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Airway Delivery of Bone Marrow–Derived Mesenchymal Stem Cells Reverses Bronchopulmonary Dysplasia Superimposed with Acute Respiratory Distress Syndrome in an Infant

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Abstract

Bronchopulmonary dysplasia (BPD), a disease affecting extremely premature infants, results from the disruption of normal pulmonary vascular and alveolar growth. Currently, there is no specific effective treatment. We report a case of a 10-mo-old female infant with BPD, who was admitted because of adenovirus pneumonia and acute respiratory distress syndrome (ARDS) with prolonged venovenous and arteriovenous extracorporeal membrane oxygenation (ECMO) support (total 125 d). The respiratory condition dramatically improved, and ECMO was removed 25 d after intratracheal delivery of maternal bone marrow-derived mesenchymal stem cells (BM-MSCs). Short tandem repeat examinations revealed that there was no maternal cells in the bronchial wash fluid. To our knowledge, this is the first human report of BM-MSC therapy reversal of the course of BPD superimposed with ARDS. We also suggest that BM-MSC therapy may not only be effective in the newborn stage but also works in infants and children with BPD.

Keywords

acute respiratory distress syndrome, bronchopulmonary dysplasia, extracorporeal membrane oxygenation, mesenchymal stem cell

Introduction

The incidence of bronchopulmonary dysplasia (BPD) is increasing with great advances in neonatal intensive care of extremely premature infants[1]. Disruption of normal pulmonary vascular and alveolar growth results in simplified pulmonary alveolar acini and vascular structures, reducing the surface area for gas exchange[2,3]. Secondary pulmonary hypertension is also common in severe cases[4]. The possible pathogenesis is altered vascular endothelial growth factor (VEGF) signaling and a decreased level of various pro-inflammatory cytokines (e.g., tumor necrosis factor-α, interleukin-1β, interleukin-6, and interleukin-16)[5,6]. Since there is no specific effective treatment, BPD remains a serious cause of morbidity and mortality in premature infants[7].

Cell therapy with mesenchymal stem cells (MSCs) provides a new hope for treatment of BPD. MSCs, one type of adult stem cells, are identified according to the following consensus of minimal criteria: (1) adherence to plastic under standard

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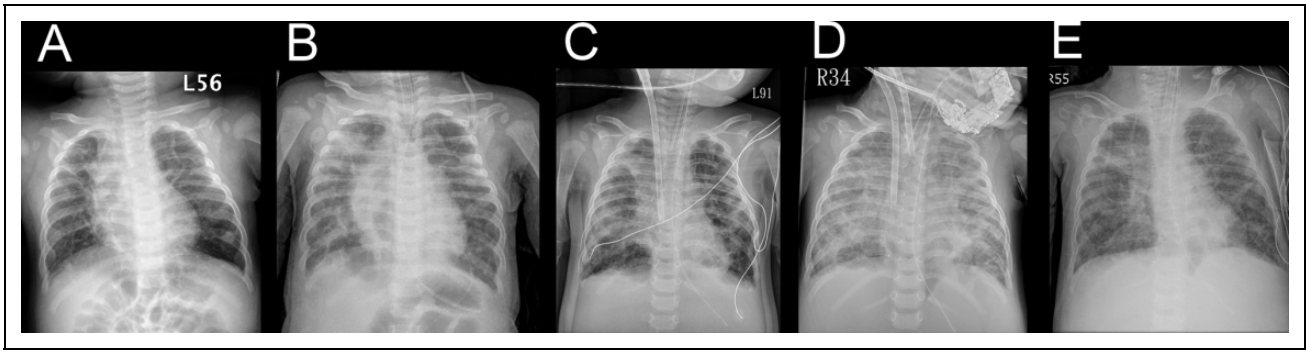

Fig. 1. Serial chest radiographs. Serial chest radiographs, anteroposterior view: (A) At 1 month before admission. (B) At the time of admission due to adenovirus pneumonia. (C) After cardiopulmonary resuscitation and venovenous ECMO implantation. (D) After conversion to venoarterial ECMO. (E) After intratracheal bone marrow–derived mesenchymal stem cell injection and extracorporeal membrane oxygenation removal. ECMO, extracorporeal membrane oxygenation.

