Improvement of Cardiac Function by Bone Marrow Cell Implantation in a Rat Hypoperfusion Heart Model

Masahiko Nishida, MD, Tao-Sheng Li, MD, Ken Hirata, MD, Masafumi Yano, MD, Masunori Matsuzaki, MD, and Kimikazu Hamano, MD

Division of Cardiovascular Surgery and Division of Cardiovascular Medicine, Department of Bioregulation, Yamaguchi University School of Medicine, Yamaguchi, Japan

Background. **Local bone marrow cell implantation can induce angiogenesis. In the present study we investigated whether angiogenesis induced by bone marrow cell implantation improves deteriorated cardiac function in a rat heart model of hypoperfusion.**

Methods. **A hypoperfusion heart model was created in Dark Agouti rats by ligating the left anterior descending artery placed against a copper wire (ϕ275 μm), then pulling out the wire immediately. The left ventricular (LV) anterior wall was injected directly at six points, each with 1×10^7 bone marrow cells in 10 μL of phosphate-buffered saline or with phosphate-buffered saline only, respectively. Echocardiography was performed to evaluate the cardiac function 7, 30, 60, and 90 days after treatment. Microvessel density and blood flow in the LV anterior wall were estimated 60 days after treatment.**

Results. **Both the increase of LV end-systolic diameter and the decrease of percent of fractional shortening caused by myocardial ischemia were attenuated effectively by bone marrow cell implantation treatment. Bone marrow cell implantation treatment also increased the levels of angiopoietin-1 and vascular endothelial growth factor in the LV anterior wall. The microvessel density, blood flow, and thickness of the LV anterior wall significantly also increased after bone marrow cell implantation treatment compared with those after phosphate-buffered saline injection.**

Conclusions. **The local implantation of autologous bone marrow cells induced angiogenesis and improved the perfusion of ischemic myocardium, thereby preventing LV remodeling and improving deteriorated cardiac function caused by myocardial hypoperfusion.**

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Therapeutic angiogenesis and vasculogenesis have the potential to improve blood flow in ischemic tissues. Numerous angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and hepatic growth factor (HGF), have been shown to promote collateral vessels in animal models of myocardial infarction or ischemic hind limbs [1–3]. Bone marrow consists of complex cell fractions, including CD34 positive cells, which can differentiate into endothelial cells and induce angiogenesis [4, 5]. Bone marrow cells also secrete many kinds of cytokines for angiogenesis. Therefore local autologous bone marrow cell implantation (BMCI) appears to be a simple and effective method of inducing therapeutic angiogenesis [6].

Our previous studies showed that the local implantation of bone marrow cells could induce angiogenesis in myocardial infarction models and ischemic hind limb models [7–9], and that this angiogenesis induced by BMCI was related to the production of angiogenic cytokines and the endothelial differentiation from bone marrow cells. Furthermore we demonstrated that the implantation of bone marrow cells improves exercise capacity, which was impaired by ischemia in a rat ischemic hind limb model [8]. These results show that therapeutic angiogenesis induced by BMCI could be an effective treatment for severe ischemic heart and limb diseases.

In this study we tried to induce therapeutic angiogenesis by BMCI in ischemic myocardium using a rat heart model of hypoperfusion. We also examined whether the angiogenesis induced by BMCI would increase the perfusion of ischemic myocardium and hence improve cardiac function.

Material and Methods

Animals

Male syngeneic Dark Agouti rats, 10 to 12 weeks old and weighing 200 to 250 g, were used for these experiments. All animals were obtained from Japan SLC Inc (Hamamatsu, Japan) and were housed under clean conditions. All experiments were approved by the Institutional Animal Care and Use Committee of Yamaguchi University School of Medicine.

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Address reprint requests to Dr Hamano, Division of Cardiovascular Surgery, Department of Bioregulation, Yamaguchi University School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan; e-mail: kimikazu@yamaguchi-u.ac.jp.

