**Adrenomedullin Regenerates Alveoli and Vasculature in Elastase-induced Pulmonary Emphysema in Mice**

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**Rationale:** Adrenomedullin, a potent vasodilator peptide, regulates cell growth and survival. However, whether adrenomedullin contributes to lung regeneration remains unknown. **Objectives:** To investigate whether adrenomedullin influences the kinetics of bone marrow cells, and whether adrenomedullin promotes regeneration of alveoli and vasculature and thereby improves lung structure and function in elastase-induced emphysema in mice. **Methods:** Adrenomedullin or vehicle was randomly administered to C57BL/6 mice for 5 days. We counted the numbers of mononuclear cells and stem cell antigen-1–positive cells in circulating blood. After intratracheal injection of elastase or saline, mice were randomized to receive continuous infusion of adrenomedullin or vehicle for 14 days. Functional and histologic analyses were performed 28 days after treatment. **Results:** Twenty-eight days after elastase injection, destruction of the alveolar walls was observed. However, adrenomedullin infusion significantly inhibited the increase in lung volume, static lung compliance, and mean linear intercept in mice given elastase. Adrenomedullin increased the numbers of mononuclear cells and stem cell antigen-1–positive cells in circulating blood. Adrenomedullin significantly increased the number of bone marrow–derived cells incorporated into the elastase-treated lung. Some of these cells were positive for cytokeratin or von Willebrand factor. Infusion of adrenomedullin after the establishment of emphysema also had beneficial effects on lung structure and function. **In vitro**, addition of adrenomedullin attenuates elastase-induced cell death in alveolar epithelial cells and endothelial cells. **Conclusions:** Adrenomedullin improved elastase-induced emphysema at least in part through mobilization of bone marrow cells and the direct protective effects on alveolar epithelial cells and endothelial cells.

**Keywords:** bone marrow cells; differentiation; mobilization; regeneration

Pulmonary emphysema, a major cause of respiratory dysfunction and death worldwide, is defined as abnormal permanent enlargement of airspaces distal to terminal bronchioles (1–3). Because many mediators with overlapping actions are involved in pulmonary emphysema, there is no effective treatment that prevents the progression of this disease. Destruction of the alveolar walls, one of the pathologic changes in pulmonary emphysema, had been considered irreversible. However, recent studies have demonstrated that bone marrow cells are mobilized into the peripheral blood to be involved in regeneration of alveoli (4, 5). Thus lung regeneration by bone marrow cells may be desirable for the treatment of pulmonary emphysema.

Adrenomedullin (AM) is a potent vasodilator peptide that was originally isolated from human pheochromocytoma (6). Immunoreactive AM has subsequently been detected in a variety of tissues, including the lungs (7, 8). The AM receptor has been shown to be expressed strongly in the basal cells of the airway epithelium and type II pneumocytes, both of which are involved in epithelial regeneration of the lung (9). Recent studies have shown that AM activates the phosphatidylinositol 3-kinase (PI3K)/Akt-dependent pathway in vascular endothelial cells, which is considered to regulate multiple critical steps in cell growth (10–13). These findings raise the possibility that AM may play an important role in the regulation of pulmonary homeostasis. However, whether AM is involved in lung regeneration remains unknown.

Thus the purposes of the present study were to (1) investigate whether infusion of AM influences the kinetics of bone marrow cells, (2) investigate the direct effects of AM on alveolar epithelial cells and endothelial cells, and (3) examine whether AM promotes regeneration of alveoli and vasculature in the lung and thereby improves lung structure and function in elastase-induced emphysema in mice.