culture conditions; (2) expression of CD105, CD73, and CD90 and lack of surface expression of CD45, CD34, CD14, CD11b, CD79, CD19, and human leukocyte antigen-antigen D related (HLA-DR); and (3) ability to differentiate into adipocytes, chondrocytes, and osteocytes in vitro[8]. MSCs have been used for treatment of soft tissue and bone injuries, autoimmune diseases, hematological diseases, diabetes mellitus, heart failure, central nervous system diseases, and lung diseases in early phase clinical trials[9]. They can be isolated from bone marrow (BM), adipose tissue, umbilical cord, and the placenta[10,11]. Four important mechanisms contribute to their therapeutic effect: (1) the ability to home to sites of inflammation following tissue injury, (2) the ability to differentiate into various cell types, (3) the ability to secrete multiple bioactive molecules capable of stimulating recovery of injured cells and inhibiting inflammation, and (4) the lack of immunogenicity and the ability to perform immunomodulatory functions[12].

We report a case of an infant with severe BPD superimposed with acute respiratory distress syndrome (ARDS). The respiratory condition dramatically improved after intratracheal delivery of BM–derived MSCs (BM-MSCs).

Case

A 10-mo-old girl (gestational age: 25 wk and 4 d, birth body weight: 778 g, corrected age 6 and a half months) was admitted to our intensive care unit. She had a history of prolonged hospitalization due to BPD. Her baseline oxygen saturation was 90% in ambient air (Fig. 1A). She was intubated due to severe adenovirus pneumonia and ARDS. Chest radiography showed widespread reticular pattern and parenchymal bands (Fig. 1B). Pulmonary hypertension was suggested by echocardiography. A trial of inhaled nitric oxide and intravenous infusion of milrinone failed to improve oxygenation. Computed tomographic (CT) scan imaging of the chest was obtained (Fig. 2A). There were diffused coarse fibrotic bands, extensively thickened interstitium, subsegmental atelectasis, air trapping, and early cavitation in both lung fields. Sudden onset profound desaturation and bradycardia happened 22 d after admission. Spontaneous circulation recovered after 1-min bag-valve-mask ventilation as well as chest compression. Her saturation could hardly be maintained above 80% even with delivery of 100% oxygen.

Therefore, venovenous extracorporeal membrane oxygenation (ECMO) was implanted (a 15F double lumen cannula, blood flow 0.4 L/min; Fig. 1C). However, the blood flow gradually became unstable due to the presence of thrombosis and hemolysis. For better support, ECMO was changed to venoarterial (a 14F A-cannula and a 14F V-cannula, blood flow 0.9 L/min) after 35 d (Fig. 1D). Chest CT on 49 d after admission revealed deterioration and diffused fibrosis of both lung fields (Fig. 2B). Since she was ECMO dependent, we sought for MSC therapy.

Materials and Methods

Informed Consent

This study was approved by the Research Ethics Committee as a compassionate use (No. C1050901). Full understanding was confirmed, and written informed consent was obtained from the parent.

Culture Protocol for MSCs

Fifty milliliters of BM was aspirated from the donor's posterior superior iliac crest. The BM sample was preserved in preservative-free heparinized Iscove's modified dulbecco's solution (Sigma-Aldrich) and filtrated through a 70-μm filter, then a 20-μm filter (Sigma-Aldrich) subsequently.

The diluted BM sample was carefully layered on Ficoll solution (Sigma-Aldrich) in a specific ratio. It was centrifuged at 4 °C, 2,400 rpm for 20 min. Mononuclear cells (MNCs) were aspirated through the interface. The MNCs were washed by low-glucose Dulbecco's modified Eagle's medium (DMEM; Gibco, Waltham, MA, USA) twice.

