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Endothelial Colony-forming Cells Attenuate Ventilator-induced Lung Injury in Rats with Acute Respiratory Distress Syndrome

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Background. Mechanical ventilation (MV) can cause ventilator-induced lung injury (VILI).

Aim of the study. This study investigated whether endothelial colony-forming cells (ECFC) could inhibit VILI in a rat model of acute respiratory distress syndrome (ARDS).

Methods. Male Wistar rats received the femoral artery and venous cannulation (sham group) or were injected intravenously with 500 μg/kg lipopolysaccharide to induce ARDS. The ARDS rats were subjected to MV. Immediately after the MV, the rats were randomized and injected intravenously with vehicle (ARDS group) or ECFC (ECFC group, $n = 8$ per group). The oxygen index, lung wet-to-dry weight (W/D) ratios, cytokine protein levels in serum or bronchoalveolar lavage fluid (BALF), neutrophil counts, neutrophil elastase and total protein levels in BALF, histology and cell apoptosis in the lung were detected. The protein levels of endothelin-1, inducible nitric oxide synthase (iNOS), endothelial NOS, matrix metalloproteinase (MMP)-9, Bax, Bcl-2, gelsolin, cleaved caspase-3, phosphorylated NF-κBp65 and myosin light chain (MLC) in the lung were analyzed.

Results. Compared with the ARDS group, treatment with ECFC significantly increased the oxygen index, and decreased the lung W/D ratios and injury, and the numbers of apoptotic cells in the lungs, neutrophils counts, total protein and elastase concentrations in BALF of rats. ECFC treatment significantly minimized the protein levels of pro-inflammatory cytokines in BALF and serum, but increased interleukin 10 in rats. Furthermore, ECFC treatment significantly reduced the protein levels of endothelin-1, iNOS, Bax, Gelsolin, MMP-9, cleaved caspase-3, phosphorylated NF-κBp65 and MLC, but enhanced eNOS and Bcl-2 in the lungs of rats.

Conclusions. Therefore, ECFC attenuated inflammation, cell apoptosis and VILI in ARDS rats.

Key Words: Endothelial progenitor cells, Ventilator-induced lung injury, Acute respiratory distress syndrome, Inflammation.

Introduction

Although the mechanical ventilation (MV) can save life for patients in critical care (1), about 24% of patients with MV develop ventilator-induced lung injury (VILI) (2). Pathologically, MV can lead to endothelial activation and inflammation in the lung (3), especially in those with pre-injured lung. VILI is characterized by focal lung inflammation with pro-inflammatory cytokine over-production and inflammatory cell infiltration in the lung tissue, leading to a lung histological changes, lung edema, the impaired lung compliance and pulmonary function (4). Patients with acute respiratory distress syndrome (ARDS), which usually has endothelium injury, are susceptible to VILI (5). Particularly, those ARDS patients usually need a large tidal

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volume to maintain oxygenation (6). Although many lung protective strategies are available for intervention of ARDS patients (7), the mortality of those patients remains substantial (8) because of severe inflammation. The endothelium injury and secondary inflammation are crucial for the development of ARDS and VILI (9). Therefore, effective reduction in lung inflammation and protection from endothelial cell activation are important strategies for management of ARDS patients with VILI.

The endothelial progenitor cells (EPC) can repair the injured endothelium, promote the vascular regeneration and regulate immune responses in different models of organ injury (10). Endothelial colony forming cells (ECFC) are late outgrowth stage of EPC and have the properties of high proliferative potency (11) and other characteristics of EPC (12). The protection of ECFC mainly depends on their high proliferative ability, differentiation into endothelial cells and promoting the post-ischemic revascularization (13). Furthermore, recent studies have indicated that ECFC protect against the reperfusion kidney injury in a paracrine manner by secreting exosomes (14). Hence, ECFC ameliorate the reperfusion injury by secreting exosomes (14) and promoting neoangiogenesis (15), and endothelium repair (16). Actually, clinical trials have shown that ECFC can attenuate the myocardium reperfusion injury (17). ECFC also have anti-apoptotic and anti-chemotactic activity (14). However, whether and how ECFC can inhibit severe inflammation and large volume ventilation-induced lung injury have not been clarified.

