow Cell Collection

The patients were divided into 2 groups by random selection without alternate assignment, and patients were then informed as to which group they belonged. In one group (patients 2–4, 8, and 10), after induction of general anesthesia for OPCAB, mononuclear bone marrow cells were obtained by aspiration from the iliac bone marrow using a syringe. In the other group (patients 1, 5–7, and 9), to achieve peripheral white blood cell counts of 25,000–30,000 by mobilization from bone marrow, granulocyte colony-stimulating factor (G-CSF; 3–5 μg/kg/d) was administered subcutaneously 3 d before OPCAB, and circulating mononuclear cells were collected by apheresis 1 d before the OPCAB. We randomly used either of these 2 techniques in patients for correcting bone marrow–derived cells because it remained to be determined which

TABLE 1
Characteristics of Patients

Patient no.	Age (y)	Sex	No. of diseased coronary vessels	Site of CABG	Site of BMCT	Method for BMCT	Follow-up period (mo)
1	69	M	2	LAD #8 (OMI)	LCX #12 (AP)	Apheresis	32 (death)
2	55	F	2	HL of LCX (AP)	RCA #4PD and #4AV (AP)	Aspiration	21 (death)
3	61	M	3	RCA #4PD (OMI + AP) LAD #8 (OMI + AP)	LCX #14 (AP)	Aspiration	27 (death)
4	66	M	3	LAD #8 (AP) LCX #12 (AP)	LAD #9 (AP) RCA #4AV (OMI + AP)	Aspiration	19
5	69	M	3	LAD #8 (AP) LCX #13 (AP)	LCX #14 (AP) RCA #4PD (OMI + AP)	Apheresis	18
6	41	M	3	LAD #8 (OMI + AP) RCA #4PD (OMI + AP) LCX #14 (AP)	RCA #4AV (OMI + AP) LCX #13 (AP)	Apheresis	18
7	12	M	1	LAD #8 (OMI + AP)	LAD #9 (OMI + AP)	Apheresis	17
8	65	M	2	LAD #8 (OMI + AP) LAD #9 (OMI + AP) LCX #12 (AP)	LAD #10 (OMI + AP) LCX #15 (OMI + AP)	Aspiration	16
9	64	M	3 (2 + left main trunk lesion)	LAD #7 (AP) LCX #14 (AP)	LCX #12 (AP)	Apheresis	5 (death)
10	66	M	3	LAD #8 (AP) LAD #9 (AP), LCX #12 (AP)	RCA #4AV (OMI + AP)	Aspiration	6

LAD = left anterior descending artery; OMI = old myocardial infarction; LCX = left circumflex artery; AP = angina pectoris; HL = high lateral branch; RCA = right coronary artery; PD = posterior descending coronary artery of RCA; AV = atrioventricular nodal artery of RCA.
Number (#) indicates coronary arterial site of lesion by American Heart Association classification.

method is more efficacious for correcting CD-positive (CD34+) cells. The bone marrow cells in phosphate-buffered saline were centrifuged at 3,000*g*, and the pellet—excluding the buffy coat—was collected, cells were counted and sorted, and their CD34 positivity was assessed by flow cytometry. The mononuclear bone marrow–derived cells, an average of $3.4 \pm 1.2 \times 10^9$ (including $5.2 \pm 1.6 \times 10^6$ CD34+ cells), were suspended in 5 mL phosphate-buffered saline just before BMCT.

OPCAB and BMCT

The OPCAB was performed in the ischemic areas that had graftable coronary vessels using arterial grafts. After that, bone marrow–derived cells were injected into the ungraftable ischemic myocardial areas in which the middle and endocardial layers were chosen using the stabilizing plate for the beating heart (0.2 mL each injection/1-cm^2 left ventricular [LV] surface, usually 25 injections for a patient, resulting in the encompassed LV surface areas of about 25 cm^2). There was no obvious leak from the site of the cell injection.

Myocardial Scintigraphy

Just before and 4 wk after OPCAB and BMCT, dipyridamole-stress and resting myocardial perfusion imaging combined with quantitative electrocardiographically (ECG) gated SPECT (QGS) at rest was performed in all patients. Forty-five minutes after intravenous injection of ^{99m}Tc-tetrofosmin (300 MBq at stress or 900 MBq at rest), cardiac imaging was started using a low-energy, high-resolution collimator connected online to a GCA 9300 (Toshiba). The polar maps of stress and resting perfusion and the data for LV function were obtained by QGS analysis. The third myocardial perfusion SPECT was performed on patients 1, 2, and 4–7, 5–6 mo after OPCAB and BMCT (data not shown). Because the color image by our scinticamera can detect a round defect of 1 and 2 cm in diameter with a depth of 1 cm (0.79 and 3.14 cm^3 in volume, respectively) in the phantom study (data not shown), we assume that the areas of BMCT sites (about 25 cm^3 as a whole) exceeded the image resolution limitation of our machine. Moreover, any changes in LV ejection fraction (LVEF) could be detected.