**METHODS**

**Animal Model**

We used 10-week-old female C57BL/6 mice. To investigate whether AM influences the kinetics of bone marrow cells, AM or saline was randomly administered to 48 mice. Transgenic mice (C57BL/6 background) that ubiquitously express green fluorescent protein (GFP) were provided by Prof. Masaru Okabe (Osaka University, Japan) (14). To assess the kinetics of bone marrow cells, 10 GFP-positive bone marrow chimeric mice were established. Four weeks after bone marrow transplantation, the chimeric mice were given intratracheal injection of porcine pancreatic elastase (200 units/kg; Sigma, St. Louis, MO). An additional 42 wild-type mice were used to examine whether AM improves lung structure and function in elastase-induced emphysema. Finally, 10 GFP-positive bone marrow chimeric mice and 42 wild-type mice were used to examine the effects of AM on established emphysema. All protocols were performed in accordance with the guidelines of the Animal Care Ethics Committee of the National Cardiovascular Center Research Institute.

**AM Preparation and Administration**

Recombinant human AM was obtained from Shionogi Co., Ltd., Osaka, Japan. The homogeneity of AM was confirmed by reverse-phase, high-performance liquid chromatography and amino acid analysis. Forty-two wild-type mice were randomly given intratracheal injection of either elastase or saline and assigned to receive continuous infusion of AM or vehicle. AM was administered by a subcutaneous osmotic minipump (Alzet minipumps #1002; Durect Corp., Cupertino, CA) with a delivery rate of 0.05 µg/kg/minute for 14 days. This protocol resulted in the creation of three groups: sham mice given vehicle (sham group, n =
Isolation of Mononuclear Cells and Flow Cytometric Analysis

AM (1, 2, or 5 μg in 100 μl saline) was randomly administered to C57BL/6 mice (n = 8 each) by intraperitoneal injection daily for 5 days. Control mice received 100 μl saline according to the same schedule (n = 8). In addition, AM or vehicle was administered to other mice (n = 8 each) by a subcutaneous osmotic minipump with a delivery rate of 0.05 μg/kg/minute for 5 days. Blood samples were obtained on Day 5 and mononuclear cells were separated using Histopaque-1083 (Sigma), as described previously (16). The mononuclear cells were counted manually and analyzed for the expression of stem cell antigen-1 (SEA-1)-fluorescein isothiocyanate (BD Pharmingen, San Diego, CA). Immunofluorescence-labeled cells were analyzed by quantitative flow cytometry with a FACSCalibur flow cytometer (BD Biosciences, Mountain View, CA). An isotype-identical antibody (BD Pharmingen) served as a control.

Bone Marrow Chimeric Mice

Bone marrow chimeric mice were created as described previously (17). In brief, wild-type recipient C57BL/6 mice were lethally irradiated (900 cGy) and transplanted with GFP mouse-derived bone marrow cells (3 × 10^6 cells/300 μl) via the tail vein. To quantify the reconstitution of mouse bone marrow, peripheral blood mononuclear cells and bone marrow cells were analyzed by flow cytometry 4 weeks after bone marrow transplantation.

Assessment of Bone Marrow Cell Homing and Differentiation

Cryosections were obtained from the lungs of GFP-positive chimeric mice 28 days after elastase injection. The number of GFP-positive cells in the alveolar walls was counted and expressed as the number per 100 alveoli. Alveolar epithelial cells were identified using a murine monoclonal antibody anti-cytokeratin 5 and 8 (Chemicon, Temecula, CA) and a goat Alexa fluor 633–conjugated antimouse antibody (Molecular Probes, Eugene, OR) (4). Vascular endothelial cells were identified using a rabbit polyclonal antibody raised against von Willebrand factor (vWF; Dako, Copenhagen, Denmark) and a goat Alexa fluor 633–conjugated antirabbit antibody (Molecular Probes). The numbers of GFP/cytokeratin double-positive cells and GFP/vWF double-positive cells were counted and expressed as the number per 100 alveoli. Histologic examinations were performed in a blinded fashion by three observers.

