produces relatively selective pulmonary vasodilation.[55] Earlier studies have shown that sildenafil improves pulmonary hemodynamics particularly in attenuating rebound pulmonary hypertension after discontinuing inhaled NO.[56,57] In addition, sildenafil has been shown to augment the effect of inhaled NO for postoperative pulmonary hypertensive crises.[58] Thus, sildenafil may have synergistic effects with inhaled NO through inhibition of cGMP degradation. A single oral dose of sildenafil is as effective and selective a pulmonary vasodilator as inhaled NO.[59] A recent study has shown that oral sildenafil is a potent pulmonary vasodilator that acts synergistically with inhaled iloprost to cause strong pulmonary vasodilatation in severe pulmonary arterial hypertension.[60] These results suggest that phosphodiesterase inhibition by sildenafil may be a novel therapeutic strategy for the treatment of PPH. It is necessary to examine whether long-term treatment with sildenafil improves survival of patients with PPH. There is an ongoing placebo-controlled trial using sildenafil in pulmonary arterial hypertension.

1.2 Anticoagulation

The presence of endothelial injury in the pulmonary vascular bed develops pulmonary thrombi. Histopathologic studies on lung biopsy specimens show *in situ* arteriolar thrombosis in one-third to one half of the patients with PPH, and these microthrombotic lesions likely contribute to progression of the disease.[61] There is no prospective, randomized, placebo-controlled study showing a beneficial effects of anticoagulation therapy. However, Fuster et al.[62] reported in a retrospective study that survival was better in 78 patients who received oral anticoagulants than in 37 patients who were not anticoagulated. Rich et al.[13] showed that in patients who do not benefit from calcium antagonists, warfarin increased survival at 1 year from 62–91% and survival at 3 years from 31–47%. Based on these two studies, warfarin should be used in all patients with PPH unless there is an absolute contraindication. The current recommendation has been to target an international normalized ratio of 2- to 2.5-fold greater than control, a level that provides effective anticoagulation with a minimal risk of bleeding. Whether heparin would be more efficacious than warfarin, based on its inhibitory effects on smooth muscle proliferation remains unknown.

1.3 Inotropic Agents and Diuretics

Efficacy of chronic long-term inotropic therapy as a treatment modality remains controversial. However, Rich et al. reported that digoxin produced a modest increase in cardiac output in patients with pulmonary hypertension and RVr failure, as well as a significant reduction in circulating norepinephrine.[63] No detectable effects of digoxin on baroreceptor responsiveness were apparent These results raise the possibility that digoxin has beneficia effects in patients with PPH, although long-term studies are neces sary to confirm a therapeutic effect of digoxin.

Diuretics may be useful in reducing the increased intravascula volume and hepatic congestion in patients with right heart failure Thus, diuretics generally afford symptom relief. However, th right ventricle is often highly dependent on preload, and care mus be taken to avoid excessive diuresis, which may lead to a decreas in cardiac output in patients with PPH.

1.4 Oxygen Therapy

Oxygen therapy has been demonstrated to improve quality o life and decrease mortality in patients with pulmonary hyperten sion secondary to chronic long-term respiratory insufficiency.[64,6 In some patients with pulmonary arterial hypertension, significan ventilation-perfusion mismatching occurs that which results i hypoxic vasoconstriction exacerbating the underlying pulmonar hypertension. Thus, supplemental low-flow oxygen may alleviat the arterial hypoxemia and attenuate pulmonary vasoconstrictio in some patients with PPH.

2. Noninvasive Assessment of Disease Severity

Accurate evaluation of both disease severity and the efficacy o vasodilator therapy is important in the management of patient with PPH. Mortality in patients with PPH is most closely asso ciated with RV hemodynamic function and can be characterize by means of an equation using three variables: mean pulmonar artery pressure, mean right atrial pressure, and cardiac index.[66,67 However, risk stratification by a simple, noninvasive, and repeat edly available method is desirable. In order to assess current statu of patients, we recommend repeated measurements of plasm brain natriuretic peptide (BNP),[68,69] serum uric acid (UA),[70,7 and the distance walked in 6 minutes.[72] Cardiopulmonary exer cise testing[73,74] and echocardiography[75,76] can also reflect dis ease severity and predict poor outcome in patients with PPH These noninvasive parameters may be helpful as part of th evaluation of treatment in patients with PPH and, in particular, as guide to the selection and timing for alternative therapies.

2.1 Plasma Brain Natriuretic Peptide Level

BNP is secreted predominantly from cardiac ventricles via constitutive pathway.[77] BNP production is enhanced by the de gree of myocardial stretch, damage, and ischemia in the ventri cle.[77,78] Thus, plasma BNP level has been used as a noninvasiv marker of left ventricular dysfunction and a prognostic indicator i a variety of patients with left-sided heart failure.[79,80] Recently, w

have shown that plasma BNP increases in proportion to the degree of RV dysfunction in pulmonary hypertension (figure 2).[68,69] Plasma BNP level correlated positively with total pulmonary resistance ($r = 0.79$, $p < 0.001$) and correlated negatively with RV ejection fraction ($r = -0.71$, $p < 0.001$). We have also shown that plasma BNP changes in association with chronic changes in hemodynamics, thereby serving as a potential indicator of the efficacy of vasodilator therapy in patients with PPH. More recently, we have demonstrated that high levels of plasma BNP, and in particular, a further increase in plasma BNP despite vasodilator therapy, may have a strong, independent association with increased mortality in patients with PPH.[69] Interestingly, patients with a supramedian level of BNP (> 180 ng/L) had a significantly lower survival rate than those with an inframedian level (figure 3). Thus, plasma BNP may serve as a noninvasive prognostic indicator of PPH, which may complement invasive standard prognostic markers.

2.2 Serum Uric Acid Level

Serum UA, the final product of purine degradation, has been shown to be increased in hypoxic states, such as chronic heart failure,[81,82] cyanotic congenital heart disease[83,84] and obstructive pulmonary disease.[85] Because tissue ischemia and hypoxia deplete adenosine triphosphate and promote degradation of adenine nucleotides to inosine, hypoxanthine, xanthine and UA,[86,87] increased serum UA levels may reflect impaired oxidative metabolism in such diseases. Recently, we and others[70,71] demonstrated that serum UA levels were significantly elevated in patients with PPH. Serum UA levels correlated negatively with cardiac output and correlated positively with total pulmonary resistance. Tissue hypoperfusion and hypoxia resulting from reduced cardiac output in severe PPH induce both overproduction of UA and impaired

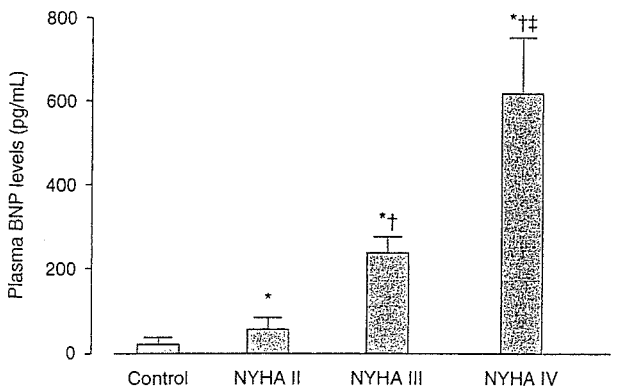

Fig. 2. Plasma BNP level in patients with PPH according to New York Heart Association (NYHA) functional class. **BNP** = brain natriuretic peptide; **PPH** = primary pulmonary hypertension. * indicates $p < 0.05$ versus control; † = $p < 0.05$ versus NYHA II; ‡ = $p < 0.05$ versus NYHA III.

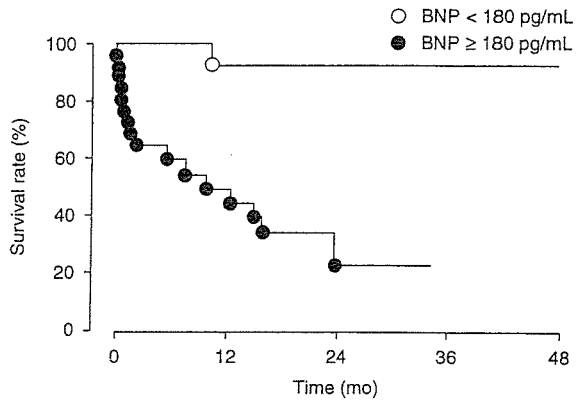

Fig. 3. Kaplan-Meier survival curves according to median value of plasma BNP levels in patients with PPH. **BNP** = brain natriuretic of peptide; **PPH** = primary pulmonary hypertension.[69]

UA excretion, leading to increased serum levels in patients with PPH. Interestingly, serum UA level significantly decreased with vasodilator therapy associated with a reduction in total pulmonary resistance. Patients with high serum UA had a significantly higher mortality rate than those with low serum UA.[70] These results suggest that serum UA increases in proportion to the clinical severity of PPH and has independent association with long-term mortality of patients with PPH.

2.3 Exercise Test

Cardiopulmonary exercise testing (CPX) allows reproducible assessment of functional capacity as well as ventilatory efficiency in patients with PPH.[73,74] Although CPX is a submaximal exercise test, most patients with PPH can safely undergo noninvasive cycle ergometer CPX to their maximal tolerance.[73] Reductions in peak O_2 uptake (peak $\dot{V}O_{2max}$), anaerobic threshold, peak O_2pulse, rate of increase in $\dot{V}O_{2max}$, and ventilatory efficiency were consistent and characteristic, and correlated well with NYHA class. Wensel et al.[74] have shown that CPX is a noninvasive prognostic substitute for hemodynamics. Patients with peak $\dot{V}O_{2max} \leq 10.4$ mL/kg/min and peak systolic blood pressure ≤120 mm Hg had poor survival rates at 12 months (23%), whereas patients with one or none of these risk factors had better survival rates (79% and 97%, respectively). These results suggest that peak $\dot{V}O_{2max}$ and peak systolic blood pressure are independent and strong predictors of survival in PPH patients.