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Preparation of Rat Bone Marrow Cell

Dark Agouti rats were killed by an overdose of pentobarbital injected intraperitoneally. Bone marrow from the femur and tibia was collected and put in phosphate buffered saline (PBS). Single bone marrow cell suspensions were prepared by gently pressing bone marrow segments through fine wire mesh. Red blood cells were removed by adding 0.14 mol/L Tris-buffered ammonium chloride (pH 7.2). An optimal number of cells were suspended in PBS for injection. The viability of bone marrow cells was more than 98% by a trypan blue staining check.

Rat Hypoperfusion Heart Model and Bone Marrow Cell Implantation

Rats were anesthetized with 50 mg/kg of pentobarbital intraperitoneally, and artificially ventilated (Harvard Rodent Ventilator, Model 683, Harvard Apparatus Inc, South Natick, MA) through an intratracheal tube. A hypoperfusion heart model was created by a method as described by Yaoita and colleagues [10]. Briefly a left thoracotomy was performed to exteriorize the heart rapidly by gentle pressure on the right side of the thorax. A copper wire (275 μm in diameter) was placed against the surface of the left anterior descending coronary artery (LAD), and the LAD was ligated together with the copper wire using 7-0 silk. After the ligation the copper wire was pulled out immediately. Rats were then randomly divided into three groups. In the ischemia group, rats were not given any treatment. In the PBS and BMCI groups, rats were injected at six points in the anterior wall of the left ventricular (LV) LAD area with a 26-gauge needle, and each point was injected with 10 μl PBS (PBS group) or with 1×10^7 of freshly collected bone marrow cells in 10 μl PBS (BMCI group). As a control for the hypoperfusion heart model, a sham operation model was made by opening the thoracic cavity and pericardium only (sham group).

Echocardiography

Transthoracic echocardiography was done to observe the changes in cardiac function 7, 30, 60, and 90 days after treatment. The rats (n = 7 in each group) were anesthetized, placed in supine position, and their chests were shaved. Using phased array technology with a spatial resolution of 0.2 mm, a 7.5 MHz commercially available pediatric transducer was connected to an echocardiographic computer console (SSD-630; ALOKA, Hoofddorp, Netherland) [11]. The interrogation depth was set at 4 cm and echocardiograms were recorded by a parasternal long axis view followed by a parasternal short axis view. We tried hard to obtain good image quality up to the midpapillary muscle level of the left ventricle in 2-dimensional echocardiography. M-mode was added for measurement of the left ventricular dimensions at end-systole and end-diastole in all rats. Regional fractional shortening was calculated by dividing the end-diastolic dimension minus the end-systolic dimension by the end-diastolic dimension times 100, expressed as a percentage [12, 13]. The total examination time usually took less than 20 minutes and rats were allowed to recover.

Measuring the Thickness of the LV Anterior Wall

The wall thickness was examined histologically 60 days after treatment in separated rats (n = 7 in each group). The heart was arrested in diastole by an intravenous injection of potassium chloride, then excised and fixed for hematoxylin and eosin staining. Specimens were coded so that measurements of wall thickness of the ischemic area were made without knowledge of hemodynamic data. The thickness of the LV anterior wall was measured using the method described by Scheinowitz and associates [14]. All measurements were done less than 10-fold magnification on two 5 μm-thick hematoxylin and eosin staining slices, each derived from cross sections of the heart maintaining a distance of 5 mm and 6 mm from the apex. The mean valve calculated from the two cross-sections was used for statistical analysis.

Histologic Assessment of Microvessels

The degree of induced angiogenesis was examined in separated rats (n = 7 in each group) by measuring the density of capillaries in sections harvested from ischemic myocardium as previously described [15]. Briefly rats were killed 60 days after treatment and tissue sections harvested from the left ventricle were embedded in Tissue-Tek O.C.T. compound (Miles Inc, Elkhart, IN), then snap-frozen in liquid nitrogen. Frozen sections were stained for alkaline phosphatase with an indoxyl tetrazolium technique to detect capillary endothelial cells, and then counterstained with eosin. A total of 10 different fields with 200-fold magnification were randomly selected, and the number of capillaries and muscles was counted by a single observer blinded to the treatment groups. The density of microvessels was estimated by the capillary to muscle fiber ratio.