Regional myocardial ^{99m}Tc uptake was measured by setting the region of interest manually, rather than automatic computerized planimetry, on these images best fit to the anatomy of the patient's coronary arteries, which was obtained by coronary cineangiography. We confirmed on the images that perfusion abnormalities, if present, appear at least on the 2 serial short-axial images. Thus, we used these 2 short-axial images best fit for targeting the risk area for measurement of myocardial tracer uptake in each patient. When the patient received OPCAB or BMCT in >2 areas each, their respective mean values were used as the uptake of the corresponding area.

We considered the sites of BMCT, OPCAB, and control (nonischemic) on the scintigraphic images by considering the cineangiographic findings and the sites of old myocardial infarction that 9 of 10 patients had. In 7 patients (patients 1–4, 7, 9, and 10 in Table 1), the sites of OPCAB and BMCT were remote, and we could easily determine those sites by considering each patient's own coronary arterial anatomy assessed by the pre- and postoperative cineangiography. Among the 5 patients (patients 2, 4–6, and 8) who had 2 areas of BMCT according to the coronary arterial anatomy, one of the 2 BMCT areas in each of the 2 patients (segment 14 of the left circumflex artery of patient 5 and segment 4 of the right coronary artery of patient 6 by American Heart Association classification (*12*)) was excluded from the assessment of ^{99m}Tc uptake because those areas were close to the site of BMCT.

Safety Assessment

In 8 patients monitored over 12 mo, 24-h ambulatory ECG monitoring and whole-body CT scanning were performed 12 mo (all 8 patients) and 24 mo (1 patient) after OPCAB and BMCT. The Lown classification was adopted for assessment of arrhythmias.

The whole-body enhanced CT scan, including 3-dimensional reconstruction, was performed before, at 4 wk (both in all patients), and at 12 mo (7 patients) and 24 mo (1 patient) after OPCAB and BMCT to assess the presence of inflammation and neoplasm.

Retinal examinations by ophthalmologists were repeated within 6 mo after OPCAB and BMCT where possible.

We screened for unfavorable events associated with OPCAB and BMCT—that is, new onset of myocardial infarction, prolonged requirement of inotropic agents (>72 h) or of respiratory assistance (>48 h) after surgery, occurrence of atrial or ventricular tachyarrythmias, cardiac tamponade, and stroke.

Histopathology at Autopsy

In patients 2, 3, and 9, who died 5–27 mo after surgery, autopsies were performed 3 h after death. Paraffin-embedded, 5-μm-thick sections were fixed with 4% paraformaldehyde and staining with hematoxylin–eosin, Azan, elastica Masson, and immunostaining for CD31 and CD34 (*13*) were performed.

The myocardial microvessel density was measured in myocardial sections stained with CD34 using the point-counting method of Weibel (*14*) in 3 autopsied patients (patients 2, 3, and 9). The eyepiece for this method (Integrationsplatte I; Zeiss) was attached to the light microscope (BX51; Olympus). This eyepiece for morphometry has 25 grid markers (points) within the field. In those 3 patients, we measured myocardial CD34+ microvessel density (the number of myocardial CD34+ microvessels [<200 μm in diameter] per 100-point count area) in a high-power field (magnification ×200) at one myocardial site. We define the vessels <200 μm in diameter as microvessels in the present study (*15*). Within each myocardial area of control (nonischemic), BMCT, and OPCAB, this measurement of myocardial CD34+ microvessel density was performed in 7 random myocardial sites (a total of 63 measures in 3 patients). In addition, in 2 patients (patients 2 and 3), who had areas of old myocardial infarction, the measurements of the CD34+ microvessel density were also done in 7 random myocardial sites within the areas of old myocardial infarction (a total of 14 measures in 2 patients).

Statistical Analysis

The data are expressed as average ± SE. One-way ANOVA was performed for multiple comparisons followed by the Bonferroni post hoc test. $P < 0.05$ was considered significant.

RESULTS

Early Outcomes

OPCAB (2.0 ± 0.3 bypass grafts) and BMCT were performed without any intraoperative events, such as ventricular arrhythmias or myocardial infarction. There was no need to prolong inotropic agents for >3 d, nor for respira-

tory assistance for >2 d, and there was no occurrence of cardiac tamponade or stroke.

There was no new occurrence of retinal lesions, such as retinopathy in 5 of 10 patients (the other 5 patients have not been examined yet), 1–6 mo after OPCAB and BMCT.

During this hospitalization, patient 6 had implantation of an implantable cardiac defibrillator. Because this patient had ischemic cardiomyopathy and ventricular tachycardia before OPCAB and BMCT, the implantation was scheduled after OPCAB and BMCT. The Lown grades of 10 patients were all 4b before OPCAB. One month after OPCAB and BMCT, 24-h ECG monitoring of all patients did not reveal any new arrhythmias (the Lown grade remained 4b). In 8 patients monitored for >12 mo after OPCAB and BMCT, the grade of arrhythmias remained 4b (similar to 1 mo after surgery).

Malignant neoplasms were not detected after OPCAB and BMCT by whole-body enhanced CT scans.