The 6-minute walk test is a submaximal exercise test which can be performed even by a patient with heart failure not tolerating maximal exercise testing.[88,89] The test is very simple, requires inexpensive equipment, and is reproducible. It is considered safe because patients are self-limited during exercise. Six-minute walking distance correlated significantly with pulmonary hemodynamics and correlated strongly with peak oxygen consumption (exer-

cise capacity) determined by cardiopulmonary exercise testing.[72] Thus, the 6-minute walk test has been used as an indicator of the efficacy of vasodilator therapy in many studies.[7,34,43] In addition, we demonstrated that 6-minute walking distance was independently related to mortality in PPH by multivariate analysis. Patients walking <332m had a significantly lower survival rate than those walking farther, assessed by Kaplan-Meier survival curves.[72] Thus, the 6-minute walk test may serve not only as a potential marker for the efficacy of vasodilator therapy but also as a prognostic indicator in patients with PPH.

2.4 Echocardiography

Echocardiography is useful for the assessment of these patients. Recent studies have demonstrated prognostic value of echocardiography. Hinderliter et al.[75] have shown that pericardial effusion was noted in 43 of 79 patients (54%) with PPH. Larger effusion was associated with hemodynamic and echocardiographic evidence of right heart failure, impaired exercise tolerance, and a poor 1-year prognosis. Raymond et al.[76] have shown that pericardial effusion, right atrial enlargement, and septal displacement are echocardiographic abnormalities that reflect the severity of right heart failure and predict adverse outcomes in patients with severe PPH. These characteristics may help identify patients appropriate for more intensive medical therapy or earlier transplantation.

3. Future Perspectives

3.1 Adrenomedullin

Adrenomedullin is a potent vasodilator peptide that was originally isolated from human pheochromocytoma.[90] The vasodilating effect is mediated by cAMP- and NO-dependent mechanisms.[91,92] Immunoreactive adrenomedullin is detected in plasma and a variety of tissues, including blood vessels, heart, and lungs.[93,94] It has been reported that there are specific receptors for adrenomedullin in the lungs.[95] We have shown that plasma adrenomedullin level increases in proportion to the severity of pulmonary hypertension, and that circulating adrenomedullin is partially metabolized in the lungs.[96,97] These findings suggest that adrenomedullin plays an important role in the regulation of pulmonary vascular tone. In 2000, a randomized, placebo-controlled study by our group was the first to investigate the therapeutic use of adrenomedullin in the treatment of pulmonary arterial hypertension.[98,99] Short-term infusion of adrenomedullin produced a 44% increase in cardiac index and a 32% decrease in pulmonary vascular resistance with a 4% reduction in mean pulmonary arterial pressure in patients with PPH. Adrenomedullin also decreased plasma aldosterone levels without significant changes in plasma renin activity. These results suggest that intravenous infusion o adrenomedullin has beneficial hemodynamic and hormonal effect in patients with PPH.

3.2 Gene Therapy

Many researchers have already successfully transfected gene into bronchial epithelium, alveolar cells, and small pulmonary arteries by intratracheal delivery of genes using viral vectors. In 1999, Champion et al.[100] demonstrated that intratracheal transfe of the endothelial nitric oxide synthase (eNOS) gene selectively reduced pulmonary vascular resistance and pulmonary vasopressor responses to ET-1, angiotensin II, and hypoxia. Recently, they have shown that intratracheal gene transfer of calcitonin gene-related peptide to bronchial epithelial cells and alveolar cells attenuates chronic hypoxia-induced pulmonary hypertension in the mouse, suggesting lung cell transduction with a vasodilator peptide may be sufficient to alter vascular function.[101]

Earlier studies have shown that prostacyclin synthase (PGIS) deficiency in the lungs and impaired prostacyclin production are linked to the development of pulmonary hypertension in patients with PPH.[102,103] We demonstrated that intratracheal transfer of the PGIS gene augmented pulmonary prostacyclin synthesis, ameliorated monocrotaline-induced pulmonary hypertension, and thereby improved survival in rats.[104] These results suggest that gene therapy may hold great promise in the treatment of PPH. However, enormous hurdles still exist in the successful use of gene therapy in humans. Sustained expression is not yet possible, and concerns remain about an inflammatory reaction to the vectors. Therefore, the initial success of gene therapy should be confirmed by long-term experiments, and extensive toxicity studies in animals are needed before clinical trials.

4. Conclusions

Oral administration of calcium channel antagonists and intravenous infusion of epoproestenol are established as treatment of PPH. The dramatic success of long-term intravenous epoproestenol is now leading to the development of prostacyclin analogs using newer drug-delivery systems. Promising drugs including ET antagonists and type V phosphodiesterase inhibitors have been developed. Furthermore, gene therapy with eNOS gene or PGIS gene may hold great promise in the treatment of PPH. Accurate evaluation of disease severity and the efficacy of vasodilator therapy is important in the management of patients with PPH. In addition to invasive assessment by cardiac catheterization, we recommend repeated measurements of plasma BNP, serum UA, and the distance walked in 6 minutes. These noninvasive parameters may be helpful as part of the evaluation of treatment in

patients with PPH and, in particular, as a guide to the selection and timing for alternative therapies.

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Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis

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Departments of [1]Regenerative Medicine and Tissue Engineering, [3]Cardiac Physiology, and [6]Biochemistry, National Cardiovascular Center Research Institute, Osaka 565-8565; Departments of [2]Internal Medicine and [7]Cardiovascular Surgery, National Cardiovascular Center, Osaka; [4]Tissue Engineering Research Center, National Institute of Advanced Industrial Science and Technology, Hyogo; and [5]Cardiovascular Division, Kansai Rosai Hospital, Hyogo 660-8511, Japan

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Nagaya, Noritoshi, Takafumi Fujii, Takashi Iwase, Hajime Ohgushi, Takefumi Itoh, Masaaki Uematsu, Masakazu Yamagishi, Hidezo Mori, Kenji Kangawa, and Soichiro Kitamura. Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis. *Am J Physiol Heart Circ Physiol* 287: H2670–H2676, 2004. First published July 29, 2004; doi:10.1152/ajpheart.01071.2003.—Mesenchymal stem cells (MSCs) are pluripotent cells that differentiate into a variety of cells, including cardiomyocytes and endothelial cells. However, little information is available regarding the therapeutic potency of systemically delivered MSCs for myocardial infarction. Accordingly, we investigated whether intravenously transplanted MSCs induce angiogenesis and myogenesis and improve cardiac function in rats with acute myocardial infarction. MSCs were isolated from bone marrow aspirates of isogenic adult rats and expanded ex vivo. At 3 h after coronary ligation, 5×10^6 MSCs (MSC group, $n = 12$) or vehicle (control group, $n = 12$) was intravenously administered to Lewis rats. Transplanted MSCs were preferentially attracted to the infarcted, but not the noninfarcted, myocardium. The engrafted MSCs were positive for cardiac markers: desmin, cardiac troponin T, and connexin43. On the other hand, some of the transplanted MSCs were positive for von Willebrand factor and formed vascular structures. Capillary density was markedly increased after MSC transplantation. Cardiac infarct size was significantly smaller in the MSC than in the control group (24 ± 2 vs. $33 \pm 2\%$, $P < 0.05$). MSC transplantation decreased left ventricular end-diastolic pressure and increased left ventricular maximum dP/dt (both $P < 0.05$ vs. control). These results suggest that intravenous administration of MSCs improves cardiac function after acute myocardial infarction through enhancement of angiogenesis and myogenesis in the ischemic myocardium.

left ventricular end-diastolic pressure; cell transplantation; differentiation; homing

INTERRUPTION OF MYOCARDIAL blood flow leads to cardiomyocyte death (20). Although myocyte mitosis and the presence of cardiac precursor cells in adult hearts have recently been reported (6, 17), death of large numbers of cardiomyocytes results in the development of heart failure (16). Thus it would be desirable to induce angiogenesis and myogenesis for the treatment of ischemic heart disease.

Mesenchymal stem cells (MSCs) are pluripotent adult stem cells residing within the bone marrow microenvironment (11, 18). In contrast to their hematopoietic counterparts, MSCs have an adherent nature and are expandable in culture. MSCs can differentiate into not only osteoblasts, chondrocytes, neurons, and skeletal muscle cells but also vascular endothelial cells (19) and cardiomyocytes (23, 24). In vitro, MSCs have the potential to induce a neovascular response in murine Matrigel angiogenesis assay (2). In vivo, local MSC implantation induces therapeutic angiogenesis in a rat model of hindlimb ischemia (1). On the other hand, MSCs directly injected into the infarcted heart have been shown to induce myocardial regeneration and improve cardiac function (21). Stem or progenitor cells have been shown to circulate in peripheral blood and home to ischemic tissues (4). These results raise the possibility that intravenously administered MSCs participate in repair of the ischemic myocardium primarily by angiogenesis, which prevents apoptosis of native cardiomyocytes, and by direct regeneration of lost cardiomyocytes. However, little information is available regarding the therapeutic potential of systemically delivered MSCs for myocardial infarction.