Regional Blood Flow Measured by Colored Microsphere Method

Regional myocardial blood flow was determined in separated rats (n = 7 in each group), using the colored microsphere method 60 days after treatment [16]. Rats were anesthetized and artificially ventilated as previously described. A thoracotomy was performed though the fourth left intercostal space, and a PE-50 catheter (Becton Dickinson, Sparks, MD) was inserted into the left atrium through the left pulmonary vein. Next, 5×10^5 colored microspheres with a diameter of 15 μm (Dye-Trak, Triton Technologies Inc, San Diego, CA) were injected into the left atrium through the catheter. The rats were killed by cutting the aorta about 30 seconds after the injection of microspheres. Tissue samples were taken from the LAD area (ischemic area) and the circumflex area (control area) and were weighted.

The tissue samples were digested with potassium hydroxide, and microspheres were reclaimed with a vacuum filter. After extracting dyes from the microspheres with dimethylformamide, the photometric absorption of

each sample was determined with a spectrophotometer (Model U-2000; Hitachi, Tokyo, Japan). Based on optical density measurements, the percent of blood flow defined as flow in the LAD area expressed as a percentage of that in the circumflex area was calculated from the equation: (optical density of the LAD area/optical density of the circumflex area) × (tissue weight of the circumflex area/ tissue weight of the LAD area) × 100 (%).

Western Blotting Analysis of Angiopoietin-1 and VEGF

To estimate the levels of angiopoietin-1 and VEGF in the ischemic myocardium of the LV anterior wall, five rats from the BMCI and PBS groups were killed 1, 3, 7, and 14 days after treatment. The myocardium of the LV anterior wall was collected and total tissue proteins were extracted as previously described [7]. Five samples containing 150 mg tissue protein were collected from each group at each time point and electrophoresed under nonreducing conditions on 10% polyacylamide gels in tris-glycine running buffer and also were electrotransferred to a 0.45 mm polyvinylene difluoride membrane. These membranes were blocked for 45 minutes with blocking buffer (5% milk in Tris-buffered saline, pH 7.5) and then incubated overnight at 4°C with the goat polyclonal antibody against angiopoietin-1 (1:200 dilution, Santa Cruz) or the rabbit monoclonal antibody against VEGF (1:200 dilution, IBL Co. Ltd, Gunma, Japan). After being washed, the membranes were incubated for 60 minutes with alkaline phosphatase conjugated secondary antibody to goat or rabbit immunoglobulin G (1:1000 dilution, Amersham, Buckinghamshire, UK). After three washes these membranes were visualized with a Western blot detection kit (Amersham), and then exposed to the high performance chemiluminescence film (Amersham). The levels of angiopoietin-1 and VEGF were analyzed quantitatively with NIH Image-Quant software (Version 1.55, NIH, WA).

Statistical Analysis

All values are expressed as means ± standard deviation. Statistical significance was evaluated by the unpaired Student's *t* test for two variables, by analysis of variance followed by Scheffe's procedure for more than two variables, and by repeated analysis of variance to test for interactions, and $p < 0.05$ was considered statistically significant.

Results

Improvement in LV Wall Motion and Cardiac Function After BMCI Treatment

To evaluate cardiac function, echocardiography was monitored until 90 days after treatment. The representative recordings of the LV short axis in M-mode, 60 days after treatment are shown in Figure 1. The anterior wall motion in the ischemia group and the PBS group was hypokinetic, but not akinetic or dyskinetic. Conversely, the anterior wall motion in the BMCI group was preserved as well as that in the rats without myocardial ischemia in the sham group.

The LV end-diastolic dimension, LV end-systolic dimension, and the percent of fractional shortening did not differ significantly among the groups before treatment. The LV end-systolic dimension increased gradually in both the PBS and ischemia groups, but did not change significantly in the BMCI or sham groups. The LV end-systolic dimension was significantly larger in the ischemia and PBS groups than in the sham and BMCI groups 30 days or more after treatment ($p < 0.01$; Fig 2A). The LV end-diastolic dimension also increased slightly with time in both the ischemia and PBS groups, but not in the BMCI and sham groups (Fig 2B).

