Intratracheal mesenchymal stem cell administration attenuates monocrotaline-induced pulmonary hypertension and endothelial dysfunction

Syed R. Baber,* Weiwén Deng, Ryan G. Master, Bruce A. Bunnell, Bradley K. Taylor, Subramanyam N. Murthy, Albert L. Hyman, and Philip J. Kadowitz

Department of Pharmacology, Tulane University Health Sciences Center, New Orleans, Louisiana

Submitted 16 February 2006; accepted in final form 31 August 2006

Baber SR, Deng W, Master RG, Bunnell BA, Taylor BK, Murthy SN, Hyman AL, Kadowitz PJ. Intratracheal mesenchymal stem cell administration attenuates monocrotaline-induced pulmonary hypertension and endothelial dysfunction. Am J Physiol Heart Circ Physiol 292: H1120–H1128, 2007. First published September 15, 2006; doi:10.1152/ajpheart.00173.2006.—The administration of mesenchymal stem cells (MSCs) has been proposed for the treatment of pulmonary hypertension. However, the effect of intratracheally administered MSCs on the pulmonary vascular bed in monocrotaline-treated rats has not been determined. In the present study, the effect of intratracheal administration of rat MSCs (rMSCs) on monocrotaline-induced pulmonary hypertension and impaired endothelium-dependent responses were investigated in the rat. Intravenous injection of monocrotaline increased pulmonary arterial pressure and vascular resistance and decreased pulmonary vascular responses to acetylcholine without altering responses to sodium nitroprusside and without altering systemic responses to the vasodilator agents when responses were evaluated at 5 wk. The intratracheal injection of 3 × 10⁴ rMSCs 2 wk after administration of monocrotaline attenuated the rise in pulmonary arterial pressure and pulmonary vascular resistance and restored pulmonary responses to acetylcholine toward values measured in control rats. Treatment with rMSCs decreased the right ventricular hypertrophy induced by monocrotaline. Immunohistochemical studies showed widespread distribution of lacZ-labeled rMSCs in lung parenchyma surrounding airways in monocrotaline-treated rats. Immunofluorescence studies revealed that transplanted rMSCs retained expression of von Willebrand factor and smooth muscle actin markers specific for endothelial and smooth muscle phenotypes. However, immunolabeled cells were not detected in the wall of pulmonary vessels. These data suggest that the decrease in pulmonary vascular resistance and improvement in response to acetylcholine in endothelium-dependent vasodilator in monocrotaline-treated rats may result from a paracrine effect of the transplanted rMSCs in lung parenchyma, which improves vascular endothelial function in the monocrotaline-injured lung.

marrow stromal cells

PULMONARY HYPERTENSION (PH) is a difficult to treat disease characterized by increased pulmonary arterial pressure, right-heart dysfunction, lung vascular remodeling, and death (8, 12, 14, 37). Although a number of therapies, including intravenous PGI₂, have proven useful in decreasing pulmonary arterial pressure, improving exercise tolerance and quality of life, the long-term outcome in this disorder is bleak (2, 9, 25, 29). Lung transplantation has proven useful in the treatment of PH; however, lung graft rejection and infection are difficult problems, and the number of donor organs is limited (25). It has been shown that interventions, including gene therapy, have a beneficial effect in experimental models of PH (5–7, 30, 34). In addition, it has been shown that cell-based therapies with pulmonary arterial smooth muscle cells and with endothelial progenitor cells alone and transfected with therapeutic genes have a beneficial effect in experimental models of PH (3, 4, 31, 32, 38, 41). However, there is a paucity of information about the effect of local administration of mesenchymal stem cells (MSCs) in the treatment of PH. MSCs, also known as marrow stromal cells, are a type of adult stem cells from bone marrow (36). It has been shown that MSCs home to bleomycin-injured lung, albeit in small numbers, adopt an epithelial cell-like morphology, and decrease lung inflammation and collagen deposition (33). The engraftment of MSCs in the irradiated lung has been demonstrated (15, 21, 22). Engrafted cells in the lung persist for a long period, exhibit a high degree of plasticity, and retain their capacity for differentiation following gene transfer (15, 23, 24). Although previous studies show that MSCs are capable of differentiating into pulmonary lineage cells in the injured lung, there is little information about the effect of intratracheally administered MSCs on pulmonary vascular resistance and endothelium-dependent responses in monocrotaline-treated rats (15, 23, 24, 33). The present study was, therefore, undertaken to investigate the effect of intratracheal administration of rat MSCs (rMSCs) in monocrotaline-treated rats with PH and impaired pulmonary endothelium-dependent responses. This study examines the hypothesis that intratracheal administration of rMSCs will improve endothelium-dependent responses and attenuate monocrotaline-induced PH in rats.