Morphologic and Functional Analyses

Twenty-eight days after elastase or saline injection, the mice were paralyzed and static lung compliance was measured using a computer-controlled small animal ventilator (flexiVent; Scireq, Montreal, PQ, Canada; n = 7 each). The lungs were removed and fixed at a constant transpulmonary pressure of 25 cm H2O for 24 hours (n = 7 each). The lung volume was measured by the method of Scherle (18). The mean linear intercept, a morphometric parameter of emphysema, was calculated by light microscopy on 20 randomly selected fields as described previously (19). Morphologic examinations were performed in a blinded fashion by three observers.

Assessment of Vascular Density

Paraffin sections were obtained from the lungs (n = 7 each). To investigate whether AM induces angiogenesis in the elastase-treated lung, tissue sections were stained for vWF (20). The number of vWF-positive vessels per millimeter squared was counted in a blinded fashion by three observers.

Assessment of Cell Proliferation

Cryosections were obtained from the lungs of GFP-positive chimeric mice. To investigate whether AM promotes cell proliferation, tissue sections were stained for Ki-67, a marker for cell proliferation, using a rat antimouse Ki-67 antibody (Dako) and a goat Alexa fluor 633–conjugated anti-rat antibody (Molecular Probes). The number of Ki-67–positive cells per millimeter squared was counted in a blinded fashion by three observers.

In Vitro Study

A549 human alveolar type II epithelial cells (American Type Culture Collection, Rockville, MD) were cultured in F-12K medium (Invitrogen Corp., Carlsbad, CA) supplemented with 10% fetal calf serum (Invitrogen Corp.) and 1% penicillin-streptomycin (Invitrogen Corp.). Human umbilical vein endothelial cells (HUVEC) (Cambrex Corp., East Rutherford, NJ) were cultured in Medium 199 (Invitrogen Corp.) supplemented with 20% serum and 1% penicillin-streptomycin. To investigate whether AM attenuates elastase-induced cell death, cells were treated with elastase (0.3 units/ml) along with various concentrations of AM (10^-10 to 10^-7 M) (21). Twenty-four hours after elastase administration, cell number and DNA fragmentation were assessed. Cell number was assessed using a CellTiter 96 aqueous one solution cell proliferation assay kit (Promega, Madison, WI) according to the manufacturer’s directions (n = 4 each). DNA fragmentation was detected by terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) assay with a commercially available kit (ApopTag; Chemicon). The cells were then mounted in medium containing 4′,6-diamidino-2-phenylindole. The ratio of TUNEL-positive cells to total cells was calculated by counting at least 300 cells/well (n = 4 each). Finally, 6 hours after elastase administration, caspase-3 activity was measured with a commercially available kit (Promega) according to the manufacturer’s directions (n = 4 each).

Assessment of Nitric Oxide Release and Detection of Soluble Kit-Ligand

In vitro, murine bone marrow stromal cells were prepared, as reported previously (26). Bone marrow stromal cells were cultured in Dulbecco’s modified Eagle medium (Invitrogen Corp.) supplemented with 10% serum and 1% penicillin-streptomycin. The cells were then treated with AM for 20 minutes with control buffer, AM, or AM plus wortmannin (5 × 10^-7 M) (21). Twenty-four hours after elastase administration, the ratio of TUNEL-positive cells to total cells was calculated by counting at least 300 cells/well (n = 4 each). Finally, 6 hours after elastase administration, caspase-3 activity was measured with a commercially available kit (Promega) according to the manufacturer’s directions (n = 4 each).

Measurement of Vascular Endothelial Growth Factor

To investigate the effect of AM on endogenous production of vascular endothelial growth factor (VEGF) in the elastase-injected mice, serum VEGF concentration was measured using a mouse VEGF ELISA kit (R&D Systems; n = 7 each).

Statistical Analysis

Numeric values were expressed as mean ± SEM unless otherwise indicated. Comparisons were made by one-way analysis of variance, followed by Newman-Keuls’ test. A value of p < 0.05 was considered statistically significant.