The BM-MNCs were added to DMEM containing 25 U/mL heparin (Sigma-Aldrich) in 1:1 ratio and plated in T25 flasks at 5×10^7 nucleated cells per flask in 5 mL regular growth

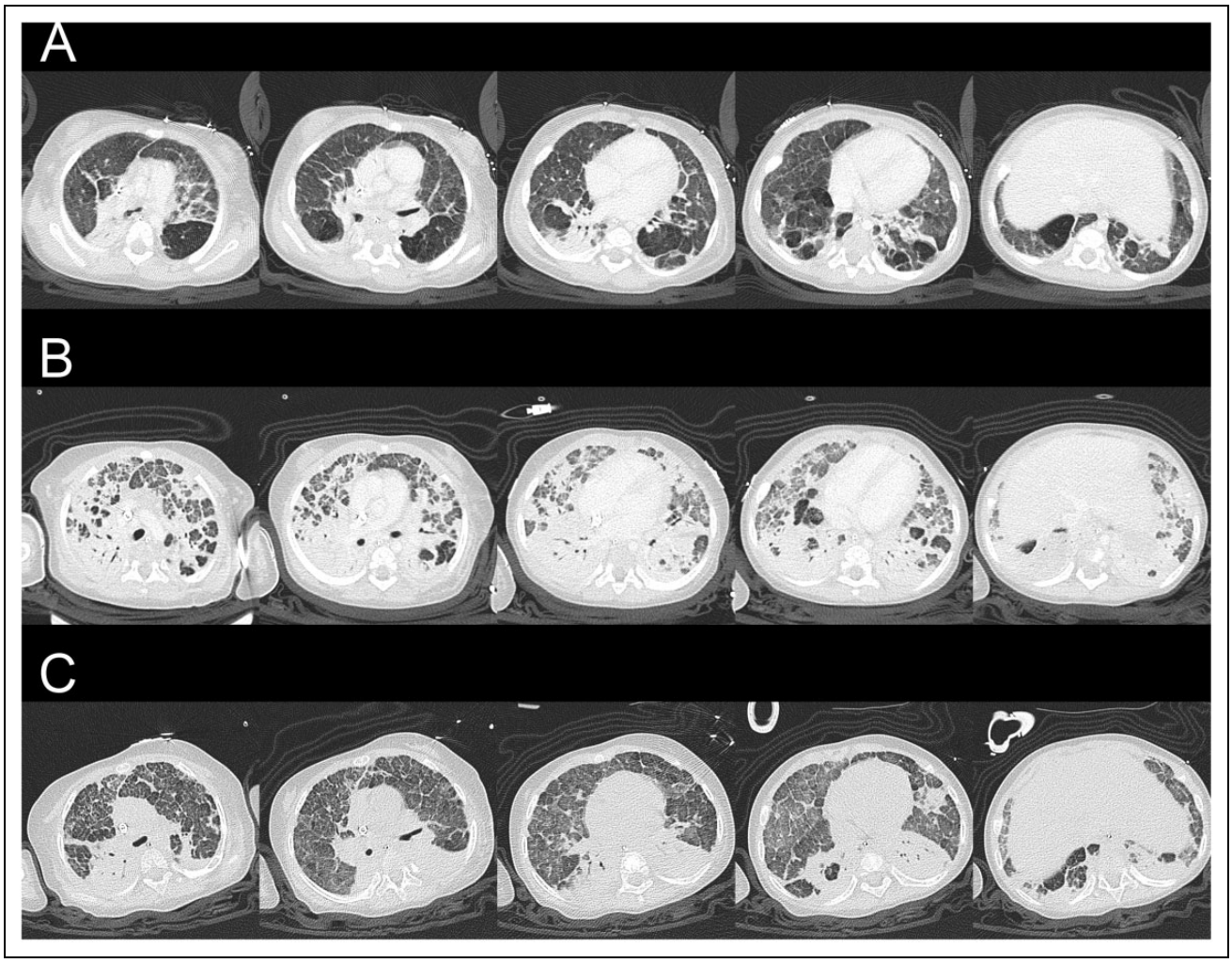

Fig. 2. Serial chest computed tomographic scan images: (A) At 17 d after admission, before venovenous ECMO implantation. (B) At 49 d after admission, before conversion to venoarterial ECMO implantation. (C) At 147 d after admission, 125 d after ECMO implantation, 25 d after intratracheal bone marrow–derived mesenchymal stem cell injection, before ECMO removal. Lung fibrosis dramatically improved after bone marrow-derived mesenchymal stem cell treatment. ECMO, extracorporeal membrane oxygenation.