MATERIALS AND METHODS

Experimental procedure. The experimental protocols used in these experiments were approved by the Institutional Animal Care and Usage Committee of Tulane University Medical Center, and all procedures were conducted in accordance with their guidelines. Monocrotaline (60 mg/kg iv, Sigma Aldrich) was injected 14 days before the rats were injected intratracheally with rMSCs. Pulmonary vascular responses to intravenous injections of acetylcholine and sodium nitroprusside were examined 3 wk after injection of the rMSCs. The rat lungs were then removed and stained in X-gal solution for β-galactosidase activity, which was used to assay the expression of the reporter gene ntlacZ in rMSCs and to determine the distribution of lacZ-labeled rMSCs in the lung of monocrotaline-treated rats. Immunofluorescence staining was used for the characterization of endothelial and smooth muscle cell-specific markers.

* S. R. Baber and W. Deng contributed equally to the work.

Address for reprint requests and other correspondence: P. J. Kadowitz, Dept. of Pharmacology SL83, Tulane Univ. Health Sciences Center, 1430 Tulane Ave., New Orleans, LA 70112 (e-mail: pkadowi@tulane.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Isolation and ex vivo expansion of rMSCs. rMSCs were isolated as previously described (10, 11). Six-week-old male Sprague-Dawley rats (Harlan) were euthanized with CO2. The femur and tibia were removed under sterile conditions and placed in culture medium for rMSCs [α-MEM ( Gibco In Vitrogen), 20% fetal bovine serum (Gibco In Vitrogen), 100 U/ml penicillin, 100 μg/ml streptomycin, 25 ng/ml amphotericin B (Atlanta Biologicals), and 2 mM L-glutamine (Gibco In Vitrogen)]. Both ends of the femur and tibia were removed, and the bone marrow was flushed out using an 18-gauge needle and a 5-ml syringe filled with rMSC culture medium. The bone marrow cells were filtered through a cell strainer with 70-μm nylon mesh (BD Bioscience), and the cells were plated in a 75-cm² culture flask. The cells were incubated in the culture medium for rMSCs at 37°C, with 5% humidified CO₂, and rMSCs were isolated by their tight adherence to cell culture flask. Fresh culture medium was added and replaced to remove nonadherent cells every 2 days. The adherent rMSCs were grown to 90% confluency (defined as rMSCs at passage 0). These were incubated for 5 min at 37°C, harvested with 0.25% trypsin/1 mM EDTA, diluted 1:3, transferred to culture flasks, and again grown to 90% confluency. The cells were then harvested with 0.25% trypsin/1 mM EDTA and diluted 1:2 per passage for further ex vivo expansion. For all the experiments rMSCs at passages 1 to 3 were used (10, 11).

Adenoviral vector. Ad5RSVntlacZ, a replication-deficient recombinant adenovirus carrying the nuclear-targeted β-galactosidase reporter gene nt lacZ under the control of Ro sv sarcoma virus (Rsv) promoter, was purchased from University of Iowa Gene Transfer Vector Core. The Ad5RSVntlacZ virus has a concentration of 1.4 × 10^12 viral particles/ml and a titer of 1.0 plaque-forming units/ml (10, 11).