During the follow-up period (range, 5–32 mo; average ± SE, 18 ± 3 mo) of the 10 patients, 2 patients died from noncardiac causes (Table 1: patient 1, shock due to gastric hemorrhage 32 mo after OPCAB and BMCT; patient 2, inappropriate use of self-insulin therapy 21 mo after OPCAB and BMCT). Patient 9 had cardiac death attributed to acute myocardial infarction, possibly caused by coronary vasospasm and spontaneous reperfusion (assumed by autopsy findings) 5 mo after OPCAB and BMCT. Another patient (patient 3) had sudden death, possibly due to ventricular arrhythmias (also assumed by autopsy findings).

Bone Marrow Aspiration and Apheresis

Patients who received apheresis tended (not significant) to have less CD34+ cells than those with bone marrow aspiration ($4.5 \pm 1.3 \times 10^6$ cells vs. $7.2 \pm 3.2 \times 10^6$ cells; not significant). There were no differences, including unfavorable events, in the postoperative clinical course.

Myocardial Perfusion SPECT Findings

The inter- and intraobserver variability of myocardial ^{99m}Tc uptake was less than 3% and 2%, respectively. Compared with before, myocardial ^{99m}Tc uptake on dipyridamole-stress was improved 1 mo after OPCAB and BMCT in both areas treated with G-CSF–based and bone marrow aspiration–based BMCT ($P < 0.05$ each; Fig. 1C), whereas it did not change in the control nonischemic untreated area (Fig. 1A). In contrast, myocardial viability assessed by ^{99m}Tc uptake at rest did not change 1 mo after OPCAB and BMCT in the corresponding areas as well as in the control area (Fig. 1B). Because there were no significant differences in scintigraphic results between the G-CSF–based and bone marrow aspiration–based BMCT (Fig. 1C), the following analyses were all done in one category.

There was no correlation on improvement of ^{99m}Tc uptake between the CD34+ cell number and the total number of mononuclear cells transplanted in 10 patients (Figs. 2A–2D). The changes in myocardial ^{99m}Tc uptake on dipyridamole-stress were positively correlated with the number of CD34+ cells transplanted: ^{99m}Tc tracer uptake after/before BMCT (ratio) = $1.091 \times (\text{CD34+ cell number } [\times 10^6])^{0.074}$ ($r^2 = 0.48$, $P < 0.05$) (Fig. 2A) but not with the total mononuclear cell number (Fig. 2B). The corresponding values at rest were not correlated with either the CD34+ or the total mononuclear cell number (Figs. 2C and 2D, respectively), suggesting that myocardial viability (*5*) did not change by BMCT.