RESULTS

Mobilization of Bone Marrow Cells

Administration of AM dose-dependently increased the number of peripheral blood mononuclear cells on Day 5 (Figure 1A).
Figure 1. (A) Effect of bolus injection of adrenomedullin (AM) on the number of peripheral blood mononuclear cells. (B) Effect of continuous administration of AM on the number of peripheral blood mononuclear cells. (C) Effect of continuous administration of AM on the number of stem cell antigen-1 (Sca-1)-positive cells. Data are mean ± SEM. White bars = vehicle; black bars = bolus injection of AM (μg); striped bars = continuous administration of AM (0.05 μg/kg/minute). *p < 0.05 versus vehicle group.

A significant change was detected from the lowest dose (1 μg/mouse: 120% of vehicle group) to the highest dose (5 μg/mouse: 140% of vehicle group). A significant increase was also detected in mice administered AM by an osmotic minipump (131% of vehicle group; Figure 1B). Furthermore, the frequency of Sca-1-positive cells was significantly increased (200% of vehicle group; Figure 1C).

Regeneration of Pulmonary Epithelium

In the chimeric mice, the proportions of GFP-positive peripheral blood mononuclear cells and bone marrow cells were 87 to 93% and 85 to 90%, respectively, indicating that most of the original stem cell population was replaced by donor cells. Twenty-eight days after elastase injection, GFP-positive cells were detected in the alveolar walls. GFP/cytokeratin double-positive cells were also observed in the alveolar walls (Figure 2A). Semiquantitative analysis demonstrated that the number of GFP-positive cells incorporated into the lung was significantly increased in the AM group compared with that in the vehicle group (Figure 2B). In addition, the number of GFP/cytokeratin double-positive cells was significantly increased in the AM group compared with that in the vehicle group (Figure 2C). Twenty-eight days after elastase injection, the development of airspace enlargement with destruction of the alveolar walls was observed in the vehicle group (Figure 3A). However, administration of AM attenuated the histologic changes in mice given elastase. The mean linear intercept in the vehicle group was significantly increased compared with that in the sham group (Figure 3B), but the increase was significantly attenuated by AM.

Regeneration of Pulmonary Vasculature

GFP/vWF double-positive cells were observed in the alveolar walls (Figure 4A), and the number of these cells in the AM group was larger than that in the vehicle group (Figure 4B). As a whole, the number of vWF-positive pulmonary vessels was decreased 28 days after elastase injection (Figures 4C and 4D). However, AM infusion significantly increased the number of vWF-positive pulmonary vessels in the elastase-treated lung.

Effect of AM on Pulmonary Function

Twenty-eight days after elastase injection, the lung volume was significantly increased in the vehicle group (Figure 5A). The increase in lung volume was significantly attenuated by AM. Static lung compliance was significantly increased in the vehicle group, and the change was significantly attenuated by AM (Figure 5B).
Figure 3. (A) Representative photographs of lung tissue stained with hematoxylin and eosin. Intratracheal injection of elastase induced the development of airspace enlargement with destruction of the alveolar walls in murine lungs (vehicle group). AM infusion attenuated the elastase-induced emphysematous changes (AM group). Original magnification ×100. (B) Semiquantitative analysis of lung tissue using the mean linear intercept, a morphometric parameter of pulmonary emphysema. Data are mean ± SEM. *p < 0.05 versus sham group; †p < 0.05 versus vehicle group.