Adenoviral transduction of rMSCs. rMSCs were transduced with adenovirus as previously described (10, 11). rMSCs were plated at a density of 10,000 cells/c㎡ in six-well plates or T75 cell culture flasks (BD Bioscience) and cultured overnight. The cells were then exposed to fresh culture medium containing Ad5RSVntlacZ at 300 multiplicities of infection (MOI, defined as plaque-forming units per cell) for 48 h. Cell viability was determined using the trypan blue exclusion method.

β-Gal staining for β-galactosidase activity. X-gal staining for β-galactosidase activity was used to assay the expression of nt lacZ in rMSCs (10, 11). Ad5RSVntlacZ-transduced rMSCs in six-well plates were rinsed with PBS, fixed in a PBS solution containing 2% formaldehyde and 0.2% glutaraldehyde (Sigma) for 5 min, washed with PBS twice, and incubated in an X-gal staining solution (5 mM K ferricyanide, 5 mM K ferrocyanide, 2 mM MgCl₂, and 1 mg/ml X-gal; Sigma) prepared in PBS at 37°C overnight in the dark. Cells were then washed with PBS, and the expression of the nt lacZ transgene in Ad5RSVntlacZ-transduced rMSCs was evaluated by light microscopic scoring of cells expressing nuclear-targeted β-galactosidase activity. The β-galactosidase-positive blue cells found in two to three microscopic fields (×25) were counted and expressed as a percentage of the total number of cells in those fields. Untreated rMSCs were used as negative controls (10, 11).

Monocrotaline administration. Monocrotaline (Sigma Aldrich) was dissolved in 1 M HCl neutralized with 0.5 N NaOH and diluted with PBS. The filtered solution was injected intravenously into the tail vein of male Sprague-Dawley rats weighing 200–250 g in a dose of 60 mg/kg.

Intratracheal injection of rMSCs and pulmonary hemodynamics. rMSCs were harvested with 0.25% trypsin/1 mM EDTA and washed with PBS, and a cell suspension of 6,000 cells/μl was prepared in PBS. The cell suspension was put on ice until the rat was anesthetized with pentobarbital sodium (30 mg/kg ip; Sigma Aldrich). Under sterile conditions, the trachea was exposed and a cell suspension of 60 μl of cell suspension was injected into the lung. Ad5RSVntlacZ-transduced rMSCs (3 × 10^6) were injected in each rat.

For histochemical studies, 3 wk after intratracheal injection of Ad5RSVntlacZ-transduced rMSCs, the rats were anesthetized with pentobarbital sodium (80 mg/kg ip; Sigma Aldrich), and the heart (right ventricle) was perfused with 200 ml of PBS. The lung was removed, fixed with 4% paraformaldehyde in PBS (USB) for 10 min, washed with PBS three times, and incubated in the X-gal staining solution at 37°C overnight in the dark. The lung was washed with PBS, fixed in 4% paraformaldehyde in PBS (USB) at 4°C overnight, and transferred to 30% sucrose in PBS (Sigma Aldrich) at 4°C and kept overnight. The tissue was embedded in OCT compound (Triangle Biomedical Sciences), snap-frozen in liquid nitrogen, and stored at −70°C. Ten-micron lung sections were prepared with a cryostat and mounted on Superfrost Plus slides (Fisher Scientific). The slides were viewed under a microscope, and β-galactosidase-positive blue cells were identified as the transplanted Ad5RSVntlacZ-transduced rMSCs that survived for 21 days and were counted in lung lobes from six rats. Lungs harvested from control rats were used as control tissue for this study.
For immunohistochemical studies, 3 wk after intratracheal injection of Ad5RSVntlacZ-transduced rMSCs, the rats were anesthetized with pentobarbital (80 mg/kg ip) and the heart (right ventricle) was perfused with 200 ml PBS. The lung was removed and fixed in 4% paraformaldehyde in PBS at 4°C overnight. The lung was then transferred to 30% sucrose in PBS at 4°C overnight. The tissue was embedded in OCT, snap-frozen in liquid nitrogen, and stored at −70°C. Forty-micron lung sections were prepared with a cryostat and double-immunostained with mouse anti-β-galactosidase monoclonal antibody (Promega) and rabbit anti-von Willebrand factor polyclonal antibody (Santa Cruz Biotechnology) or mouse anti-smooth muscle actin monoclonal antibody (Sigma) and rabbit anti-β-galactosidase polyclonal antibody (Cortex Biochem) at a dilution of 1:200 each. FITC-conjugated horse anti-mouse IgG (Vector Laboratories) and Rhodamine-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories) at a dilution of 1:200 each were used as secondary antibodies, and the sections were analyzed using a deconvoluted fluorescent microscope (Nikon TE 200E) and Metamorph software (Universal Imaging).