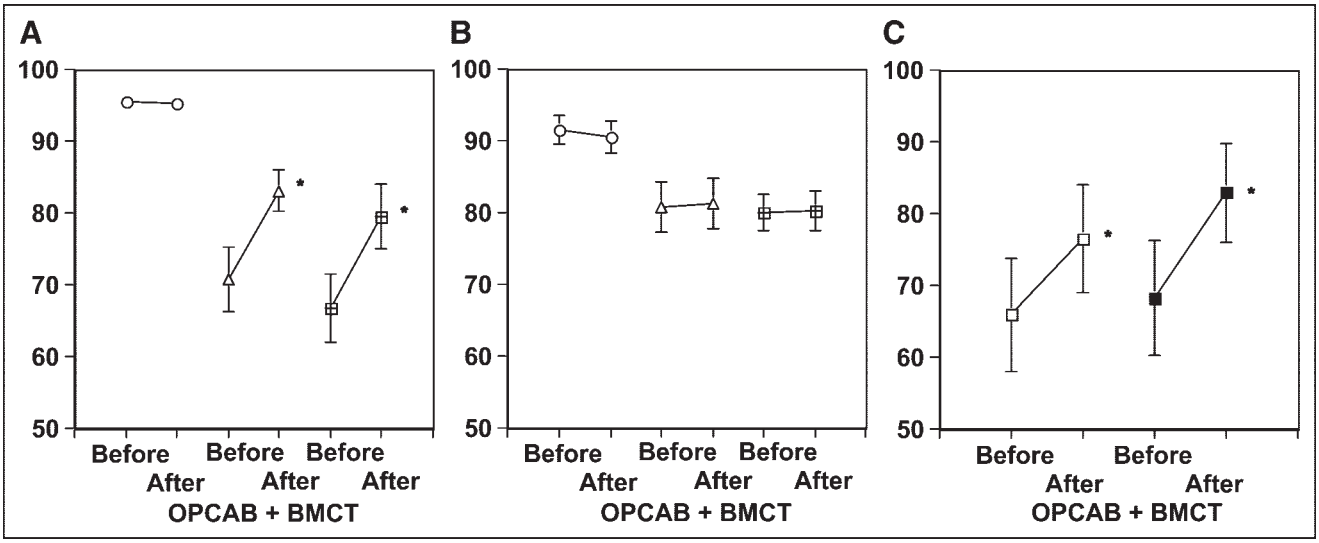

FIGURE 1. Myocardial uptake of ^{99m}Tc-tetrofosmin on dipyridamole-stress (A) and at resting condition (B) before and 1 mo after OPCAB and BMCT in regions of no ischemia (control), OPCAB, and BMCT treatment. ○, Control sites; △, OPCAB sites; ⊞, BMCT sites ($n = 10$ each). *$P < 0.05$ vs. before OPCAB + BMCT. (C) Comparison of myocardial uptake of ^{99m}Tc-tetrofosmin on dipyridamole-stress among different methods for bone marrow–derived mononuclear cell correction by G-CSF and apheresis or by bone marrow aspiration. □, Sites of BMCT by G-CSF and apheresis; ■, sites of BMCT by bone marrow aspiration ($n = 5$ each). *$P < 0.05$ vs. before OPCAB + BMCT.

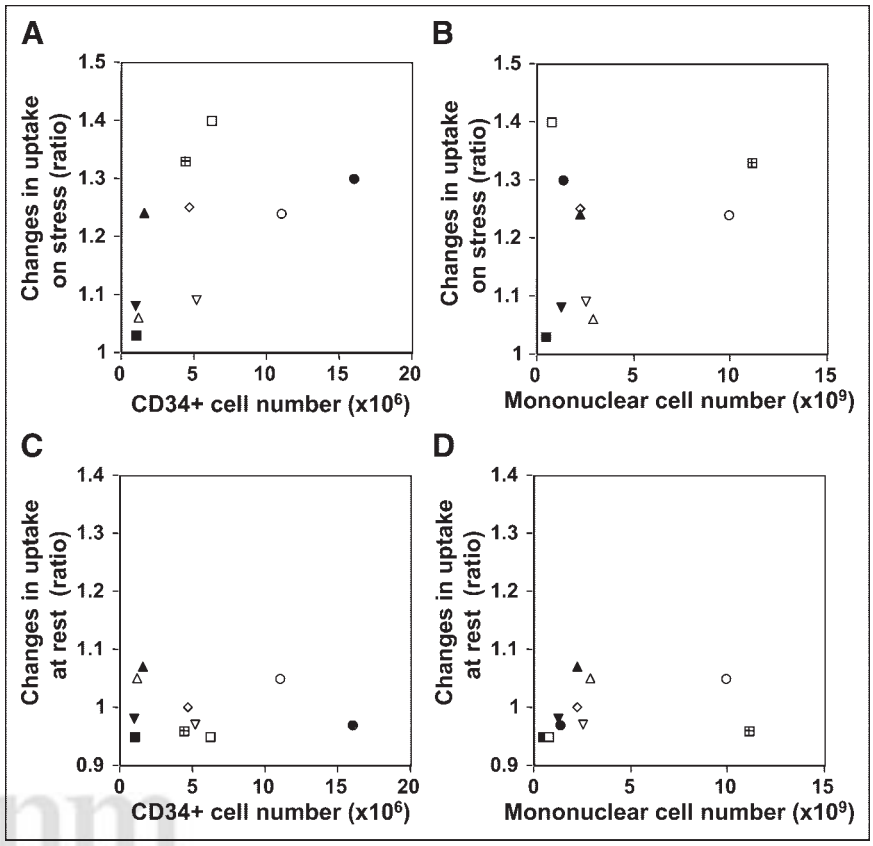

FIGURE 2. Relation between CD34+ (A and C) or total number of mononuclear cells (B and D) and changes (ratios) in myocardial uptake (myocardial ^{99m}Tc uptake 1 mo after BMCT/myocardial ^{99m}Tc uptake before BMCT) on dipyridamole-stress (A and B) and at rest (C and D) in 10 patients. Plots in A–D are from the same patient. Only A showed a significant relation between *x*- and *y*-axes: Changes in myocardial uptake on stress = 1.091 × (CD34+ cell number [×10^6])$^{0.074}$ (r^2 = 0.48).

LV end-diastolic volume tended (not significant) to be lower 1 mo after (138 ± 18 mL) compared with before (160 ± 22 mL) OPCAB and BMCT, whereas LVEF did not change (from 0.34 ± 0.04 to 0.35 ± 0.04).

Cineangiographic Findings

Cineangiography documented that all coronary artery bypass grafts were patent after OPCAB but that new coronary arteries were not apparent at the site of BMCT (data not shown).

Scintigraphic Images

The polar maps of dipyridamole-stress and resting ^{99m}Tc myocardial perfusion scintigraphy in the patient (patient 2 in Table 1) are shown in Figure 3. The myocardial perfusion during dipyridamole-stress improved in the inferior wall of BMCT as well as in the lateral wall of OPCAB.

Histopathology at Autopsy

The myocardial CD34+ microvessel density decreased in the OPCAB and the BMCT areas ($P < 0.05$ each) and in the scar area because of old myocardial infarction ($P < 0.0001$) compared with that of the control (nonischemic) area (Fig. 4). The myocardial microvessel density in the BMCT area was greater than that of the OPCAB area ($P < 0.05$) and that of ($P < 0.0001$) the scar area due to old myocardial infarction. There was a patchy light scar formation in the OPCAB and BMCT sites, possibly reflecting some tissue damage attributed to previous chronic ischemia before the interventions.