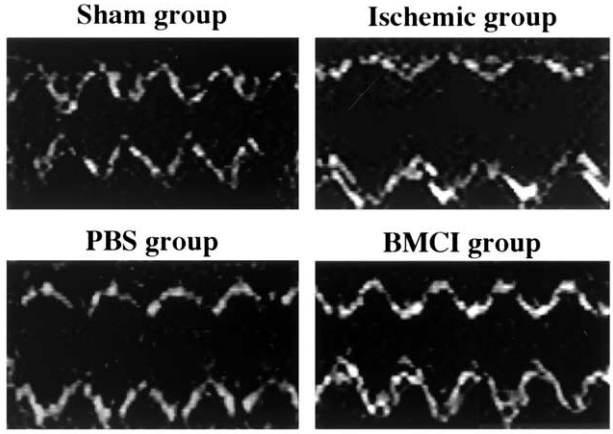

Fig 1. Representative M-mode echocardiogram done 60 days after treatment. Two-dimensional left ventricular short-axis echocardiograms were taken at the head of the papillary muscle. The upper white lines *show the motion of the anterior wall. The wall motion in the ischemia and phosphate buffered saline (*PBS*) groups was hypokinetic, but that in the bone marrow cell implantation (*BMCI*) group was similar to that in the sham group.*

The percent of fractional shortening increased gradually in the ischemia and PBS groups within the first 60 days after treatment from approximately 55% at 7 days after treatment to about 40% at 60 days after treatment, and it remained unchanged thereafter (Fig 2C). However, the percent of fractional shortening in the BMCI group remained at about 60% throughout the experimental periods There was no significant difference in percent of fractional shortening between the BMCI and sham groups. The percent of fractional shortening in the sham and BMCI groups was significantly higher than that in the ischemia and PBS groups 30, 60, and 90 days after treatment ($p < 0.001$). Because cardiac function in the ischemic rats did not change beyond 60 days after LAD ligation, we selected 60 days after treatment as the time point for evaluation in the following experiments.

Interstitial Fibrosis and Thickness in the LV Anterior Wall

Severe fibrosis of the myocardium was observed in the ischemia and PBS groups, but only slight fibrosis was seen in the BMCI group (Fig 3, upper panel) 60 days after treatment. The LV wall was significantly thicker in the BMCI group at 2.53 ± 0.18 mm than in the ischemia or PBS groups at 2.16 ± 0.20 mm and 2.25 ± 0.19 mm, respectively ($p < 0.001$) 60 days after treatment (Fig 3, lower panel). No significant difference was observed between the BMCI and sham groups (2.53 ± 0.16 mm).

Developed Microvessels and Increased Regional Blood Flow in the Ischemic Myocardium After BMCI Treatment

The capillary to muscle fiber ratio in the ischemic myocardium is shown in Figure 4. The capillary to muscle fiber ratio was 1.54 ± 0.21 in the BMCI group, which was significantly higher than that in any of the other groups, (ie, 0.99 ± 0.12 in the sham group [$p < 0.0001$], 0.94 ± 0.10 in the ischemia group [$p < 0.01$], and 1.09 ± 0.16 in the PBS group [$p < 0.05$]. The percent of blood flow of the ischemic LAD decreased to 62.6% and 69.8% in the ischemia and PBS groups, respectively, which was signif-