Figure 4. (A) Representative examples of bone marrow cell differentiation into endothelial lineage. Green fluorescence indicates GFP; red fluorescence indicates von Willebrand factor (vWF), a marker for endothelial cells. Original magnification ×400. (B) Semiquantitative analysis of endothelial differentiation. The number of GFP/vWF double-positive cells was significantly higher in the AM group than in the vehicle group. (C) Immunohistochemical analysis of vWF. Although vWF-positive vessels were less frequently observed in elastase-injected mice given vehicle, they were increased by AM infusion. Original magnification ×100. (D) Semiquantitative analysis demonstrated that the number of vWF-positive vessels was decreased 28 days after elastase injection (vehicle group). However, AM infusion significantly increased the number of vWF-positive vessels in elastase-treated lung (AM group). Data are mean ± SEM. *p < 0.05 versus sham group; †p < 0.05 versus vehicle group.
Figure 5. Effects of AM on lung volume (A) and static lung compliance (B) in mice 28 days after elastase injection. Elastase injection significantly increased lung volume and compliance in mice (vehicle group). AM infusion significantly suppressed the changes in elastase-injected mice (AM group). Data are mean ± SEM. *p < 0.05 versus sham group; †p < 0.05 versus vehicle group.

Protective Effects of AM on Epithelial and Endothelial Cells

In vitro, exposure of elastase significantly decreased the numbers of A549 cells (Figure 6A) and HUVEC (Figure 6B). However, AM dose-dependently attenuated elastase-induced cell death. In addition, elastase increased the frequency of TUNEL-positive cells in both A549 cells (Figure 6C) and HUVEC (Figure 6D). However, AM (10−8 M) significantly decreased the frequency of these TUNEL-positive cells (A549 cells: elastase alone, 26.5 ± 2.2%, elastase + AM, 20.0 ± 1.5%; p < 0.05; HUVEC: elastase alone, 27.3 ± 2.3, elastase + AM, 19.8 ± 1.6%; p < 0.05). Elastase induced caspase-3 activation in both A549 cells (Figure 6E) and HUVEC (Figure 6F). However, AM (10−7 M) significantly attenuated elastase-induced caspase-3 activation in both type of cells.

In vivo, Ki-67–positive cells were observed in both GFP-positive and GFP-negative populations after elastase injection (Figure 7A). Semiquantitative analysis demonstrated that the number of Ki-67–positive cells in GFP-negative populations was significantly increased in the AM group compared with that in the vehicle group (Figure 7B).

Mechanism of Effect of AM on Bone Marrow Cell Mobilization

In vitro, administration of AM (10−8 M) stimulated NO release from bone marrow stromal cells (Figure 8A). The effect of AM on NO release was attenuated by pretreatment with wortmannin, a PI3K inhibitor (5 × 10−9 M). In vivo, continuous infusion of AM for 5 days significantly increased the concentration of soluble kit-ligand in bone marrow (Figure 8B).

VEGF Concentration

There was no significant difference in circulating VEGF concentration between the sham group (11.7 ± 1.0 pg/ml) and vehicle group (13.8 ± 1.5 pg/ml). However, AM infusion significantly increased the VEGF concentration (19.7 ± 2.0 pg/ml; p < 0.05 vs. the vehicle group).

Delayed Therapy

Morphometric analysis demonstrated that delayed AM therapy slightly, but significantly attenuated the increase in mean linear intercept in elastase-injected mice (Figures 9A and 9B). Delayed AM therapy significantly attenuated the increases in lung volume (Figure 9C) and static lung compliance (Figure 9D). In addition, the number of bone marrow–derived cells (GFP-positive cells) incorporated into the lung was significantly increased in the AM group in established emphysema (16.2 ± 0.9 vs. 11.5 ± 0.7 in 100 alveoli; p < 0.05). The numbers of bone marrow–derived epithelial cells (GFP/cytokeratin double-positive cells) and bone marrow–derived endothelial cells (GFP/αWF double-positive cells) were also significantly increased in the AM group in established emphysema (5.1 ± 0.3 vs. 2.2 ± 0.2, 3.8 ± 0.2 vs. 1.7 ± 0.1 in 100 alveoli, respectively; p < 0.05).

DISCUSSION

In the present study, we demonstrated in vivo that (1) infusion of AM increased the numbers of mononuclear cells and Sca-1–positive cells in circulating blood, (2) AM increased the number of bone marrow–derived cells incorporated into the elastase-treated lung, and (3) AM promoted regeneration of alveoli and vasculature in the lung. We also demonstrated in vitro that (4) AM improved cell survival after elastase exposure. Finally, we demonstrated that (5) AM improved lung structure and function in mice given elastase.