Pentobarbital was administered (80 mg/kg iv), the hearts were removed and dissected, and the right ventricle and left ventricle plus the septum were weighed (5, 6). The hemodynamic data are expressed as means ± SE. The data were analyzed by paired or group analysis or an analysis of variance with Dunnet’s post hoc test. The criterion for statistical significance was a P < 0.05.

RESULTS

Isolation and gene modification of rMSCs. Phase-contrast microscopic images or rMSCs isolated from Sprague-Dawley rats are shown in Fig. 1. Control rMSCs are shown in Fig. 1A, control rMSCs stained in X-gal solution are shown in Fig. 1B, and rMSCs transduced with Ad5RSVntlacZ at 300 MOI for 48 h are shown in Fig. 1C.

To determine whether markers specific for smooth muscle and endothelial cell phenotypes were expressed, the Ad5RSVntlacZ-transduced rMSCs were double immunostained. The results of
these experiments show that reporter gene transduced rMSCs stained positive for von Willebrand factor and for smooth muscle actin. The merged images in Fig. 2 show that rMSCs stain positive for von Willebrand factor and for β-galactosidase (β-gal) and the endothelial cell-specific marker von Willebrand factor (vWF) or for the smooth muscle cell-specific marker, smooth muscle actin (SMA). As shown in the merged images in the bottom panels, rMSCs express both nuclear targeted β-gal and vWF or SMA.

Effect of rMSCs on the response to monocrotaline. The protocol used in these experiments is shown in Fig. 3, and the effect of intratracheal administration of rMSCs on the pulmonary hypertensive response to monocrotaline is shown in Fig. 4. The injection of monocrotaline caused a significant increase in pulmonary arterial pressure when measured 5 wk after administration of the alkaloid. Mean pulmonary arterial pressure in monocrotaline-treated rats was 44 ± 6 and 18 ± 4 mmHg in untreated control rats at 5 wk (Fig. 4). Cardiac output decreased significantly and pulmonary vascular resistance was increased significantly in monocrotaline-treated rats at 5 wk (Fig. 4). Right ventricular weight was increased significantly 5 wk after administration of monocrotaline (Fig. 4). The intratracheal administration of $3 \times 10^6$ rMSCs 2 wk after injection of monocrotaline significantly attenuated the monocrotaline-induced increase in pulmonary arterial pressure and pulmonary vascular resistance when evaluated 5 wk after administration of the plant alkaloid (Fig. 4). The increase in right ventricular weight was significantly smaller in rats treated with rMSCs and monocrotaline than in animals treated with monocrotaline alone (Fig. 4).

Effect of monocrotaline and rMSCs on responses to vasodilator agents. The effect of monocrotaline treatment and intratracheal administration of rMSCs on pulmonary vascular responses to acetylcholine and sodium nitroprusside was investigated, and these data are shown in Fig. 5. In control rats, intravenous injections of acetylcholine and sodium nitroprusside in doses of 0.1–1.0 μg/kg caused dose-related decreases in pulmonary arterial pressure when baseline pulmonary arterial pressure was increased to 40–45 mmHg by U-46619 infusion.
The injections of acetylcholine and sodium nitroprusside (0.1–1 μg/kg iv) caused dose-related decreases in pulmonary arterial pressure in monocrotaline-treated rats when baseline pulmonary arterial pressure was 45 ± 6 mmHg and in monocrotaline plus rMSC-treated rats when baseline pulmonary arterial pressure was increased to 39–44 mmHg by U-46619 infusion. The decreases in pulmonary arterial pressure in response to intravenous injection of acetylcholine were significantly smaller in rats 5 wk after treatment with monocrotaline compared with responses in control animals when baseline pulmonary arterial pressure was increased to similar values (Fig. 5). The decreases in pulmonary arterial pressure in response to intravenous injection of acetylcholine were significantly greater in rats treated with monocrotaline and with rMSCs 2 wk after the alkaloid than in rats treated with monocrotaline alone when responses were compared at 5 wk (Fig. 5). Decreases in pulmonary arterial pressure in response to intravenous injections of sodium nitroprusside (0.1–1.0 μg/kg) were not significantly different in the three groups of rats when evaluated at 5 wk (Fig. 5).