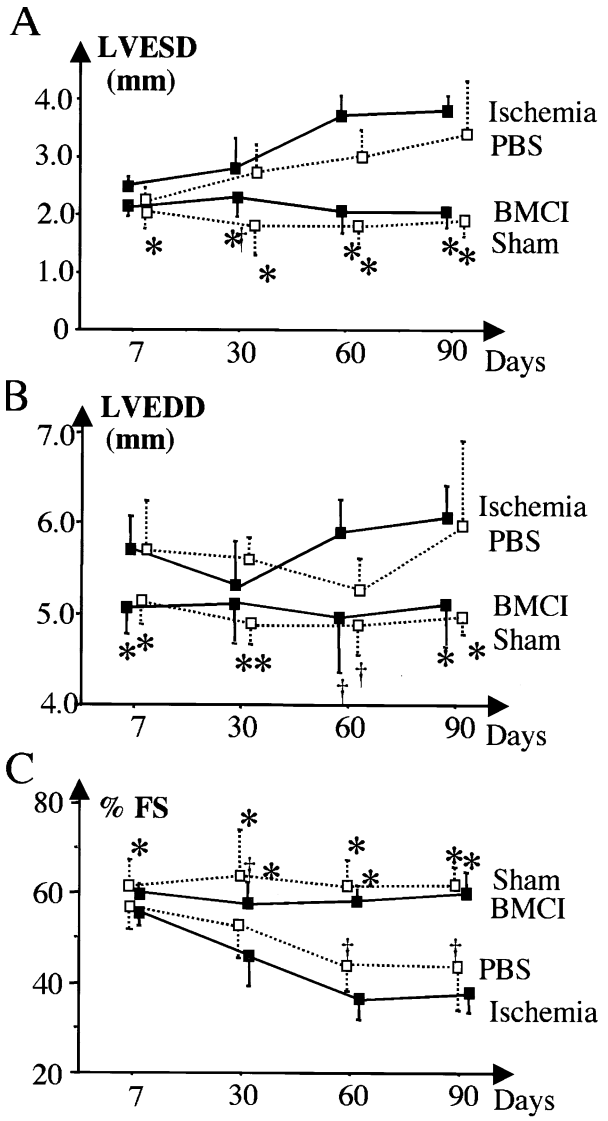

*Fig 2. The time course of the changes of left ventricular (LV) end-systolic dimension (LVESD), LV end-diastolic dimension (LVEDD), and percent of fractional shortening (%FS) at 7, 30, 60, and 90 days after treatment. (A) The LVESD increased, but (B) the LVEDD dilated with time after treatment in the ischemia and phosphate buffered saline (PBS) groups. These valves did not change significantly in the bone marrow cell implantation (BMCI) and sham groups. (C) The %FS in the PBS and ischemia groups continued to decrease for 60 postoperative days, but the %FS in the BMCI and sham groups remained stable preoperatively and postoperatively (*p < 0.001 versus PBS and ischemia groups; †p < 0.01 versus sham group).*

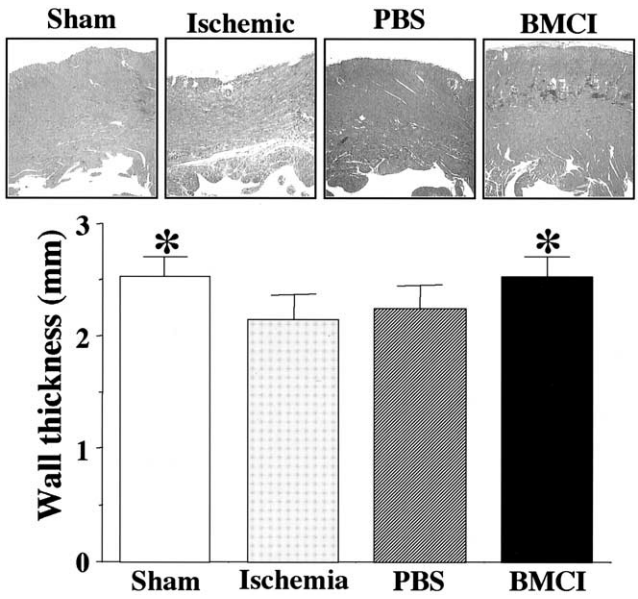

*Fig 3. Wall thickness of the left ventricular (LV) anterior wall 60 days after the operation. Hematoxylin-eosin staining (upper panel) showed visible fibrosis in the ischemia and phosphate buffered saline (PBS) groups, but minimal or no fibrosis was found in the bone marrow cell implantation (BMCI) and sham groups. The LV anterior wall was significantly thinner in the ischemia and PBS groups than in the BMCI and sham groups (*p < 0.01 versus PBS and ischemia group).*