Thus the purpose of this study was to investigate whether *1*) intravenously administered MSCs are able to engraft in the ischemic myocardium, *2*) transplanted MSCs induce angiogenesis and myogenesis after myocardial infarction, and *3*) transplantation of MSCs decreases infarct size and improves cardiac function.

METHODS

Animals. Male Lewis rats ($n = 70$) weighing 220–250 g were used in this study. These isogenic rats ($n = 8$) served as donors and recipients of MSCs to simulate autologous implantation. The Animal Care Committee of the National Cardiovascular Center approved the experimental protocol.

Model of myocardial infarction and cell transplantation. Fifty-one rats underwent ligation of the left coronary artery to produce myocardial infarction, as described previously (15). Briefly, after rats were anesthetized by injection of pentobarbital sodium (30 mg/kg body wt ip), they were artificially ventilated using a volume-regulated respirator. The heart was exposed via a left thoracotomy, and the left coronary artery was ligated 2–3 mm from its origin between the pulmonary artery conus and the left atrium using a 6-0 Prolene suture.

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At 3 h after coronary ligation, 40 rats survived (78% survival rate): 30 were randomized to receive an intravenous injection of MSCs (MSC group, $n = 14$) or PBS (control group, $n = 16$), and 10 received fluorescence-labeled MSCs for examination of MSC differentiation ($n = 5$) and incorporation ($n = 5$). Eleven rats underwent a sham operation consisting of thoracotomy and cardiac exposure but without coronary artery ligation. At 3 h after coronary ligation, we administered 5×10^6 MSCs/100 μl in PBS or PBS alone through a catheter inserted into the left jugular vein in ~30 s. The subsequent mortality for 4 wk was 25% in the control group and 14% in the MSC group. This protocol resulted in the creation of three groups: normal rats given PBS (sham group, $n = 11$), myocardial infarction rats given PBS (control group, $n = 12$), and myocardial infarction rats given MSCs (MSC group, $n = 12$).

Expansion of bone marrow MSCs. MSC expansion was performed according to previously described methods (18). Briefly, we killed the male Lewis rats and harvested the bone marrow by flushing the cavity of the femurs and tibias with PBS. Bone marrow cells were introduced into 100-mm dishes and cultured in α-MEM supplemented with 10% FBS and antibiotics. A small number of cells developed visible symmetrical colonies by *day 5–7*. Nonadherent hematopoietic cells were removed, and the medium was replaced. The adherent, spindle-shaped MSC population expanded to $>5 \times 10^7$ cells by approximately four to five passages after the cells were first cultured.

Flow cytometry. Adherent cells were analyzed by fluorescence-activated cell sorting (FACS SCAN flow cytometer, Becton Dickinson). Cells were incubated for 30 min at 4°C with the FITC-conjugated mouse monoclonal antibodies against rat CD34 (clone ICO-115, Santa Cruz Biotechnology) and CD45 and CD90 (clones OX-1 and OX-7, respectively, Becton Dickinson). FITC-conjugated hamster anti-rat CD29 monoclonal antibody (clone Ha2/5, Becton Dickinson) and rabbit anti-rat c-Kit polyclonal antibody (clone C-19, Santa Cruz Biotechnology) were used. Isotype-identical antibodies served as controls.

Echocardiographic studies. Echocardiographic studies were performed by an investigator blinded to treatment allocation 4 wk after coronary ligation. Two-dimensional targeted M-mode traces were obtained at the level of the papillary muscles using an echocardiographic system equipped with a 7.5-MHz phased-array transducer (SONOS 5500, Hewlett-Packard, Andover, MA). Anterior and posterior end-diastolic wall thickness and left ventricular (LV) end-diastolic and end-systolic dimensions were measured by the American Society for Echocardiology leading-edge method from at least three consecutive cardiac cycles. LV fractional shortening was calculated as follows: $(LVD_d - LVD_s)/LVD_d \times 100$, where LVD_d is LV diastolic dimension and LVD_s is LV systolic dimension. LV volume and ejection fraction were calculated on the basis of the Teichholtz formula.

Hemodynamic studies. Hemodynamic studies were performed 4 wk after coronary ligation. A 1.5-Fr micromanometer-tipped catheter (Millar Instruments) was inserted in the right carotid artery for measurement of mean arterial pressure. Then the catheter was advanced into the LV for measurement of LV pressure. Hemodynamic variables were measured using a pressure transducer (model P23 ID, Gould) connected to a polygraph. After completion of these measurements, the left and right ventricles were excised and weighed. Infarction size was determined as a percentage of the entire LV area, as reported previously (8). Briefly, incisions were made in the LV, so that the tissue could be pressed flat. The circumference of the entire flat LV and the visualized infarcted area, as judged from the epicardial and endocardial sides, was outlined on a clear plastic sheet. The difference in weight between the two marked areas on the sheet was used to determine infarction size and was expressed as a percentage of LV surface area.

Histological examination. To detect fibrosis in cardiac muscle, the LV myocardium ($n = 5$ each group) was fixed in 10% formalin, cut transversely, embedded in paraffin, and stained with Masson's trichrome. To detect capillary endothelial cells in the peri-infarct area, samples of the harvested muscle ($n = 5$ each) were embedded in OCT compound (Miles Scientific), snap frozen in liquid nitrogen, and cut into transverse sections. Tissue sections were stained for alkaline phosphatase with an indoxyltetrazolium method. The number of capillary vessels was counted in the peri-infarct area using a light microscope at ×200 magnification. The numbers in five high-power fields were averaged and expressed as the number of capillary vessels. These morphometric studies were performed by two examiners who were blinded to treatment.

An additional five rats were used to examine whether transplanted MSCs differentiated into cardiomyocytes or vascular endothelial cells. Suspended MSCs were labeled with fluorescent dyes with a PKH-26 red fluorescent cell linker kit (Sigma Chemical, St. Louis, MO) before implantation, as reported previously (13). Fluorescence-labeled MSCs were intravenously administered 3 h after coronary ligation. This subgroup of rats was killed 4 wk after coronary ligation. After the LV was excised and dissected free, muscle samples were embedded in OCT compound, snap frozen in liquid nitrogen, and cut into sections. Immunofluorescent staining for cardiac and endothelial cell markers was performed using monoclonal mouse antidesmin (Dako), anti-cardiac troponin T (Novo), anticonnexin43 (Sigma Chemical), and polyclonal rabbit anti-von Willebrand factor (Dako). FITC-conjugated IgG antibody (BD Pharmingen and Molecular Probes) was used as a secondary antibody.

At 24 h after intravenous administration of PKH-26-labeled MSCs, cardiac muscle was embedded in OCT compound and snap frozen in liquid nitrogen. Then the cardiac muscle from base to apex was

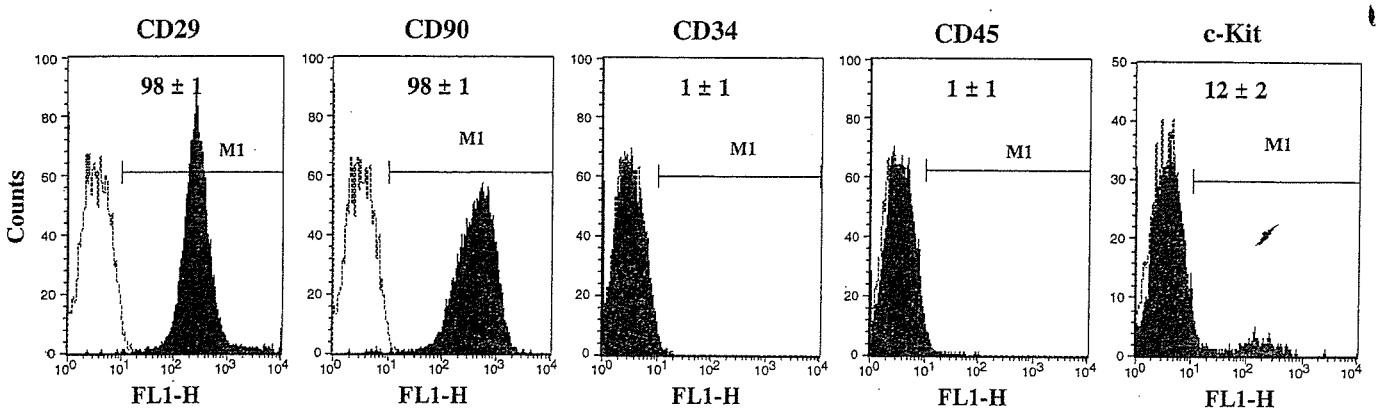

Fig. 1. Flow cytometric analysis of adherent, spindle-shaped mesenchymal stem cell (MSC) population expanded to 4–5 passages. Most of the cells expressed CD29 and CD90 but were negative for CD34 and CD45. Some cells were positive for c-Kit. MI, myocardial infarction.