medium (Gibco). During the first 2 wk incubation, 5 mL of fresh growth medium was added once weekly for cell adherence and initial expansion. After adherent cells reached approximately 60% to 70% confluence, they were detached with 0.25% trypsin-ethylenediamineteraacetic acid (Gibco) and replated at 1:3 in regular growth medium to allow for continuing passing. Cultured MSCs were harvested at the end of the second passage.

Quality Control

Sterility and contamination: Aerobe, anaerobe, and fungus cultures were performed for sterility.

Immunophenotyping: The harvested cells were stained with phycoerythrin-conjugated antibodies (Becton Dickinson, Franklin Lakes, NJ, USA) against human antigens CD10, CD13, CD14, CD29, CD34, CD44, CD90/Thy-1, CD117/c-kit, CD166, AC133, human leukocyte antigens (HLA) A, B, C loci, HLA-DR, and glycophorin A and analyzed by a Becton Dickinson FACS Caliber flow cytometry system.

Differentiation: (1) Adipogenic differentiation: Adipogenic medium consisted of high-glucose DMEM, (Gibco) supplemented with 0.5 mM isobutyl-1-methylxanthine (IBMX; Sigma-Aldrich, St. Louis, MO, USA), 1 μM dexamethasone (Sigma-Aldrich), 10 ng/mL insulin (Sigma-Aldrich), 50 μM indomethacin (Sigma-Aldrich), and 10% fetal bovine serum (FBS, Gibco). To induce adipocyte differentiation, hMSCs were transferred into a 15-mL polypropylene tube and centrifuged at 1,000 rpm for 5 min, to form a pelleted micromass at the bottom of the tube. The cells were then treated with adipogenic medium (Gibco) for 4 wk with medium changes twice weekly. Adipogenic differentiation was assessed by the cellular

accumulation of neutral lipid vacuoles after cells were fixed with 4% formaldehyde (Sigma-Aldrich), stained with oil-red O (Sigma-Aldrich). (2) Chondrogenic differentiation: Chondrogenic medium consisted of low-glucose DMEM, (Gibco) supplemented with 1 mM sodium pyruvate (Sigma-Aldrich), 0.1 μM dexamethasone (Sigma-Aldrich), 0.1 mM acetylsalicylic acid (AsA, Sigma-Aldrich), 10 ng/mL transforming growth factor-β (TGF-β1; R&D Systems, Minneapolis, MN, USA), and 1× ITS premix (Sigma-Aldrich; 5 μg/mL insulin, 5 μg/mL transferrin [Sigma-Aldrich], and 25 ng/mL selenius acid). Similar MSC processing was used as in adipogenic differentiation. Chondrogenic differentiation was evaluated after pellets had been transferred into 96-well plates, fixed with 4% formaldehyde (Sigma-Aldrich), and stained with 1% Alcian blue (Sigma-Aldrich).

Administration

After harvest, concentrated BM-MSCs were diluted to 20 mL with 0.9% saline. The patient was put in supine position and was deeply sedated. A 3-mm flexible bronchoscope was inserted through the endotracheal tube. The diluted BM-MSCs were equally administered into both bronchi from the working channel. After administration, sputum suctioning was avoided for 24 h.

Follow-Up

ECMO and ventilation parameters were adjusted according to clinical conditions. Blood gas was checked and recorded daily. Short tandem repeat (STR) examination of bronchial wash fluid was used to evaluate engraftment after obvious improvement in ventilation function. Chest CT was performed to evaluate lung condition.