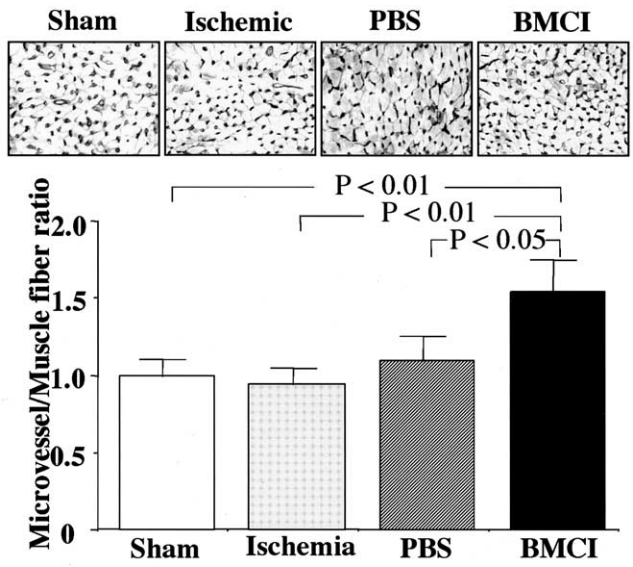

Fig 4. Density of the microvessels in the left ventricular (LV) anterior wall 60 days after treatment (upper panel). Quantitative analysis showed that the capillary to muscle fiber ratio in the bone marrow cell implantation (BMCI) group was significantly higher than that in any other group. (PBS = phosphate buffered saline.)

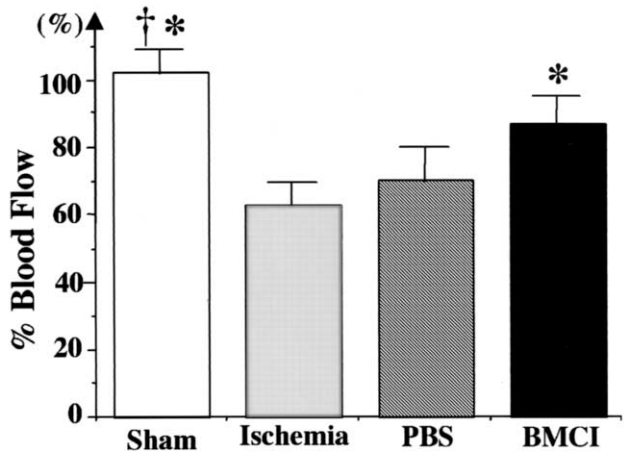

*Fig 5. Percent of blood flow of the left anterior descending coronary artery to the circumflex perfusion area 60 days after treatment. The regional blood flow in the bone marrow cell implantation (*BMCI*) group was significantly better than that in the ischemia and phosphate buffered saline (*PBS*) groups, although it was slightly less than that in the sham group (*$p < 0.001$ versus PBS and ischemia groups; †$p < 0.05$ versus BMCI group).*

icantly lower than that in the BMCI and sham groups ($p < 0.01$; Fig 5). There was no significant difference in percent of blood flow between the BMCI and sham groups, although it decreased slightly to 87.0% ± 8.2% in the BMCI-treated rats.

Improvement in Angiopoietin-1 and VEGF in the Myocardium of the LV Anterior Wall After BMCI Treatment

The levels of angiopoietin-1 in the myocardium of the LV anterior wall were significantly higher in the BMCI group than in the PBS group at 1, 3, 7, and 14 days after treatment (Fig 6A). The levels of VEGF were also significantly higher in the BMCI group than in the PBS group at 1, 3, and 7 days after treatment (Fig 6B). Both angiopoietin-1 and VEGF were significantly increased 1 day after treatment in the PBS group, which was considered a response to acute ischemia of the left ventricle.

Comment

Before this study we investigated therapeutic angiogenesis induced by BMCI using the rat myocardial infarction and ischemic hind limb models. We found that BMCI induced angiogenesis and improved the physiologic function in the rat ischemic hind limb model [8]. In the present study we examined the effect of BMCI treatment on angiogenesis and cardiac function in the hypoperfusion rat heart model.

A reliable model of cardiac ischemia has not yet been established in a small animal. Therefore we tried to create a rat heart model of hypoperfusion by modifying the method of LAD ligation as described in Material and Methods. We ligated the LAD placed against a copper wire, then pulled out the wire, causing the space left by the copper wire to result in the LAD stenosis, but not occlusion. Transmural infarction, necrosis, and progressive remodeling usually occurs in the anterior wall after complete LAD occlusion [17, 18]. However, a hypoperfusion heart model differs from the LAD occlusion model, in that both blood flow and wall motion in the ischemic anterior wall decreased to around 60% of the normal state and the LAD became hypokinetic, resulting in a decrease in percent of fractional shortening to about 40%. Furthermore, histologic findings showed that myocardium still survived in the LAD area even if fibrotic change were also seen in the hypoperfusion model, but it showed that transmural infarction and myocardium in the anterior wall of the left ventricle were replaced by scar tissue in the LAD ligation model [7]. Echocardiography also showed a decrease in contractility and dilatation of the left ventricle, both of which are characterized as LV remodeling in the ischemic heart.