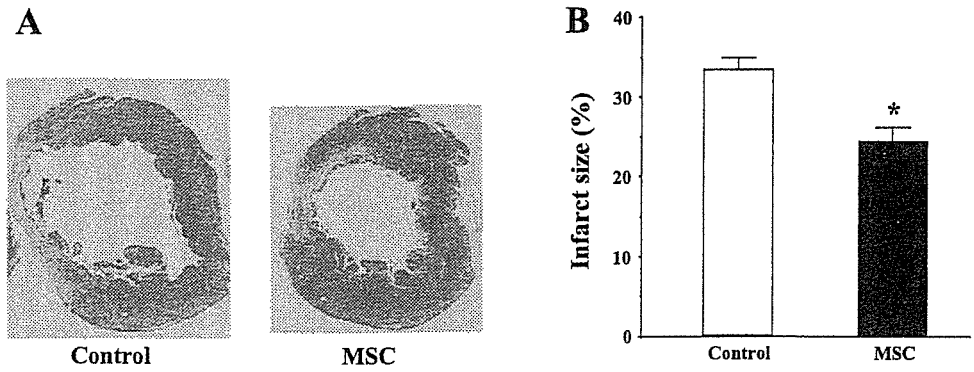

Fig. 2. Effect of MSC transplantation on myocardial infarct size 4 wk after myocardial infarction. *A*: representative Masson's trichrome-stained myocardial sections from control and MSC groups. *B*: quantitative analysis demonstrating that MSC transplantation significantly decreased infarct size. Values are means ± SE. $*P < 0.05$ vs. control.

transversely cut into 5-μm slices for calculation of the numbers of transplanted MSCs in the heart ($n = 5$).

Statistical analysis. Numerical values were expressed as means ± SE unless otherwise indicated. Comparisons of parameters among the three groups were made using one-way analysis of variance (ANOVA) followed by Scheffé's multiple comparison test. Comparisons of parameters between two groups were made by unpaired Student's *t*-test. $P < 0.05$ was considered significant.

RESULTS

Characterization of cultured MSCs. Most of cultured adherent cells expressed CD29 and CD90 (Fig. 1). In contrast, a majority of adherent cells were negative for CD34 and CD45. A small fraction of the adherent cells expressed c-Kit. Thus we confirmed that the major population of adherent cells was MSCs.

Reduction of myocardial infarct size after MSC transplantation. Moderate-to-large infarcts were observed in Masson's trichrome-stained myocardial sections 4 wk after coronary ligation (control group; Fig. 2*A*). However, MSC transplantation markedly decreased the infarct size after myocardial infarction (MSC group). Quantitative analysis also demonstrated that cardiac infarct size was significantly smaller in the MSC than in the control group: 24 ± 2 vs. 33 ± 2% ($n = 12$ each, $P < 0.05$; Fig. 2*B*).

Hemodynamic effects of MSC transplantation. At 4 wk after coronary ligation, hemodynamic studies were performed in the sham ($n = 11$), control ($n = 12$), and MSC ($n = 12$) groups. LV end-diastolic pressure showed a marked elevation in the control group (18 ± 1 mmHg); the elevation was significantly attenuated in the MSC group (13 ± 1 mmHg, $P < 0.05$; Fig. 3*A*). LV maximum dP/d*t* was significantly higher in the MSC than in the control group (Fig. 3*B*). LV minimum dP/d*t* tended to be lower in the MSC than in the control group (Fig. 3*C*). Although mean arterial pressure was significantly lower in the control than in the sham group, no decrease was observed in the MSC group (Table 1). Heart rate did not significantly differ among the three groups.

LV diastolic dimension was significantly smaller in the MSC than in the control group (Table 2). Fractional shortening was significantly greater in the MSC than in the control group (Fig. 3*D*). LV ejection fraction was also higher in the MSC than in

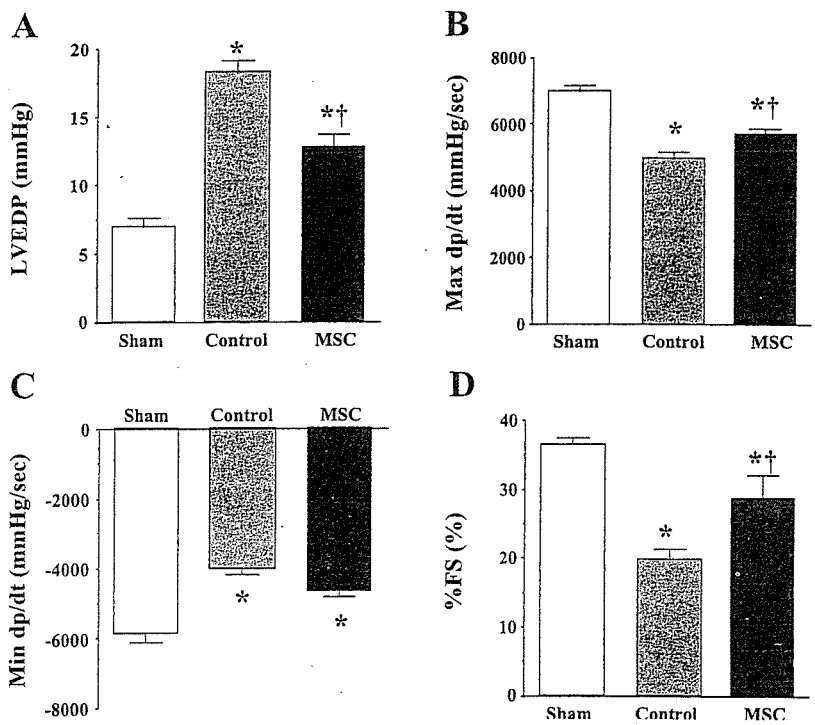

Fig. 3. Effects of MSC transplantation on hemodynamic parameters. LVEDP, LV end-diastolic pressure (*A*); max dP/d*t*, LV maximum dP/d*t* (*B*); Min dP/d*t*, LV minimum dP/d*t* (*C*); %FS, LV fractional shortening (*D*). Values are means ± SE. $*P < 0.05$ vs. sham. $†P < 0.05$ vs. control.

Table 1. *Characterization of animals*

	Sham (n = 11)	Control (n = 12)	MSC (n = 12)
Body wt, g	331±4	301±7*	321±7†
LV wt/body wt, g/kg	1.83±0.11	2.22±0.10*	2.17±0.09*
RV wt/body wt, g/kg	0.55±0.02	0.83±0.04*	0.71±0.03*†
Heart rate, beats/min	404±15	428±17	418±15
Mean arterial pressure, mmHg	128±2	113±4*	119±3

Values are means ± SE. Sham, sham-operated rats given vehicle; control, myocardial infarction rats given vehicle; MSC, myocardial infarction rats given mesenchymal stem cells; LV, left ventricle; RV, right ventricle. *P < 0.05 vs. sham. †P < 0.05 vs. control.

Table 2. *Echocardiographic data*

	Sham	Control	MSC
LVD_d, mm	6.3±0.1	8.6±0.2*	7.5±0.3*†
LVD_s, mm	4.0±0.1	6.9±0.3*	5.5±0.5*†
%FS, %	37±1	20±2*	29±3*†
LVEF, %	65±1	39±3*	53±5*†
AWT diastole, mm	1.6±0.1	1.1±0.1*	1.4±0.1†
PWT diastole, mm	1.6±0.1	1.7±0.1	1.7±0.1

Values are means ± SE. LVD_d, LV diastolic dimension; LVD_s, LV systolic dimension; %FS, LV fractional shortening; LVEF, LV ejection fraction; AWT, anterior wall thickness; PWT, posterior wall thickness. *P < 0.05 vs. sham. †P < 0.05 vs. control.

the control group (Table 2). Diastolic anterior wall thickness was significantly attenuated in the MSC group compared with the control group.

Myogenesis and angiogenesis induced by MSCs. Red fluorescence-labeled MSCs were intravenously administered 3 h after coronary ligation (n = 5). Semiquantitative analysis demonstrated that ~3% of the transplanted MSCs were incorporated into the heart 24 h after transplantation. At 4 wk after transplantation (n = 5), MSCs were incorporated predominantly into the border zone of infarcts (Fig. 4), whereas few MSCs were detected in the noninfarcted myocardium. Immunofluorescence analyses demonstrated that the engrafted MSCs were positive for desmin (Fig. 4), cardiac troponin T (Fig. 5*A*), and connexin43 (Fig. 5*B*). These results suggest the ability of MSCs to engraft in the ischemic myocardium and differentiate into cardiomyocytes. On the other hand, some of the transplanted MSCs were positive for von Willebrand factor and formed vascular structures (Fig. 6). Alkaline phosphatase staining of the ischemic myocardium showed marked augmentation of neovascularization in the MSC group (Fig. 7*A*). Quantitative analysis demonstrated that capillary density was significantly higher in the MSC than in the control group (n = 5 each; Fig. 7*B*).

DISCUSSION

In the present study, we demonstrated that intravenously administered MSCs were capable of engraftment in the ischemic myocardium and that the engrafted MSCs differentiated into cardiomyocytes and vascular endothelial cells, resulting in myogenesis and angiogenesis. We also demonstrated that MSC transplantation decreased myocardial infarct size and improved cardiac function after acute myocardial infarction in rats.