Results

Intratracheal administration of BM-MSCs (6.25×10^6 cells/kg; total dose: 5×10^7 cells, body weight: 8.0 kg) was performed 100 d after ECMO implantation.

Dramatic improvement in oxygen demand and tidal volume was obvious under similar settings of peak inspiratory pressure, positive end-expiratory pressure, and mean airway pressure (Fig. 3). A much improved lung condition could be seen in the chest CT (Fig. 2C). STR examination 23 d after delivery revealed no maternal cell in bronchial wash fluid. ECMO was removed 25 d after delivery of BM-MSCs (Fig. 1E). She received tracheostomy and was discharged for long-term care. The delivered oxygen fraction before discharge was only 0.3.

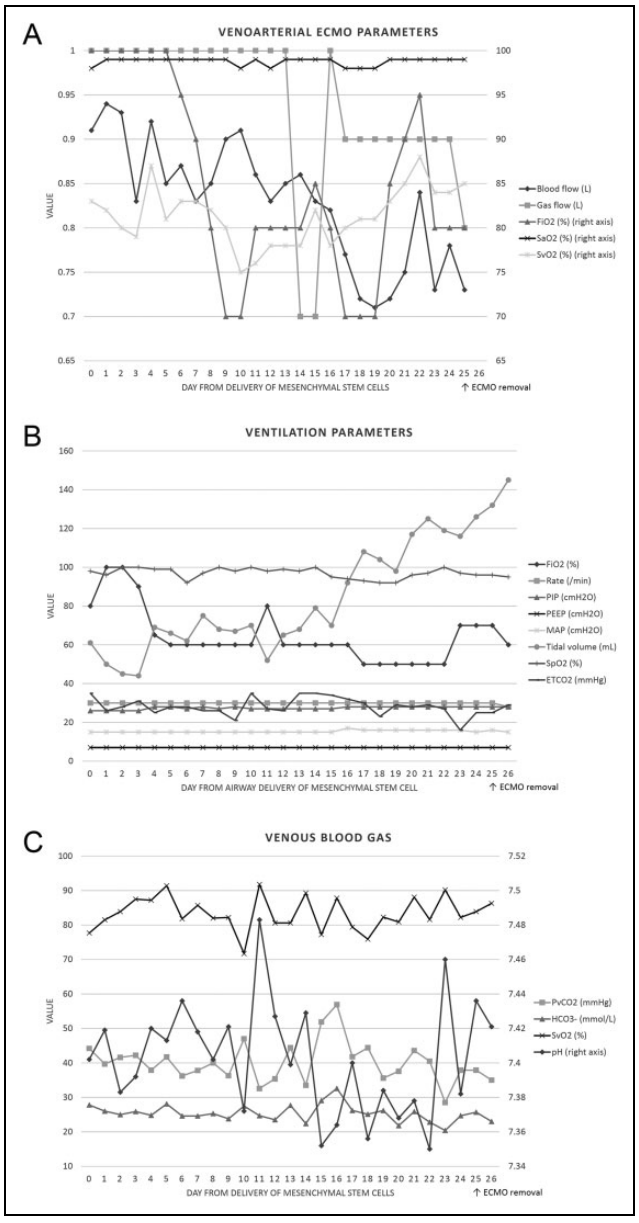

Fig. 3. Venoarterial extracorporeal membrane oxygenation, ventilation, and venous blood gas parameters after delivery of bone marrow–derived mesenchymal stem cells. (A) Venoarterial ECMO parameters: FiO_2 and blood flow gradually decreased after delivery of MSCs. (B) Ventilation parameters: FiO_2 was 100% after delivery of MSCs but could be adjusted to 60% to 70% after 5 d. Tidal volume improved from 40 to 60 mL to over 140 mL in similar settings of PIP, PEEP, and MAP. (C) Venous blood gas parameters: Blood gas values remained stable during venoarterial ECMO support. ECMO, extracorporeal membrane oxygenation; $ETCO_2$, end-tidal carbon dioxide; FiO_2, fraction of inspired oxygen; HCO_3^-, bicarbonate; MAP, mean airway pressure; MSC, mesenchymal stem cell; PEEP, positive end-expiratory pressure; PIP, peak inspiratory pressure; $PvCO_2$, partial pressure of venous carbon dioxide; SaO_2, arterial oxygen saturation; SpO_2, peripheral oxygen saturation; SvO_2, venous oxygen saturation.