AM has a variety of effects on the vasculature that include vasodilation, inhibition of endothelial cell apoptosis, and regulation of smooth muscle cell proliferation (6, 10, 23–28). Recently, AM has been shown to induce angiogenesis through the PI3K/Akt-dependent pathway (12, 13). However, the effect of AM on bone marrow cell mobilization has remained unknown. In the present study, infusion of AM significantly increased the numbers of mononuclear cells and Sca-1–positive cells in circulating blood. Circulating endothelial progenitor cells have been shown to exist in a fraction of Sca-1–positive mononuclear cells (29). Thus, AM might induce release of progenitor cells from the bone marrow into circulation. Earlier studies have shown that VEGF and stromal-derived factor-1 mobilize endothelial progenitor cells from the bone marrow (16, 30). Bone marrow cell mobilization has been shown to be dependent on local secretion of matrix metalloproteinase-9 and subsequent release of soluble kit-ligand, a key molecule for stem cell recruitment (31). A recent study has shown that NO activates matrix metalloproteinase-9 by S-nitrosylation (32). Moreover, endothelial NO synthase has been shown to be essential for mobilization of stem and progenitor cells (22). In the present study, AM stimulated NO release from bone marrow stromal cells, and the effect of AM on NO release was attenuated by pretreatment with wortmannin, a PI3K inhibitor. These findings suggest that infusion of AM stimulates NO release from bone marrow stromal cells at least in part through the PI3K/Akt-dependent pathway. In vivo, continuous infusion of AM significantly increased expression of soluble kit-ligand in murine bone marrow plasma. Taken together, AM may mobilize bone marrow cells at least in part through NO production and release of soluble kit-ligand.

Recent studies have shown that bone marrow progenitor cells contribute to regeneration of alveoli and vasculature (4, 16, 33, 34). These progenitor cells mobilized from the bone marrow have been shown to migrate to the site of damage and differentiate into alveolar epithelium or vascular endothelial cells. In the present study, to observe the behavior of bone marrow cells during tissue regeneration, we generated a chimeric mouse in which the bone marrow is reconstituted with GFP-positive cells. During tissue regeneration, we generated a chimeric mouse in which the bone marrow is reconstituted with GFP-positive cells. Some of these GFP-positive cells were flat and stained for cytokeratin, a marker of epithelial cells. Interestingly, in the present study, AM infusion increased the number of GFP-positive cells in the alveolar walls. Moreover, the number of GFP/cytokeratin double-positive cells was significantly increased after AM infusion. These results suggest that AM promotes lung regeneration by increasing the number of bone marrow–derived alveolar epithelial cells in elastase-treated lung. It has been well
established that intratracheal injection of proteolytic enzymes induces emphysema in laboratory animals (35). In fact, in the present study, intratracheal injection of elastase induced emphysematous changes in the lung, as indicated by lung function (increased lung volume and static lung compliance) and morphologic findings (increased mean linear intercept). These findings were consistent with the results from earlier studies (36, 37). Interestingly, 14-day infusion of AM significantly attenuated the increase in lung volume, static lung compliance, and mean linear intercept in mice given elastase. Taken together, AM infusion
may improve elastase-induced emphysema at least in part through regeneration of alveoli.

In the progression of emphysema, the capillary net cannot escape being destroyed because capillaries make up a substantial fraction of the volume of the alveolar walls. Accordingly, to repair emphysematous changes, it is important to regenerate not only alveoli, but also vasculature. In the present study, infusion of AM increased the number of GFP/vWF double-positive cells, suggesting that AM increases bone marrow–derived vascular endothelial cells. Furthermore, recent studies have shown that AM exerts angiogenic activities through activation of Akt, mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2, and focal adhesion kinase in endothelial cells (12, 13). Expectedly, in the present study, AM significantly increased the number of pulmonary vessels in the mice given elastase. Thus AM-induced angiogenesis in the lung may contribute to pulmonary regeneration in mice given elastase. AM infusion significantly increased the circulating VEGF level. Considering that VEGF signaling is required for cell growth and survival, it is interesting to speculate that VEGF upregulated by AM also induces angiogenesis in the lung.