**Fate of injected Ad5RSVnlacZ-transduced rMSCs.** The fate of rMSCs in the lung was investigated in rats treated with monocrotaline and 3 × 10⁶ Ad5RSVnlacZ-transduced rMSCs 2 wk after intravenous injection of the plant alkaloid. The rats were euthanized 3 wk later, and the lung was removed and stained in X-gal solution. The surface of lung from rats treated with the reporter gene-labeled rMSCs was stained blue and 1–8 β-galactosidase-positive rMSCs were detected in sections from lung lobes examined (2–3 sections from lobes of 6 rats) (Fig. 6). X-gal staining was not seen on the lung surface or in lung sections obtained from untreated control rats (Fig. 6).

**Immunofluorescence studies for endothelial and smooth muscle markers.** Immunofluorescence studies were carried out on lung tissue from monocrotaline-treated rats to identify lacZ-positive rMSCs staining positive for von Willebrand factor.
factor and for smooth muscle actin (Fig. 7). When Ad5RSVnlacZ-transduced rMSCs were injected into the trachea 2 wk after monocrotaline treatment and the rats were euthanized 3 wk later, widespread distribution of lacZ-positive rMSCs or small clusters of cells was seen in lung lobes. lacZ-positive rMSCs showed positive staining for von Willebrand factor and for smooth muscle actin (Fig. 7). The merged images in Fig. 7 show colocalization of lacZ and von Willebrand factor and colocalization of smooth muscle actin and lacZ, which is represented by the yellow color in Fig. 7, bottom (indicated by white arrows in the merged images).

**DISCUSSION**

The results of the present study show that intratracheal administration of rMSCs attenuates monocrotaline-induced PH in the rat. The mechanism by which rMSCs administration attenuates monocrotaline-induced PH is uncertain. However, 3 wk after injection of Ad5RSVnlacZ-transduced rMSCs, β-galactosidase-positive blue staining rMSCs were identified in sections from various areas of the lung. There was widespread distribution of reporter gene-labeled rMSCs and small clusters of cells after intratracheal injection, indicating that the transplanted cells are capable of surviving in the monocrotaline-injured lung for at least 21 days. Monocrotaline treatment has been reported to impair endothelium-dependent responses in isolated lung arteries and in the isolated perfused rat lung, although this has not been demonstrated in the intact animal (20, 26–28). In the present study, monocrotaline treatment attenuated the decrease in pulmonary arterial pressure in response to intravenous injections of acetylcholine when responses were compared with those in control animals at similar levels of baseline pulmonary arterial pressure. The observation that acetylcholine responses were decreased while responses to sodium nitroprusside were not altered in monocrotaline-treated animals when compared with responses in control rats suggests that pulmonary endothelial function is impaired (18). The administration of rMSCs 3 wk before pulmonary responses were evaluated in monocrotaline-treated rats resulted in a significant improvement in the response to acetylcholine. The data showing that decreases in pulmonary arterial pressure in response to acetylcholine in monocrotaline-treated rats are restored toward values measured in control animals while responses to sodium nitroprusside were not altered provide evidence in support of the hypothesis that a beneficial effect of rMSC treatment may involve an improvement in pulmonary vascular endothelial function (27, 28).