The important novel findings of this study, in accordance with our previous reports on the rat LAD occlusion heart model and ischemic hind limb model [7–9], are that BMCI significantly accelerated the development of microvessels resulting in enhanced regional blood flow, and that the angiogenesis induced by BMCI treatment could prevent LV remodeling and restore normal cardiac function in the ischemic heart.

Angiopoietin-1 and VEGF were the most critical

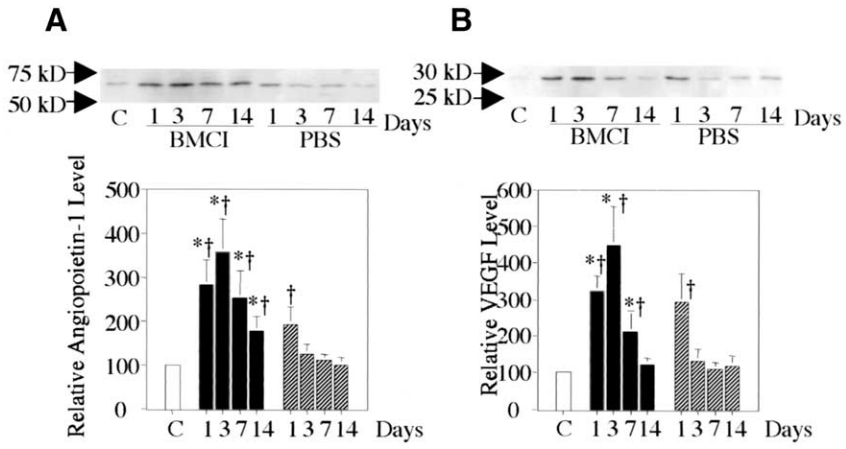

*Fig 6. Western blot analysis of (A) angiopoietin-1 and (B) vascular endothelial growth factor (VEGF) in the myocardium of the left ventricular anterior wall after treatment. Both were significantly higher in the bone marrow cell implantation (*BMCI*) group (*dark bars*) than in the phosphate buffered saline (*PBS*) group (*shaded bars*) after treatment. *$p < 0.01$ versus PBS group; †$p < 0.01$ versus normal myocardium. (C = control of normal myocardium.)*

growth factors for inducing angiogenesis. In this study, both the angiogenic growth factors were higher after BMCI treatment than after the injection of PBS alone. We assumed that the production of multiple angiogenic growth factors and the endothelial differentiation from these implanted bone marrow cells accelerated the development of microvessels, resulting in the enhanced regional blood flow. We also considered that the enhanced cardiac function after BMCI treatment mainly contributed to the increased blood flow in the LV anterior wall. Although previous studies found that cardiomyocytes were differentiated from bone marrow stem cells [19, 20], this differentiation would be insufficient to affect any increase in cardiac function.

According to past reports, impaired cardiac function can be improved by the implantation of bone marrow cells cultured with 5-azacytidine, but not by that of fresh bone marrow cells, although the implantation of fresh bone marrow cells induces angiogenesis [21]. These conflicting results could be related to differences in the experimental design or implanted cell number.

In conclusion, the implantation of autologous bone marrow cells is an effective method of inducing therapeutic angiogenesis in ischemic heart disease. BMCI is a simple treatment and easy to perform in clinical trials compared with other methods such as the direct injection or the gene transfer of angiogenic cytokines [22, 23].