Earlier studies showed that MSCs directly injected into the myocardium or those injected into coronary arteries improve cardiac function after myocardial infarction. However, little information is available regarding the therapeutic potential of systemically delivered MSCs for myocardial infarction. This study demonstrated that intravenous administration of MSCs

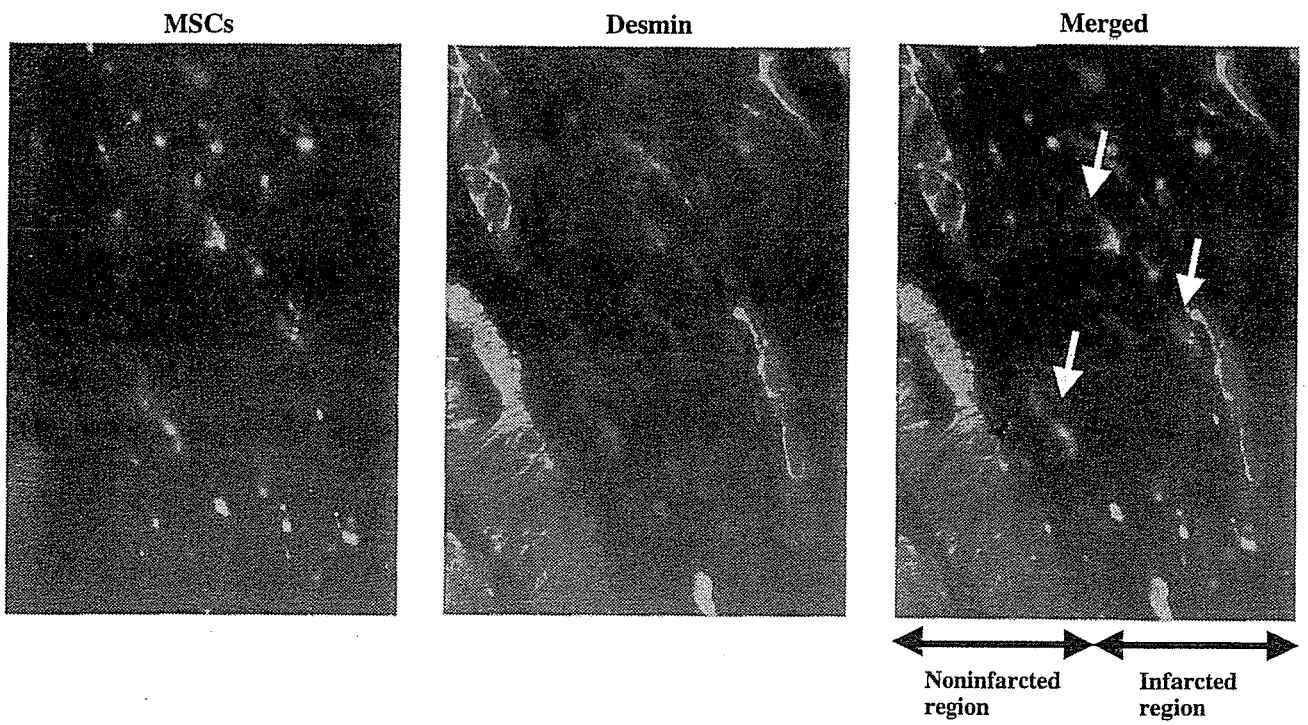

Fig. 4. Distribution of intravenously administered MSCs in myocardium after acute myocardial infarction. Red fluorescence-labeled MSCs were incorporated into ischemic boundary zone of the heart. These cells were positive for desmin (arrows), a cardiac marker. Magnification ×400.

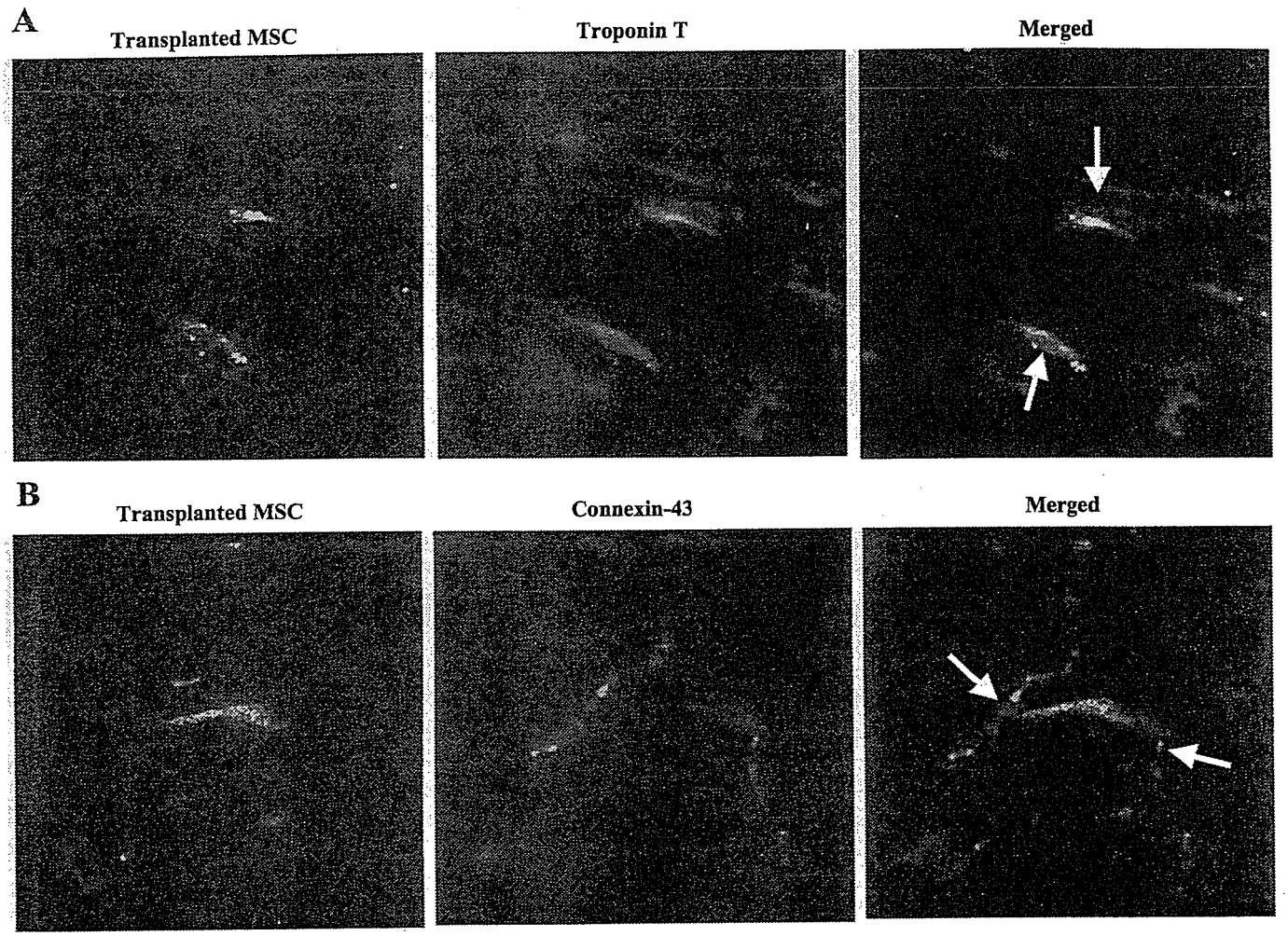

Fig. 5. Differentiation of transplanted MSCs in ischemic myocardium. Engrafted MSCs were positive (arrows) for cardiac troponin T (*A*) and connexin43 (*B*). Magnification ×400.

improves cardiac function after acute myocardial infarction through enhancement of angiogenesis and myogenesis in the ischemic myocardium.

Earlier studies showed that endothelial progenitor cells are mobilized from bone marrow into the peripheral blood in response to tissue ischemia and home to and incorporate into sites of neovascularization (21). Similar to epithelial progenitor cells, in the present study, transplanted MSCs were preferentially attracted to and retained in the border zone of infarcts. This is consistent with recent findings in the ischemic heart (5)

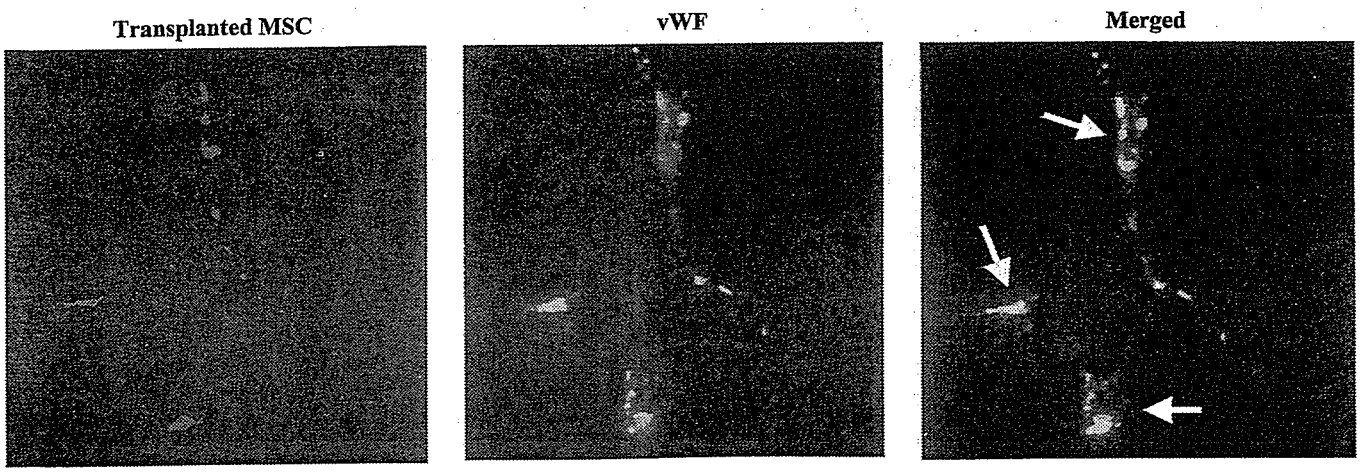

Fig. 6. Transplanted MSCs were positive for von Willebrand factor (vWF) and formed vascular structures. Magnification ×400.