In patient 9, who died of acute myocardial infarction—probably due to coronary vasospasm—that was assumed from the findings on the autopsy, there were no organic stenoses, thrombi, or obstructions in the coronary arteries and bypass grafts, but contraction band necrosis spread in the anterior wall. In this patient, changes in the dipyridamole-stress myocardial perfusion by BMCT were negligible (108% of that before BMCT), and there were no obvious findings of new coronary vessel formation in the BMCT area.

In patient 2 (noncardiac death), myocardial sections showed an old myocardial infarction in the endocardial side of the posterior wall that corresponded to chronic total occlusion of the right coronary artery (Fig. 5A). There was the intramyocardial space with loose connective tissue in the middle layer close to the lateral border zone of the old infarction but discrete from the old infarction by Azan staining. High-power fields of Azan (Figs. 5B and 5C) and immunostaining for CD34 to detect vascular endothelium (Figs. 5D and 5E) showed sparse small vessels in the old infarction area (Fig. 5E) but an increase in small vessels in the middle layer (Fig. 5D), compared with nonischemic (non–cell transplanted) anterior wall (Fig. 5F).

In patient 3 (sudden death), macroscopic (Fig. 6A) and microscopic (Fig. 6C) myocardial sections showed an old myocardial infarction (avascular area) in the inferior wall perfused by the right coronary artery where BMCT was not performed. In the posterolateral wall perfused by the left circumflex artery (segment 14 according to the American

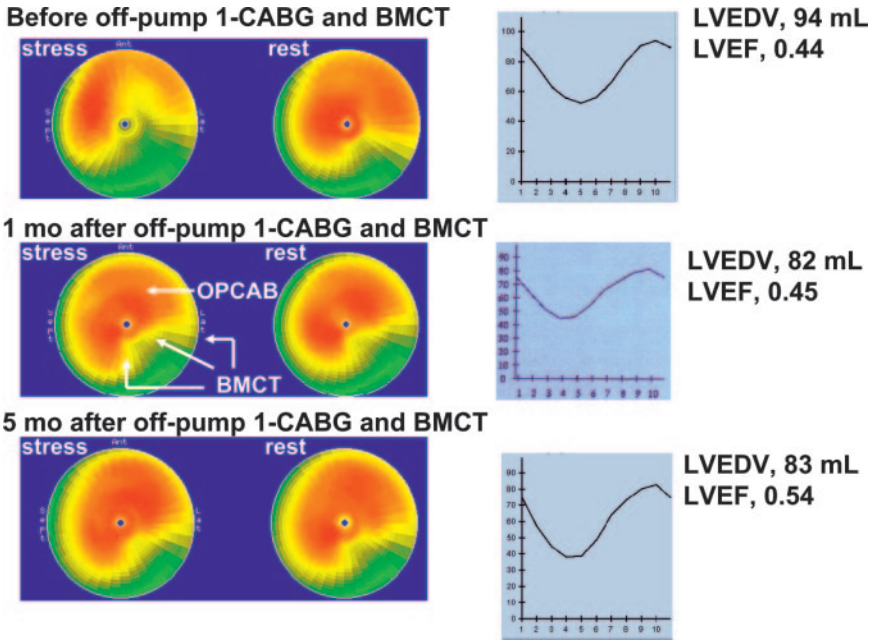

FIGURE 3. A representative case: patient 2. Polar maps on myocardial ^{99m}Tc perfusion SPECT during dipyridamole-stress and at rest, and time–volume curve (volume [mL]/interval) on QGS (from left to right) before and 1 and 5 mo after OPCAB and BMCT. Compared with before, after OPCAB and BMCT, myocardial perfusion on dipyridamole-stress was ameliorated in posterolateral wall (BMCT site) and anterolateral wall (OPCAB site), although myocardial perfusion at rest was unchanged. LVEF increased 5 mo after compared with before OPCAB and BMCT. LVEDV = LV end-diastolic volume.

Heart Association classification (*12*)) where BMCT was performed, intramyocardial focal sites with the connective tissue and with proliferation of coronary microvessels (filled with red blood cells due to congestion) were observed (Figs. 6B and 6D). Compared with before BMCT, the ^{99m}Tc tracer uptake during dipyridamole infusion but not at rest improved 1 mo after BMCT (image not shown). These findings in patient 3 are similar to those in patient 2 described earlier.

DISCUSSION

Feasibility and Safety

The assessment of feasibility and safety of BMCT in combination with OPCAB was one of the aims of this study. Our initial results underscored BMCT feasibility. With regard to safety, 4 patients died at a mean 24 mo after BMCT, and in 2 of them cardiac events (acute myocardial infarction in one and worsened heart failure in the other) were the causes of death. Because our follow-up periods were short, we need to assess the mid-term and long-term safety of BMCT in the future.