Discussion

MSCs are regulators of lung growth[13]. Exogenous MSCs are home to injured tissues and exert their immunomodulatory ability with the secretion of cytokines (e.g., VEGF, interferon-γ, interleukin-10, hepatocyte growth factor),

stimulating angiogenesis and anti-inflammation, and tissue repair[14]. Several hyperoxia animal model studies proved the beneficial effect of BM-MSCs with different routes of administration, intratracheally, intravenously, or even intraperitoneally, in treatment of BPD[15–23]. BM-MSC therapy for BPD has never been reported in clinical trial. There was only 1 small-scale phase 1 dose escalation clinical trial using human umbilical cord–derived MSC therapy for BPD in Korea[16]. Intratracheal injection was performed in 9 extremely preterm infants (3 with 1×10^7 cells/kg, 6 with 2×10^7 cells/kg) at a mean of 10.4 d after birth. There was no adverse effect. BPD severity was lower in the recipients.

In addition, preclinical data support the use of MSCs for treatment of ARDS[24]. Early phase clinical trials are ongoing[25]. MSC therapy for refractory ARDS on ECMO was previously reported in 2 adult patients (2×10^6 cells/kg), whose conditions subsequently improved with resolution of respiratory, hemodynamic, and multiorgan failure[26]. The potential of MSC therapy in lung diseases is promising.

After intratracheal delivery of BM-MSC treatment, a delay in recovery was noticed (Fig. 3). Tidal volume improved slightly at day 4 (from 40 to 60 mL before treatment to 60 to 80 mL during day 4 to 14) and dramatically increased after day 14 (from 80 mL at day 14 to 145 mL at day 26). This phenomenon is similar to the finding of the only phase 1 clinical trial for BPD: respiratory severity score only mildly improved during the first 7 d after transplantation of MSCs and differed much more obviously at day 14[16].

MSCs do not engraft and have no risk of rejection or malignancy[15,18]. Previous studies demonstrated that delivery of conditioned medium of MSCs is also therapeutic[15,19]. It is compatible with our finding that no maternal cell was found in STR analysis. The effect of MSCs is likely to be paracrine[27].

Delivery of MSCs is safe. There is no difference in acute infusional toxicity and fever, organ system–related adverse events (cardiovascular, gastrointestinal, renal, pulmonary, neurological, hematological, infection, and malignancy), or death, in comparison to the control group in a systematic review and meta-analysis of clinical trials[18]. However, it is still a serious concern that a relatively large volume (20 mL) of 0.9% saline is needed to dilute the MSCs for passing and flushing the narrow working channel of a 3 mm bronchoscope in an infant with poor lung condition (tidal volume 40 to 60 mL at the day of delivery). Full ECMO support of respiratory function facilitates this procedure in our case. No desaturation happened during or soon after delivery. MSCs that are more concentrated might be used for patients without ECMO support.

This is only a case study of MSC treatment. Although the results are promising, other contributing factors should still be considered, including successful control of infection, restricting fluid intake, and lung protective ventilation strategy. Further clinical trials are needed to validate the safety and efficacy.

Conclusion

To our knowledge, this is the first human report of BM-MSC therapy in treatment of BPD superimposed with ARDS. We also suggest that MSC therapy may not only be effective in the newborn stage but also works in infants and children with BPD.

Ethical Approval

This study was approved by our institutional review board as a compassionate use (No. C1050901).

Statement of Human and Animal Rights

The study was performed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2005.

Statement of Informed Consent

Written informed consent was obtained from the parent.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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