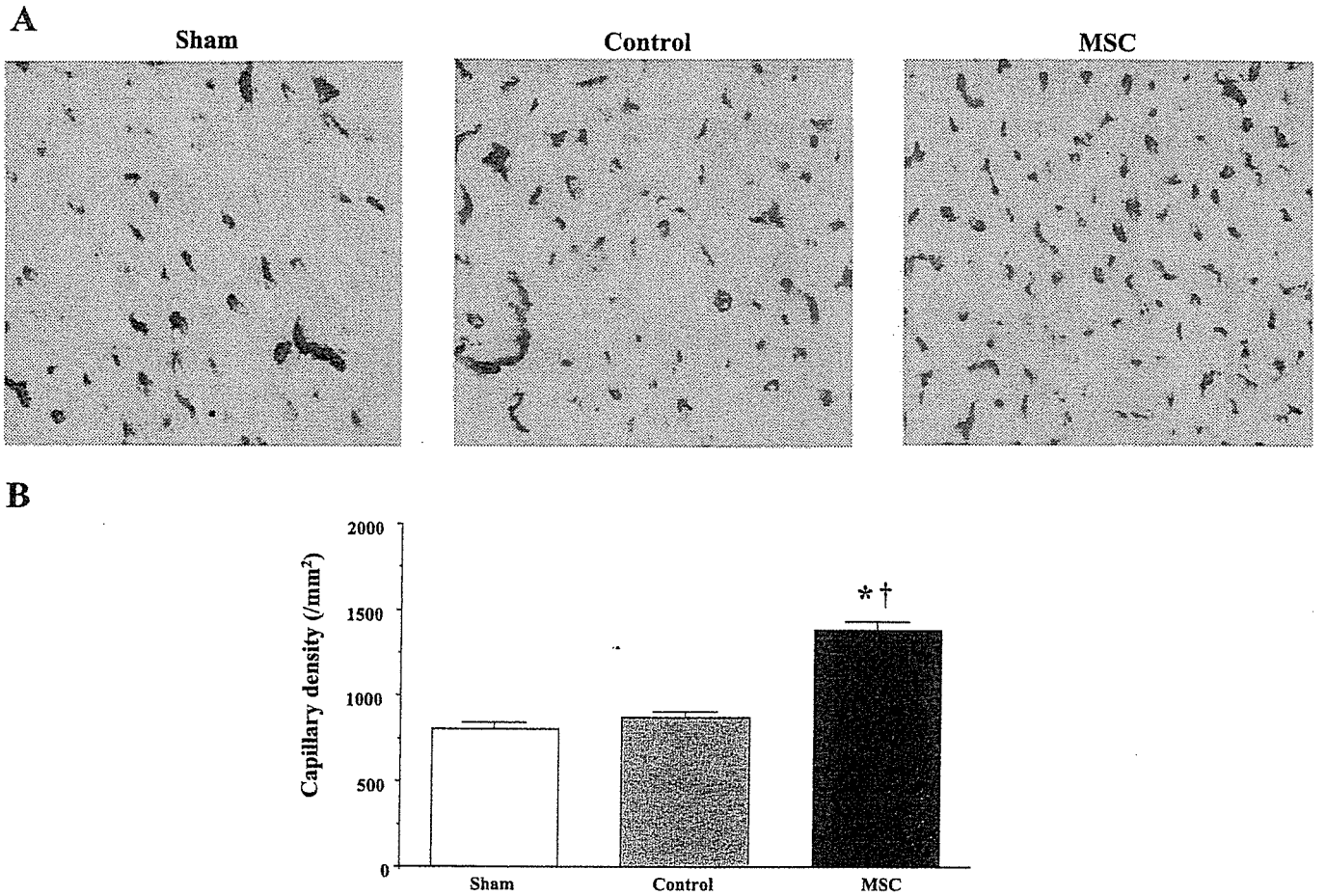

Fig. 7. *A*: representative samples of alkaline phosphatase staining in peri-infarct area. Magnification ×200. *B*: quantitative analysis of capillary density in peri-infarct area. Values are means ± SE. $*P < 0.05$ vs. sham. $\dagger P < 0.05$ vs. control.

or brain (7). Although the underlying mechanisms remain unclear, ischemic tissue may express specific receptors or ligands to facilitate trafficking, adhesion, and infiltration of MSCs to ischemic sites.

In the present study, some of the engrafted MSCs were stained by cardiac proteins such as desmin and cardiac troponin T. Transplanted MSCs also expressed connexin43, a gap junction protein, at contact points with native cardiomyocytes. These results suggest that MSCs differentiated into cardiomyocytes in the ischemic myocardium and formed connections with native cardiomyocytes. In contrast to skeletal myoblasts, which have been used as a tool for myocardial repair, MSCs may have the capacity for electromechanical coupling. Earlier studies demonstrated the importance of the microenvironment for cardiomyogenic differentiation. Possible factors might include direct cell-cell contact (9), electrical and mechanical stimulation (10), and unknown growth factors. On the other hand, recent studies showed that stem cells may fuse with existing native cells (22, 25). Although the mechanisms by which MSCs develop into cardiomyocyte-like cells remain unclear, it is possible that the direct attachment with host cardiomyocytes in the ischemic myocardium contributes to the cardiogenic differentiation of transplanted MSCs. Further studies are necessary to investigate whether engrafted MSCs are actually becoming contractile.

In the present study, some of the transplanted MSCs were positive for an endothelial cell marker and participated in vessel formation. MSC transplantation significantly increased the capillary density in ischemic myocardium. The recently reported phenotypic plasticity of MSCs to transform into endothelial-like cells provides a rationale for their potential role in neovascularization. Hypoxia has been shown to induce MSC migration and capillary-like structure formation by upregulation of membrane type 1 matrix metalloproteinase (3). MSC implantation has been shown to induce therapeutic angiogenesis in a rat model of chronic hindlimb ischemia (1). These findings support the theory that intravenously administered MSCs are able to differentiate into vascular endothelial cells in the ischemic myocardium. Interestingly, MSCs enhance angiogenesis partly by increasing endogenous levels of vascular endothelial growth factor and vascular endothelial growth factor type 2 receptor (7). Together, these findings suggest that MSCs may contribute to neovascularization in the ischemic myocardium not only through their ability to generate capillary-like structures and but also through growth factor-mediated paracrine regulation.

The present study showed that MSC transplantation significantly reduced infarct size and attenuated wall thinning after acute myocardial infarction. Cardiomyocyte apoptosis during ischemia is one of the major contributors to the development of myocardial infarcts (16, 20). It is possible that newly formed vessels after MSC transplantation improve tissue perfusion around the ischemic boundary zone, resulting in functional recovery after acute myocardial infarction. We also demonstrated that transplanted

MSCs differentiated into cardiomyocytes in the ischemic myocardium. These results suggest that the decrease in infarct size and the increase in wall thickness may be attributable not only to MSC-induced neovascularization but also to myocardial regeneration. In the present study, MSC transplantation improved cardiac function after acute myocardial infarction, as indicated by a significant decrease in LV end-diastolic pressure, a tendency for an increase in maximum LV dP/d*t*, and a decrease in minimum LV dP/d*t*. Thus MSC-induced angiogenesis and myogenesis and the resultant reduced infarct size may have contributed to the hemodynamic improvement after acute myocardial infarction.

The low percentage of MSC migration to the heart is in agreement with some previous studies (5, 14). The present study also showed that only a small percentage of transplanted MSCs were incorporated into the heart. This may be explained by MSC apoptosis (12), tracking in the lung (5), and a dilution of the fluorescent dyes as the cells reproduce. Nevertheless, when MSCs were intravenously administered in an acute phase of myocardial infarction, MSCs induced angiogenesis and myogenesis and modestly, but significantly, improved cardiac function. Thus systemic delivery of MSCs may be beneficial for the treatment of myocardial infarction.

A limitation of this study is that the cell population may be mixed, rather than limited to MSCs, although cell surface markers of cultured cells were consistent with those of previously reported MSCs (12, 18).

In conclusion, intravenously administered MSCs were preferentially attracted to the infarcted myocardium and differentiated into vascular endothelial cells and cardiomyocytes. MSC transplantation decreased the infarct size and improved cardiac function after acute myocardial infarction through enhancement of angiogenesis and myogenesis. Thus MSC transplantation may be a new therapeutic strategy for the treatment of myocardial infarction.

GRANTS

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Review

Adrenomedullin in the treatment of pulmonary hypertension

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Abstract

Adrenomedullin (AM) is a potent, long-lasting pulmonary vasodilator peptide. Plasma AM level is elevated in patients with primary pulmonary hypertension (PPH), and circulating AM is partially metabolized in the lungs. These findings suggest that AM plays an important role in the regulation of pulmonary vascular tone and vascular remodeling. We have demonstrated the effects of three types of AM delivery systems: intravenous administration, inhalation, and cell-based gene transfer. Despite endogenous production of AM, intravenously administered AM at a pharmacologic level decreased pulmonary vascular resistance in patients with PPH. Inhalation of AM improved hemodynamics with pulmonary selectivity and exercise capacity in patients with PPH. Cell-based AM gene transfer ameliorated pulmonary hypertension rats. These results suggest that additional administration of AM may be effective in patients with pulmonary hypertension. AM may be a promising endogenous peptide for the treatment of pulmonary hypertension.