In the present study, exposure of elastase reduced the numbers of cultured alveolar epithelial cells and vascular endothelial cells. Furthermore, elastase induced DNA fragmentation and caspase-3 activation in both type of cells. Interestingly, AM attenuated elastase-induced cell death in alveolar epithelial cells and vascular endothelial cells. Recently, AM has been shown to promote vascular endothelial cell proliferation (38). In the present study, AM significantly increased the number of Ki-67–positive cells even in GFP-negative populations, implying that AM may also induce proliferation of host lung cells. Thus, not only mobilization of bone marrow cells but also protective effects of AM on alveolar epithelium and endothelium may contribute to improvement in lung structure and function in elastase-injected mice. An inadequate repair process contributes to the development of emphysema (39, 40). Moreover, apoptosis of alveolar cells may be involved in pathogenesis of emphysema (41, 42). These findings raise the possibility that AM may prevent loss of alveoli via modulation of alveolar repair and inhibition of alveolar cell apoptosis.

Finally, to confirm the effect of AM on elastase-induced emphysema via its bone marrow action, we administered AM to mice with established emphysema. AM slightly but significantly improved lung structure and function in mice given elastase. In addition, AM increased the number of bone marrow–derived alveolar epithelial cells and vascular endothelial cells in GFP-positive bone marrow chimeric mice. Thus, continuous infusion

Figure 8. (A) Effects of AM (10^{-8} M) on nitric oxide (NO) release from murine bone marrow stroma cells. Pretreatment with 5 \times 10^{-8} M wortmannin, a PI3K inhibitor, attenuated the effect of AM. Data are mean ± SEM. *p < 0.05 versus control (medium alone); †p < 0.05 versus AM alone. (B) Soluble kit-ligand level in bone marrow plasma. Continuous infusion of AM significantly increased soluble kit-ligand expression. Data are mean ± SEM. *p < 0.05 versus vehicle group.

Figure 9. Effects of AM on established emphysema. AM administration was started 25 days after elastase injection. (A) Representative photographs of lung tissue. (B) Semiquantitative analysis of lung tissue using the mean linear intercept. (C) Lung volume. (D) Static lung compliance. Data are mean ± SEM. *p < 0.05 versus sham group; †p < 0.05 versus vehicle group.
of AM may also improve established emphysema at least in part through mobilization of bone marrow cells and their differentiation into alveolar epithelial cells and vascular endothelial cells.

This study includes a study limitation. We used an elastase-induced model of emphysema to demonstrate that AM has the capacity to promote regeneration of alveoli and vasculature. An elastase-induced model is often used because of its relative simplicity and particularly useful for investigating mechanisms of lung repair. However, the artificiality of putting a large amount of elastase into the animal lung limits the usefulness of this model in answering questions relating to mechanisms of human emphysema. Moreover, cigarette smoke exposure causes a variety of other abnormalities that are not observed with simple intratracheal instillation of elastase. Thus, the results obtained from the elastase model may not be predictive of response to therapy in human cigarette smoke–induced pulmonary emphysema. Therefore, the initial success of AM therapy reported here should be confirmed by further studies using other animal models of pulmonary emphysema before clinical trials.

In conclusion, continuous infusion of AM improved elastase-induced emphysema. These beneficial effects of AM may be mediated at least in part through mobilization of bone marrow cells and the direct protective effects on alveolar epithelial cells and endothelial cells. Thus, this may be a new therapeutic strategy for the treatment of pulmonary emphysema.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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