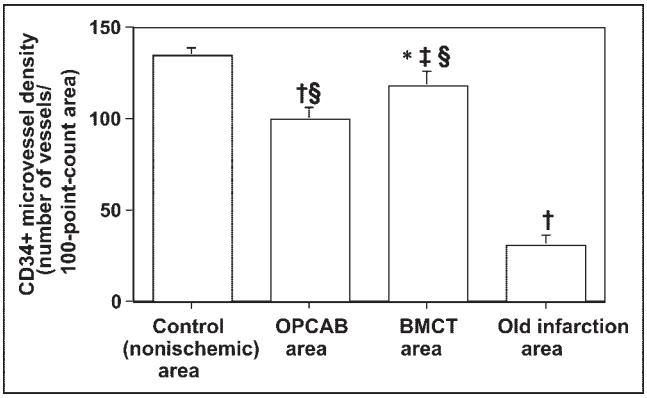

FIGURE 4. Myocardial CD34+ vessel densities in areas of control (nonischemic), OPCAB, BMCT, and old myocardial infarction. There were 3 patients each in control, OPCAB, and BMCT (total $n = 63$) areas and 2 patients in old infarction area ($n = 14$). *$P < 0.05$ vs. control area; †$P < 0.0001$ vs. control area; ‡$P < 0.05$ vs. OPCAB area; §$P < 0.0001$ vs. old infarction area.

The arrhythmogenic property is one of the major concerns of cell transplantation therapies (*2,7–10,16–19*). Eight clinical reports on transplantation of autologous skeletal myoblasts or bone marrow cells in a total of 134 patients documented that 11 patients had sudden death, supraventricular tachycardia, or ventricular tachycardia during the follow-up periods of 3–17.5 mo. In the present study, 1 of 10 patients received implantation of the internal defibrillator, although ventricular tachycardia predated BMCT. Thus, long-term follow-up on the arrhythmogeneity of BMCT is needed to accurately judge the safety of this therapy.

Therapeutic Effects of BMCT on Ischemic Myocardium

In this study, pharmaceutical-stress myocardial perfusion SPECT documented amelioration of myocardial perfusion in areas of autologous BMCT comparable to the areas of OPCAB (Fig. 1). A lack of improvement of LVEF after OPCAB and BMCT by QGS analysis suggests that a longer time may be needed for functional recovery after relieving chronic myocardial ischemia in our patients. On the basis of the fact that cineangiography after BMCT showed no evidence of new coronary vessels in spite of increased myocardial flow in myocardial perfusion SPECT study, we assume that small coronary vessels (<200 μm in diameter (*15*)) may have a role in improving myocardial perfusion by BMCT.

Histopathologic findings revealed that myocardial microvessel density increased by BMCT compared with the CABG sites. That the microvessel densities in the sites of

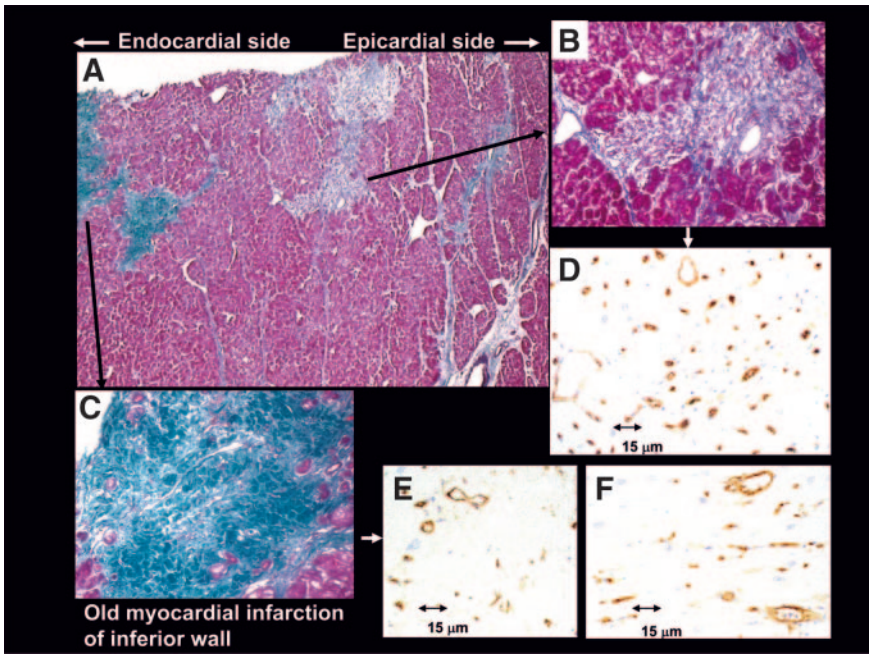

FIGURE 5. Histopathogic findings at autopsy: patient 2. Light microscopic findings by Azan staining of posterolateral wall (A, ×40), of local areas corresponding to BMCT (B, ×100), and of old myocardial infarction (C, ×100). Light microscopic findings by CD34 immunostaining of area of BMCT (D, ×200), of old infarction (E, ×200), and of control (nonischemic) anterior wall (F, ×200).