Keywords: Adrenomedullin; Pulmonary hypertension; Inhalation; Gene therapy

Contents

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1. Introduction

Primary pulmonary hypertension (PPH) is a rare but life-threatening disease characterized by progressive pulmonary hypertension, ultimately producing right ventricular failure, and death [42,43]. Median survival is considered to be 2.8 years from the time of diagnosis. Because the presence of endothelial injury in the pulmonary vascular bed develops pulmonary vasoconstriction, smooth muscle cell proliferation, and in situ thrombosis [1], a variety of vasodilators, anti-proliferative agents, and anticoagulants have been proposed as therapeutic agents of PPH [3,10,23,45]. Despite therapeutic medical advances including prostacyclin therapy [3,23,45], some patients ultimately require heart–lung or lung transplantation [38,41]. Thus, a novel therapeutic strategy is desirable for the treatment of pulmonary hypertension including PPH.

Adrenomedullin (AM) is a potent, long-lasting vasodilator peptide that was originally isolated from human pheochromocytoma [19]. The peptide consists of 52 amino acids with an intramolecular disulfide bond, sharing slight homology with calcitonin gene-related peptide and amylin. Immunoreactive AM has subsequently been detected in plasma and a variety of tissues including blood vessels and lungs [13,47]. AM is metabolized by neutral endopeptidase protein in the kidney and by receptor binding in a variety of tissues. The half-life of AM is approximately 15 min. Earlier studies have shown that plasma AM level is increased in patients with hypertension [14] or heart failure [34]. Taking together its potent vasodilatory effect [19] and diuretic and natriuretic effects [21], AM may be involved in the regulation of the body fluid and thus in the cardiovascular homeostasis. We have shown that plasma AM level is elevated in patients with PPH, and that the plasma AM level increases in proportion to the severity of pulmonary hypertension [16]. It has been reported that there are abundant binding sites for AM in the lungs [37]. In fact, circulating AM is partially metabolized in the lungs [52]. These findings suggest that AM plays an important role in the regulation of pulmonary vascular tone and vascular remodeling. Earlier studies have shown that AM has a variety of biological effects, which are necessary for the treatment of pulmonary hypertension (Table 1). These actions of AM are mediated by calcitonin receptor-like receptor (CRLR) which functions as a selective AM receptor depending on the expression of the subtypes 2 and 3 of a family of receptor-activity-modifying proteins (RAMPs) [22]. AM acts through some signaling pathways: the cyclic adenosine 3′, 5′-monophosphate (cAMP), cyclic guanosine 3′, 5′-monophosphate (cGMP), phosphatidylinositol 3-kinase (PI3K)/Akt, and etc. These actions are induced by 0.01∼0.1 ug/(kg min) in vivo and by 10^{-10} to 10^{-7} M in vitro. This article will summarize the therapeutic potential of AM for the treatment of pulmonary hypertension.

2. Intravenous administration of AM

In vivo studies have shown that intravenously administered AM causes vasodilation, diuresis, and a positive inotropic effect in an experimental model of heart failure [40]. In humans, intravenous administration of AM decreases systemic and pulmonary vascular resistance and increases cardiac output in patients with congestive heart failure, together with slight increases in urine volume and urinary sodium excretion [31]. Endogenous AM production is enhanced in a variety of cardiovascular diseases through a compensatory mechanism [29]. Nonetheless, additional supplementation of AM has beneficial effects in these diseases [27]. These results suggest that endogenous AM level is not sufficient enough to improve deteriorated conditions in spite of the increased AM production.

Experimental studies have shown that intralobar arterial infusion of AM causes dose-related decreases in pulmonary vascular resistance under conditions of high pulmonary vascular tone [9,20,36]. The vasodilatory effect is mediated by cAMP-dependent and nitric oxide-dependent mechanisms [15,32]. Thus, AM is known to be one of the most potent endogenous vasodilators in the pulmonary vascular bed. However, little information is available regarding the hemodynamic effects of intravenously administered AM in patients with pulmonary hypertension. Accordingly, we examined the hemodynamic and hormonal responses to intravenous infusion of AM (0.05 μg/kg/min) or placebo, were examined in 13 patients with pulmonary arterial hypertension including PPH [28]. Because AM-induced hypotension

Table 1
Beneficial effects of adrenomedullin for the treatment of pulmonary hypertension

Biological activity	Second messenger or signal
1. Potent pulmonary vasodilation	cAMP, NO/cGMP, PI3K/Akt
2. Inhibition of endothelial cell apoptosis	PI3K/Akt
3. Inhibition of smooth muscle cell proliferation and migration	cAMP, Ca^{2+}
4. Positive inotropic effect	cAMP, protein kinase C, Ca^{2+} release or influx
5. Diuresis and natriuresis	NO/cGMP, cAMP
6. Suppression of aldosterone production	Ca^{2+}
7. Induction of angiogenesis	PI3K/Akt, MEK/ERK
8. Anti-inflammation	cAMP

cAMP: cyclic adenosine 3′, 5′-monophosphate, cGMP: cyclic guanosine 3′, 5′-monophosphate, PI3K: phosphatidylinositol 3-kinase, NO: nitric oxide, ERK: extracellular signal-regulated kinase, MEK: mitogen-activated protein ERK kinase.

may cause adverse effects in patients with pulmonary hypertension, we used a relatively low dose of AM. Intravenous infusion of AM increased plasma AM level in patients with pulmonary hypertension (15 ± 1 to 48 ± 8 fmol/ml, cf. 10 ± 1 fmol/ml in healthy subjects). Infusion of AM significantly decreased pulmonary vascular resistance by 32%. In addition, AM decreased systemic vascular resistance without inducing a marked hypotension. The hemodynamic effects of AM lasted at least 15 min after the end of infusion. These results suggest that AM has potent, relatively long-lasting pulmonary vasodilator activity in patients with pulmonary hypertension. We have shown that administered AM increases plasma cAMP, but not cGMP, in patients with pulmonary hypertension, in association with its hemodynamic effects. The increase in cAMP in smooth muscle cells by AM activates protein kinase A, resulting in the decrease in caicium content in smooth muscle cells. It is therefore possible that AM may relax vascular smooth muscle through a cAMP/protein kinase A-dependent mechanism. On the other hand, Nossaman et al. [36] have shown that AM regulates pulmonary vascular tone in rats through an endothelium-derived nitric oxide-dependent mechanism. Nishimatsu et al. [35] have shown that AM induces Akt activation in the endothelium via the Ca^{2+}/calmodulin-dependent pathway and that this is implicated in the production of nitric oxide, which in turn induced endothelium-dependent vasodilation. Because the vascular effects of AM are known to vary with species and vascular regions, further studies are necessary to elucidate the mechanisms responsible for pulmonary vasodilator activity of AM in humans.

Intravenous infusion of AM markedly increased cardiac index in patients with pulmonary hypertension [28], consistent with our previous results from left sided heart failure [31]. Considering the strong vasodilator activity of AM in the systemic and pulmonary vasculature, the significant decrease in cardiac afterload may be responsible for increased cardiac index with AM. On the other hand, a previous binding study has shown abundant, specific binding sites for AM in ventricular myocardium [37]. AM has been shown to increase cardiac cAMP [33], which is known to mediate the positive inotropic action of beta-adrenergic stimulants. Alternatively, AM has been shown to produce a positive inotropic action through cAMP-independent mechanisms [49]. These findings suggest that the increase in cardiac index may be attributable not only to a fall in cardiac afterload but also to the direct positive inotropic action of AM.

Infusion of AM significantly decreased plasma aldosterone, although there was no significant change in plasma renin activity. In vitro, AM has been shown to inhibit Ang II-induced secretion of aldosterone from dispersed rat adrenal zona glomerulosa cells [51]. Therefore, the inhibition of plasma aldosterone by AM was probably due to a direct effect on adrenal gland, as is the case for atrial natriuretic peptide [46].

It appears that a number of similarities in pharmacologic actions, i.e. vasodilatation, cardiac effect, and cAMP production, exist between AM and prostacyclin that is used for reducing pulmonary resistance in PPH. Unlike prostacyclin, however, AM has diuretic and natriuretic activities. AM inhibits inflammation and aldosterone production [7,51]. These biological effects may be the advantages of AM over prostacyclin in respect of therapeutic effectiveness. Exogenously administered AM at a pharmacologic level increased plasma cAMP in association with hemodynamic effects. Thus, additional administration of AM may be effective in patients with pulmonary hypertension.

3. Inhalation of AM

The goal of vasodilator therapy for patients with PPH is to reduce pulmonary vascular resistance without producing systemic hypotension, and improve quality of life and survival. We have shown that intravenous administration of AM markedly decreases pulmonary vascular resistance in patients with PPH [28]. Nevertheless, systemically administered AM decreases systemic arterial pressure, which may be harmful in treating patients with PPH. Recently, inhalation of aerosolized prostacyclin and its analogue, iloprost, has been shown to cause pulmonary vasodilation without systemic hypotension in patients with PPH [11,53]. In addition, inhalant application of vasodilators does not impair gas exchange because the ventilation-matched deposition of drug in the alveoli causes pulmonary vasodilation matched to ventilated areas. In clinical settings, inhalation therapy may be more simple, noninvasive, and comfortable than continuous intravenous infusion therapy. Thus, the purpose of this study was to investigate the effects of AM inhalation on hemodynamics and exercise capacity in patients with PPH.