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References

1. Schultz A, Lavie L, Hochberg I, et al. Interindividual heterogeneity in the hypoxic regulation of VEGF. Circulation 1999;100:547–52.
2. Lee SH, Wolf PL, Escudero R, Deutsch R, Jamieson SW, Thistlethwaite PA. Early expression of angiogenesis factors in acute myocardial ischemia and infarction. N Engl J Med 2000;342:626–33.
3. Brizzi MF, Formato L, Bonamini R. The molecular mechanisms of angiogenesis: a new approach to cardiovascular disease. Ital Heart J 2001;2:81–92.
4. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endotherial cells for angiogenesis. Science 1997;275:964–7.
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. Hamano K, Li TS, Kobayashi T, Kobayashi S, Matsuzaki M, Esato K. Angiogenesis induced by the implantation of self-bone marrow cells: a new material for therapeutic angiogenesis. Cell Transplant 2000;9:439–43.
7. Kobayashi T, Hamano K, Li TS, et al. Enhancement of angiogenesis by the implantation of self bone marrow cells in a rat ischemic heart model. J Surg Res 2000;89:189–95.
8. Ikenaga S, Hamano K, Nishida M, et al. Autologous bone marrow implantation induced angiogenesis and improved deteriorated exercise capacity in a rat ischemic hindlimb model. J Surg Res 2001;96:277–83.
9. Hamano K, Li TS, Kobayashi T, et al. The induction of angiogenesis by the implantation of autologous bone marrow cells: a novel and simple therapeutic method. Surgery 2001;130:44–54.
10. Yaoita H, Sakabe A, Maehara K, Maruyama Y. Different effects of carvedilol, metoprolol, and propranolol on left ventricular remodeling after coronary stenosis or after permanent coronary occlusion in rats. Circulation 2002;105:975–80.
11. Schwarz ER, Pollick C, Meehan WP, Kloner RA. Evaluation of cardiac structures and function in small experimental animals: transthoracic, transesophageal, and intraventricular echocardiography to assess contractile function in rat heart. Basic Res Cardiol 1998;93:477–86.
12. Litwin SE, Katz SE, Morgan JP, Douglas PS. Serial echocardiographic assessment of left ventricular geometry and function after large myocardial infarction in the rat. Circulation 1994;89:345–54.
13. Litwin SE, Raya TE, Anderson PG, Litwin CM, Bressler R, Goldman S. Induction of myocardial hypertrophy after coronary ligation in rats decreases ventricular dilatation and improves systolic function. Circulation 1991;84:1819–27.
14. Scheinowitz M, Kotlyar A, Zimand S, et al. Basic fibroblast growth factor induces myocardial hypertrophy following acute infarction in rats. Exper Physiol 1998;83:585–93.
15. Takeshita S, Isshiki T, Ochiai M, et al. Endothelium-dependent relaxation of collateral microvessels after intramuscular gene transfer of vascular endothelial growth factor in a rat model of hindlimb ischemia. Circulation 1998;98:1261–3.
16. Kowallik P, Schulz R, Guth BD, et al. Measurement of regional myocardial blood flow with multiple colored microspheres. Circulation 1991;83:974–82.
17. Pfeffer MA, Pfeffer JM, Fishbein MC, et al. Myocardial infarct size and ventricular function in rats. Circ Res 1979;44:503–12.
18. Pfeffer JM, Pfeffer MA, Fletcher PJ, Braunwald E. Progressive ventricular remodeling in rat with myocardial infarction. Am J Physiol 1991;260:H1406–14.
19. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001;410:701–5.
20. Jackson KA, Majka SM, Wang H, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 2001;107:1395–402.
21. Tomita S, Li RK, Weisel RD, et al. Autologous transplantation of bone marrow cells improves damaged heart function. Circulation 1999;100(Suppl II):247–56.
22. Losordo DW, Vale PR, Symes JF, et al. Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia. Circulation 1998;98:2800–4.
23. Sellke FW, Laham RJ, Edelman ER, Perlman JD, Simons M. Therapeutic angiogenesis with basic fibroblast growth factor: technique and early results. Ann Thorac Surg 1998;65:1540–4.

INVITED COMMENTARY

The study by Nishida and colleagues complements other studies published by this group and that of other groups which have examined the effects of implantation of bone marrow cells into the ischemic myocardium or ischemic hind limb of rats. The distinguishing aspect of the present study is that a novel method was used to create ischemic but not infarcted tissue. The authors used a thin copper wire as a guide to place a ligature around the left

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