either OPCAB or BMCT were lower than in those of the nonischemic control myocardium seemed to be related to patchy fibrosis in previously ischemic myocardium. Histopathology reports of human stem cell therapies are rare except for reports on neovascularization in the vascular wall of the aorta, peripheral arteries in a patient with acute radiation syndrome (*20*), and cases of skeletal myoblast transplantation (*21,22*). To the best of our knowledge, this is the first report of histologic evidence in human myocardial BMCT. Our results suggest that autologous BMCT in patients with IHD may contribute to improving myocardial perfusion, especially in ungraftable ischemic myocardial areas.

Mechanistic Insights into Myocardial BMCT

Some clinical studies have assessed the effects of cardiac regenerative therapies using scintigraphy. Intracoronary infusion of autologous bone marrow–derived cells using a catheter in a subacute phase of myocardial infarction (*10*) or their transendocardial injection using a NOGA catheter (Biosense-Webster, Johnson & Johnson) in chronic ischemic heart failure (*7*) reduced reversible perfusion defect areas of the infarcted left ventricle. Our results are compatible with various studies with differences in the therapeutic approach. Although our study had fewer patients and they were not randomized, we believe it revealed for the first time that the changes in myo-

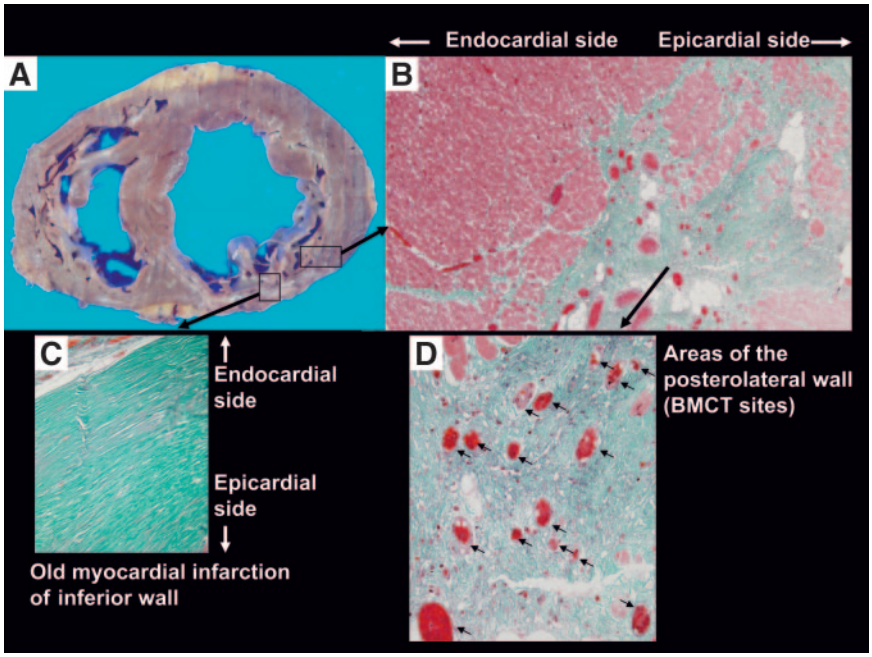

FIGURE 6. Histopathologic findings at autopsy: patient 3. Macroscopic finding of short-axial section of ventricles (A), light microscopic findings by elastica Masson staining of posterolateral wall where BMCT was performed (B, ×40; D, ×100), and light microscopic findings of old myocardial infarction where BMCT was not performed (C, ×150). Proliferating vessels filled with red blood cells are shown by arrows in D.

cardial ^{99m}Tc tracer uptake on dipyridamole-stress after BMCT were positively correlated with the number of CD34+ cells transplanted. Taken together with other findings indicating that the myocardial microvessel density was increased by BMCT compared with the OPCAB area and that there were no increases in ^{99m}Tc uptake on the resting image, our results suggest that the therapeutic effects of this BMCT method may be attributed to coronary angiogenesis—in which transplanted CD34+ cells are significantly involved—rather than to regeneration of cardiomyocytes.

Limitations

This study has limitations. First, the number of patients enrolled was quite small and they were not randomized. Second, as a control we did not have the OPCAB group without BMCT. Instead, the area of simple OPCAB without BMCT was used as a control area in each patient. This makes it difficult to determine whether the improvement of myocardial perfusion by BMCT is specifically related to BMCT and not to OPCAB. The areas of BMCT and OPCAB were sometimes close to each other to distinguish between the effects of BMCT and OPCAB. In this respect, we set the region of interest on myocardial scintigraphic images carefully in accordance with each patient's coronary arterial anatomy on cineangiography. Together with a positive correlation with the number of CD34+ cells transplanted and improvement of myocardial perfusion assessed by SPECT, we assume that the improvement of myocardial perfusion in the areas of BMCT cannot be due solely to the effect of OPCAB. Third, we have not assessed the long-term effects of BMCT on prognosis. Fourth, although we did not find an apparent leak of the phosphate-buffered saline containing bone marrow cells to be associated with its epicardial injection, the leak rate was not quantified. In addition, we do not know the efficacy of regeneration—that is, how many transplanted stem cells differentiated into cells that constitute treated myocardium. Even with histopathologic investigation, the tracking of stem cells (*23*) transplanted into the myocardium is difficult in a human study. Fifth, our parameters for assessment of myocardial perfusion are the relative values obtained from SPECT but they are not absolute. The assessment of absolute values and changes by interventions using PET is one future direction for precise interpretation of the study results.