In the present study, responses to acetylcholine and sodium nitroprusside were compared at similar levels of pulmonary arterial pressure in the three groups of rats. It has been shown that responses to vasodilator agents are dependent on the existing level of baseline tone in the pulmonary vascular bed (18). It is therefore important to compare responses at similar levels of tone. Although monocrotaline and U-46619-induced increases in pulmonary arterial pressure may be mediated by different mechanisms, the observation that sodium nitroprusside produced similar decreases in pulmonary arterial pressure in monocrotaline and U-46619-treated animals suggests that responses to both interventions involve an active increase in vasoconstrictor tone and not an obstructive process in the pulmonary vascular bed.

The mechanism of improvement of pulmonary vascular endothelial function is unknown. It is, however, possible that transplanted rMSCs may repair injured vascular endothelium.
by an action involving the release of factors that improve endothelial function or stimulate vascular growth in the monocrotaline-injured lung (17, 21, 22, 24, 36, 38). To provide information on the localization of rMSCs, Ad5RSVnlacZ-transduced rMSCs were injected into the trachea of monocrotaline-treated rats and the lung was removed 3 wk later. The analysis of immunostained lung sections 21 days after administration demonstrated that the transplanted rMSCs in lung parenchyma surrounding airways stained positive for lacZ also stained positive for von Willebrand factor and for smooth muscle actin. The colocalization of stained images represented by yellow merged images for lacZ and for von Willebrand factor and lacZ and smooth muscle actin suggests that transplanted rMSCs retain expression of markers specific for endothelial and smooth muscle cell phenotypes (15, 21, 24). However, immunostained rMSCs were not detected in the wall of

Fig. 6. Histochemical examination for the presence of β-galactosidase staining of transduced rMSCs on day 35 in the lung of a control rat (A and C) and in a rat treated with monocrotaline on day 0 (B and D). 3 × 10^6 Ad5RSVnlacZ-transduced rMSCs were injected intratracheally on day 14 in both control and in monocrotaline-treated rats, and the lungs were harvested and examined on day 35. β-gal-positive staining rMSCs were counted in three sections from lobes in three control and six monocrotaline-treated rats.

Fig. 7. Immunohistochemical analysis of rMSCs in the rat lung. On day 35 after injection of monocrotaline and 21 days after intratracheal injection of 3 × 10^6 Ad5RSVnlacZ-transduced rMSCs into the rat with monocrotaline-induced pulmonary hypertension, the animals were euthanized, and lung sections were double-immunostained with antibodies for β-gal and for vWF or SMA. The deconvoluted microscopic images show that lacZ-positive rMSCs stain positive for vWF or SMA in the lung. Merged images in the bottom panels demonstrate colocalization of β-gal and vWF (bottom left) or SMA and β-gal (bottom right). The arrows denote merged images from double-positive stained cells.
pulmonary vessels, suggesting a potential role for a paracrine mechanism rather than for differentiation into vascular cells in the arterial wall. Although these experiments do not address issues, such as cell fusion, the present data suggest that the transplanted cells by an undefined mechanism may improve endothelial function by releasing factors which have not been identified that aid in the functional repair or regeneration of endothelial cells and improve endothelium-dependent responses (21, 22, 24, 33, 38–40).

Although it is our hypothesis that a paracrine mechanism in which constitutive release of factors from transplanted rMSCs in the lung microenvironment improves endothelial function, it is also possible that other mechanisms are involved. An alternative hypothesis may be that the rMSCs become “endothelialized” and release a vasodilator substance such as nitric oxide consequent to acetylcholine administration and the vasodilator substance diffuses to the media of the vessel.

The observation that intravenous injection of sodium nitroprusside can reduce pulmonary arterial pressure to near control value in monocrotaline-treated rats is similar to the observation in our laboratory showing that the nitric oxide donor can reverse the pulmonary hypertensive response to the nitric oxide synthase inhibitor N\(^{\text{G}}\)-nitro-L-arginine methyl ester (l-NMMA) (unpublished data). The present data with nitroprusside injections suggest that the pulmonary hypertensive response to monocrotaline can be reversed at the time it was examined and is similar to observations in l-NMMA-treated rats. The present data are consistent with the hypothesis that endothelial dysfunction resulting in decreased NO release may contribute to monocrotaline-induced PH in the rat (27, 28).