In this study, we employed a rat model of severe inflammation and large volume ventilation-induced lung injury to test the hypothesis that ECFC can reduce the VILI in ARDS rats and determined the potential mechanisms.

Materials and Methods

Male Wistar rats at 6−8 weeks of age (about 200−250 g) were obtained from the Animal Care facility of Harbin Medical University and housed in a specific pathogen-free facility with free access of food and water *ad libitum*. This study was approved by the Institutional Animal Care and Use Committee of the Second Affiliated Hospital of Harbin Medical University.

Isolation and Culture of ECFC

Peripheral blood mononuclear cells were isolated from Wistar rats by density gradient centrifugation using the Ficoll-Plaque Plus (Amersham Pharmacia Biotech, Uppsala, Sweden). The mononuclear cells were cultured in endothelial growth medium (EGM)-2 (Lonza, Basel, Switzerland) supplemented with 2% fetal bovine serum (FBS) in six-well plates that had been coated with human fibronectin at 37°C, 5% CO_2. The cells were exposed to fresh medium daily and cultured for 21 d. The adherent cells (known as ECFC) were harvested by trypsinization and characterized.

Characterization of ECFC

ECFC were treated in triplicate with DiI-acetyl-low density lipoprotein (LDL, Invitrogen, Carlsbad, USA) and fluorescein isothiocyanate (FITC)-ulexeuropaeus agglutinin-1 (UEA-1, Sigma-Aldrich, Saint Louis, USA) for 2 h. The ECFC were examined under a fluorescence confocal microscope (A1R, Nikon, Japan). The cells with dual green UEA-1 and red DiI-labeled acetyl-LDL were identified as differentiating ECFC. Furthermore, the ECFC (10^6 cells/tube) were stained with FITC-anti-VEGFR-2 (Abcam, Cambridge, UK), PE-anti-CD34 (Santa Cruz Biotechnology, Santa Cruz, USA), PE-anti-CD11b, ALEXA 647-conjugated anti-14 and anti-CD45 (Abcam) and PE-conjugated anti-mouse CD105 (Biolegend, San Diego, USA) and analyzed by a flow cytometer (FACScan, Becton Dickinson, USA).

VILI Model

A rat model of ARDS was established, as described previously (18). Wister rats were randomized into the sham, ARDS and ECFC groups ($n = 8$ per group). All rats were anesthetized intraperitoneally with 3% pentobarbital sodium (30 mg/kg) and randomized. The sham group of rats received the femoral artery and venous cannulation only. The rats in the ARDS and ECFC groups were intubated with 14-G catheter and injected intravenously with 500 μg/kg lipopolysaccharide (LPS, Escherichia coli endotoxin, 0111:B4, Sigma) to induce ARDS (18,19). Thirty min after injection with lipopolysaccharide, the rats were injected intravenously with 0.6 mg/kg rocuronium and subjected to the MV for 4 h (tidal volume: 17 mL/kg, respiratory rate: 50 min, inspiratory expiratory ratio: 1:1 and fraction of inspiratory oxygen 50%) to induce VILI (18). Immediately after the beginning of MV, the ARDS rats were randomized and injected intravenously with 1 mL of vehicle PBS (ARDS group) or 10^6 ECFC (ECFC group). The rats in the ARDS and ECFC groups were injected intraperitoneally with 3% pentobarbital sodium (10 mg/kg) and rocuronium (0.6 mg/kg) hourly. When the rats in the ARDS and ECFC groups recovered spontaneous breath, they were extubated. All rats were sacrificed at 24 h post ventilation and the bronchoalveolar lavage fluid (BALF), artery blood samples and lung tissues were collected.

Analysis of Arterial Blood Gases

Arterial blood samples were collected before injection of lipopolysaccharide, before and 24 h after the MV. The arterial blood gases were analyzed using the Bayer Rapidlab 348 (Bayer Diognostics, Germany). The ratios of partial