CONCLUSION

Our clinical study revealed the feasibility of BMCT in combination with OPCAB in patients who undergo CABG. In addition, improvement of myocardial ^{99m}Tc tracer uptake in the sites of BMCT on dipyridamole-stress perfusion SPECT was positively correlated with the CD34+ cell number implanted. Although long-term follow-up of the therapeutic outcome is required, BMCT seems to improve myocardial perfusion in ungraftable but ischemic myocardial areas via CD34-dependent development of coronary microcirculation.

REFERENCES

1. Britten MB, Abolmaali ND, Assmus B, et al. Infarct remodeling after intracoronary progenitor cell treatment in patients with acute myocardial infarction (TOPCARE-AMI): mechanistic insights from serial contrast-enhanced magnetic resonance imaging. *Circulation.* 2003;108:2212–2218.
2. Fuchs S, Satler LF, Kornowski R, et al. Catheter-based autologous bone marrow myocardial injection in no-option patients with advanced coronary artery disease: a feasibility study. *J Am Coll Cardiol.* 2003;41:1721–1724.
3. Hamano K, Nishida M, Hirata K, et al. Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart disease: clinical trial and preliminary results. *Jpn Circ J.* 2001;65:845–847.
4. 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;22:1804–1810.
5. Matsunari I, Fujino S, Taki J, et al. Quantitative rest technetium-99m tetrofosmin imaging in predicting functional recovery after revascularization: comparison with rest-redistribution thallium-201. *J Am Coll Cardiol.* 1997;29:1226–1233.
6. Orlic D, Kajstura J, Chimenti S, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci USA.* 2001;98:10344–10349.
7. Perin EC, Dohmann HF, Borojevic R, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation.* 2003;107:2294–2302.
8. Pompilio G, Cannata A, Peccatori F, et al. Autologous peripheral blood stem cell transplantation for myocardial regeneration: a novel strategy for cell collection and surgical injection. *Ann Thorac Surg.* 2004;78:1808–1812.
9. Stamm C, Westphal B, Kleine HD, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet.* 2003;361:45–46.
10. Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation.* 2002;106:1913–1918.
11. World Medical Association, Inc. World Medical Association Declaration of Helsinki: recommendations guiding physicians in biomedical research involving human subjects. *Cardiovasc Res.* 1997;35:2–3.
12. Austen WG, Edwards JE, Frye RL, et al. A reporting system on patients evaluated for coronary artery disease: report of the Ad Hoc Committee for Grading of Coronary Artery Disease, Council on Cardiovascular Surgery, American Heart Association. *Circulation.* 1975;51(4 suppl):5–40.
13. Vidal S, Kovacs K, Lloyd RV, Meyer FB, Scheithauer BW. Angiogenesis in patients with craniopharyngiomas: correlation with treatment and outcome. *Cancer.* 2002;94:738–745.
14. Weibel ER. Principles and methods for morphometric study of the lung and other organs. *Lab Invest.* 1963;12:131–155.
15. Davidson CJ, Bonow RO. Cardiac catheterization. In: Zipes DP, Libby P, Bonow RO, Braunwald E, eds. *Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine.* 7th ed. Philadelphia, PA: Elsevier Saunders; 2004:447–448.
16. Menasche P, Hagege AA, Vilquin JT, et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. *J Am Coll Cardiol.* 2003;41:1078–1083.
17. Smits PC, van Geuns RJ, Poldermans D, et al. Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure: clinical experience with six-month follow-up. *J Am Coll Cardiol.* 2003;42:2063–2069.
18. Schachinger 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;44:1690–1699.
19. Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. *Lancet.* 2003;361:47–49.
20. Suzuki T, Nishida M, Futami S, et al. Neoendothelialization after peripheral blood stem cell transplantation in humans: a case report of a Tokaimura nuclear accident victim. *Cardiovasc Res.* 2003;58:487–492.
21. Hagege AA, Carrion C, Menasche P, et al. Viability and differentiation of autologous skeletal myoblast grafts in ischemic cardiomyopathy. *Lancet.* 2003;361:491–492.
22. Pagani FD, DerSimonian H, Zawadzka A, et al. Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans. *J Am Coll Cardiol.* 2003;41:879–888.
23. Boersma HH, Tromp SC, Hofstra L, Narula J. Stem cell tracking: reversing the silence of the lambs. *J Nucl Med.* 2005;46:200–203.