The pulmonary vascular response to sodium nitroprusside 5 wk after monocrotaline treatment suggests that smooth muscle cells are capable of responding to nitric oxide as shown previously (27). The defect in monocrotaline-induced PH has been reported to involve endothelial dysfunction and low nitric oxide bioavailability, and it has been shown that chronic nitric oxide donor aerosol administration attenuates monocrotaline-induced PH in the rat (16, 27, 28).

Cell-based therapies have been shown to have a beneficial effect in monocrotaline-induced PH (3, 4, 31, 32, 38, 41). In most studies, endothelial progenitor cells or smooth muscle cells have been administered by intravenous injection (3, 4, 31, 32, 41). In one study, endothelial progenitor cells were directly injected into the parenchyma of the lower lobes of the dog by using a bronchoscope-guided modified 27-gauge needle (38). The improvement in pulmonary vascular resistance caused by transplantation of endothelial progenitor cells in the dog lung was attributed to neovascularization (38). In studies in monocrotaline-treated rats, fluorescent-labeled endothelium-like progenitor cells engrafted in distal pulmonary arterioles and were incorporated into the endothelial lining of the vessel (3, 4, 41). The bone marrow-derived cells that incorporated into the pulmonary microvasculature prevented the increase in right ventricular systolic pressure (3, 4, 41). In addition, when bone marrow-derived cells were transfected with the endothelial nitric oxides synthase gene, a more significant reduction in right ventricular pressure was observed (41). In another study, unmodified human umbilical cord mononuclear cells and adenomethullin-modified cells were injected intravenously in monocrotaline-treated immunodeficient rats (31, 32). These cells were incorporated into the lung vasculature and produced a significant reduction in pulmonary vascular resistance (31, 32). In the present investigation, which to our knowledge is the first study to use airway administration of well-characterized rMSCs for the treatment of monocrotaline-induced PH, the rMSCs injected into the trachea were transplanted into lung parenchyma surrounding the airways. This treatment produced a significant decrease in pulmonary vascular resistance and improved pulmonary endothelium-dependent responses. The experiments in the present study utilize a treatment protocol in which the rMSCs were administered 2 wk after administration of monocrotaline at which time it has been reported that there is a significant increase in pulmonary arterial pressure and right ventricular weight (27, 28) and a significant decrease in the vasodilator response to acetylcholine in pulmonary arteries (26–28). It would be interesting to carry out future experiments in which monocrotaline and rMSCs are administered at the same time to determine whether rMSCs instillation halts, slows, or reverses the progression of monocrotaline-induced PH.

In conclusion, results of the present study show that intratracheal administration of syngeneic rMSCs decreases pulmonary arterial pressure and pulmonary vascular resistance and improves endothelium-dependent responses in monocrotaline-treated rats. The present data show widespread distribution of rMSCs and small clusters of cells in lung parenchyma and that the transplanted cells are capable of surviving in the monocrotaline-injured lung for at least 21 days. The transplanted rMSCs in lung parenchyma retain smooth muscle and endothelial cell markers. It is hypothesized that improvement in pulmonary vascular function may result from an undefined paracrine action of the transplanted cells, since lacZ-labeled rMSCs were not identified in the wall of pulmonary vessels. The release of growth factors or cytokines has been described when stem cells are transplanted into the microenvironment of injured tissue and serve as biological catalysts for tissue repair and regeneration (16, 21). Future studies with transplanted rMSCs in the lung will provide new information about the mechanism by which stem cell therapy may have a beneficial effect in the treatment of PH disorders (7, 13, 19, 30, 34, 35).

ACKNOWLEDGMENTS

The authors thank Janice Ignarro for editorial assistance.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grants HL-62000 and HL-77421.

REFERENCES

5. Champion HC, Bivalacqua TJ, D’Souza FM, Ortiz LA, Jeter JR, Toyoda K, Heistad DD, Hyman AL, Kadowitz PJ. Gene transfer of


21. Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, Epstein SE. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res 94: 678–685, 2004.