Interestingly, Champion et al. [5] have shown that intratracheal gene transfer of calcitonin gene-related peptide (CGRP), a member of the same peptide family as AM, to bronchial epithelial cells attenuates chronic hypoxia-induced pulmonary hypertension in the mouse. These results raise the possibility that intratracheal delivery of a vasodilator peptide may be sufficient to alter pulmonary vascular function. In fact, inhalation of AM significantly decreased pulmonary vascular resistance in patients with pulmonary hypertension, whereas it did not alter systemic arterial pressure or systemic vascular resistance [26]. The ratio of pulmonary vascular resistance to systemic vascular resistance was significantly reduced by AM inhalation. These results suggest that inhaled AM improves hemodynamics with pulmonary selectivity. This is consistent with earlier findings that inhaled prostacyclin or its analogue, iloprost, acts transepithelially with pulmonary selectivity and improves pulmonary hypertension.

We examined the long-term effects of inhaled AM in monocrotaline (MCT)-induced pulmonary hypertension rats [30]. AM or saline was inhaled as an aerosol using an ultrasonic nebulizer, for 30 min, four times a day. Repeated

inhalation of AM for three weeks markedly decreased mean pulmonary arterial pressure and pulmonary vascular resistance in MCT rats without systemic hypotension. The potent, long-lasting pulmonary vasodilator effect of inhaled AM may contribute to the strong inhibition of the development of pulmonary hypertension. In addition, considering intermittent delivery of AM to the lungs, the chronic effects of inhaled AM appear to go beyond acute pulmonary vasodilation. Inhalation of AM inhibited an increase in the medial wall thickness of peripheral pulmonary arteries of MCT rats. In vitro studies have shown that AM inhibits the migration and proliferation of vascular smooth muscle cells [12,17]. Given the known potent vasoprotective effects of AM such as vasodilation and inhibition of smooth muscle cell migration and proliferation, it is interesting to speculate that AM trapped in the bronchial epithelium or alveoli leaks to the pulmonary arteries to maintain pulmonary vascular integrity in MCT rats. Importantly, Kaplan–Meier analysis demonstrated that the 6-week survival rate for MCT rats treated with aerosolized AM was significantly high (70%) as compared with 10% in those given saline [30]. Thus, treatment with aerosolized AM may be an alternative approach for severe pulmonary hypertension that is refractory to conventional therapy.

We have demonstrated that inhalation of AM has beneficial hemodynamic effects in animals and humans [26,30]. Recently, pulmonary delivery of a dry-powder insulin has been shown to improve glycemic control without adverse pulmonary effects [48]. Although further studies are necessary to maximize the efficiency and reproducibility of pulmonary AM delivery, combining AM inhalation therapy with other modalities that have a different mode of action may have beneficial effects in patients with PPH.

4. Cell-based AM gene transfer

The pulmonary endothelium plays an important role in the regulation of pulmonary vascular tone through the release of vasoactive substances such as nitric oxide and prostacyclin [6]. Dysfunction of the endothelium may play a role in the pathogenesis of pulmonary hypertension including PPH [4]. Thus, pulmonary endothelial cell may be a therapeutic target for the treatment of pulmonary hypertension. Recently, endothelial progenitor cells have been discovered in adult peripheral blood [2]. EPCs are mobilized from bone marrow into the peripheral blood in response to tissue ischemia or traumatic injury, migrate to sites of injured endothelium, and differentiate into mature endothelial cells in situ [8,18,50]. These findings raise the possibility that transplanted EPCs may serve not only as a tissue-engineering tool to reconstruct the pulmonary vasculature, but also as a vehicle for gene delivery to injured pulmonary endothelium. Thus, we investigated whether cell (EPCs)-based AM gene transfer ameliorates MCT-induced pulmonary hypertension in rats.

We obtained EPCs from cultured human umbilical cord blood mononuclear cells and constructed AM plasmid DNA. We used cationic gelatin to produce ionically linked DNA–gelatin complexes. Interestingly, EPCs phagocytosed plasmid DNA–gelatin complexes, which allowed nonviral, highly efficient gene transfer into EPCs [24]. Recently, intravenously administered hematopoietic cells have been shown to be attracted to sites of cerebral injury [39]. Intravenously injected EPCs accumulate in ischemic myocardium after acute myocardial infarction [18]. These findings suggest that progenitor cells have the capability to sense injured tissues. In fact, intravenously administered gene-modified EPCs were incorporated into pulmonary arterioles and capillaries in MCT rats and differentiated mature endothelial cells [25]. MCT injures endothelial cells of small arteries and capillaries in the lungs, resulting in pulmonary hypertension [44]. Taking these findings together, transplanted EPCs may circulate in the blood and attach to injured pulmonary endothelia in MCT rats. Thus, EPCs may serve not only as a vehicle for gene delivery to injured pulmonary endothelia, but also as a tissue-engineering tool in restoring intact pulmonary endothelium. Transplantation of EPCs without gene modification slightly, but significantly decreased pulmonary vascular resistance in MCT rats [25]. EPCs have been shown to express endothelial nitric oxide synthase and produce nitric oxide [24]. We showed that EPCs produce AM even when its gene is not transduced. These results suggest that vasodilator substances secreted from EPCs contribute to improvement in pulmonary hypertension. We also investigated whether transplantation of gene-modified EPCs causes further improvement in pulmonary hemodynamics and survival in MCT rats [25]. Interestingly, EPCs cultured with AM DNA–gelatin complexes markedly secreted AM protein for more than 2 weeks. These results suggest relatively long-lasting AM secretion from EPCs. The consequence of this synthesis in MCT rats was a marked decrease in mean pulmonary arterial pressure and pulmonary vascular resistance. Histological examination revealed that transplantation of AM-expressing EPCs inhibited an increase in medial wall thickness of pulmonary arteries. Expectedly, transplantation of AM-expressing EPCs caused significantly greater improvement in pulmonary hypertension and vascular remodeling than transplantation of EPCs alone. Given the known potent vasoprotective effects of AM such as vasodilation and inhibition of smooth muscle cell proliferation [12,17], it is interesting to speculate that AM secreted from EPCs may act not only as a circulating factor but also as a autocrine/paracrine factor in the regulation of pulmonary vascular tone and vascular remodeling in MCT rats. Importantly, a single transplantation of AM-expressed EPCs improved survival in MCT rats as compared with administration of EPCs alone or culture medium. These results suggest that ex vivo gene transfer into EPCs greatly enhances therapeutic effects of EPCs transplantation. Further studies are necessary to examine whether repeated administration of EPCs produces an even greater effect than single transplantation.

5. Summary

This article described the therapeutic potential of AM for the treatment of pulmonary hypertension. Baseline plasma AM is significantly higher in patients with pulmonary arterial hypertension. Nevertheless, exogenously administered AM at a pharmacologic level induces hemodynamic improvement. This suggests that an additional administration of AM may be effective in patients with pulmonary hypertension. We have demonstrated the effects of three types of AM delivery systems: intravenous administration of AM peptide, inhalation of AM peptide, and cell-based AM gene transfer. Further studies are necessary to examine which delivery system is the best in clinical settings. AM induces potent pulmonary vasodilation and has vasoprotective effects beyond vasodilation. Thus, AM is a promising endogenous peptide for the treatment of pulmonary arterial hypertension.

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Adrenomedullin enhances therapeutic potency of bone marrow transplantation for myocardial infarction in rats

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angiogenesis; apoptosis; mononuclear cell

DESPITE THE RECENT REMARKABLE progress in medical and surgical treatment for ischemic heart disease, this disease remains a major cause of death worldwide (5). Bone marrow-derived mononuclear cells (MNC) contain various kinds of cell lineages and numerous cytokines that contribute to neovascularization (1, 15). In fact, autologous transplantation of bone marrow cells has been shown to enhance angiogenesis and improve cardiac function in an animal model of cardiac ischemia (6, 9, 10). Recent human studies have demonstrated beneficial effects of transplanted MNC in patients with ischemic heart disease (23, 25). However, some patients fail to respond to this cell therapy. Thus a novel therapeutic strategy to enhance the angiogenic property of MNC is desirable.

Adrenomedullin (AM) is a potent vasodilator peptide that was originally isolated from human pheochromocytoma (8). We have shown that infusion of AM has beneficial hemodynamic and renal effects in patients with heart failure (17). On the other hand, AM has been shown to activate the phosphatidylinositol 3-kinase (PI3-kinase)/Akt-dependent pathway in vascular endothelial cells, which is considered to regulate multiple critical steps in angiogenesis including endothelial cell proliferation, migration, and capillary-like formation (14, 22). In fact, we have shown that AM gene transfer induces therapeutic angiogenesis in a rabbit model of hindlimb ischemia via activation of Akt (24). These findings suggest that AM may play an important role in the regulation of vascular regeneration. In addition, AM has been shown to exert an antiapoptotic effect on a variety of cells including vascular endothelial cells (7, 20). Taking these findings together, combination therapy with MNC transplantation and AM infusion may have additional or synergetic effects on therapeutic angiogenesis for the treatment of ischemic heart disease.

Thus the purposes of this study were *1*) to investigate whether infusion of AM enhances the angiogenic potency of MNC transplantation in a rat model of myocardial infarction, and *2*) to investigate the effects of AM on survival and differentiation of the transplanted MNC to examine the underlying mechanisms of the effects induced by AM.

MATERIALS AND METHODS

Animal model. Myocardial infarction was produced in male Lewis rats weighing 200–220 g by left coronary ligation. In brief, after rats were anesthetized by intraperitoneal injection of pentobarbital sodium (30 mg/kg body wt), they were ventilated artificially. The heart was exposed via left thoracotomy, and the left coronary artery was ligated 2–3 mm from its origin between the pulmonary artery conus and the left atrium using a 6-0 proline suture. Finally, the heart was restored to its normal position, and the chest was closed. The Animal Care Committee of the National Cardiovascular Center approved this experimental protocol.

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