Comparative Effects of Umbilical Cord Mesenchymal Stem Cell Treatment via Different Routes on Lipopolysaccharide-Induced Acute Lung Injury

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Abstract

Background: Although umbilical cord mesenchymal stem cell (UCMSC) infusion has been proposed as a promising strategy for the treatment of acute lung injury (ALI), the parameters of UCMSC transplantation, such as infusion routes and doses, need to be further optimized. Methods: In this study, we compared the therapeutic effects of UCMSCs transplanted via intravenous injection and intratracheal instillation on lipopolysaccharide-induced ALI using a rat model. Following transplantation, levels of inflammatory factors in serum; neutrophils, total white blood cells, and lymphocytes in bronchoalveolar lavage fluid (BALF); and lung damage levels were analyzed. Results: The results indicated that UCMSCs administered via both intravenous and intratracheal routes were effective in alleviating ALI, as determined by analyses of arterial blood gas, lung histopathology, BALF contents, and levels of inflammatory factors. Comparatively, the intratracheal instillation of UCMSCs was found to result in lower levels of lymphocytes and total proteins in BALF, whereas greater reductions in the serum levels of tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) were detected in rats receiving intravenously injected stem cells. Conclusions: Our findings in this study provide convincing evidence to indicate the efficacy of UCMSC therapy in the treatment of ALI mediated via different delivery routes, thereby providing a reliable theoretical basis for further clinical studies. Moreover, these findings imply that the effects obtained using the two assessed delivery routes for UCMSC transplantation are mediated via different mechanisms, which could be attributable to different cellular or molecular targets.

Keywords: acute lung injury; inflammation; intravenous injection; intratracheal instillation; UCMSCs

1. Introduction

Mesenchymal stem cells (MSCs) are a type of pluripotent adult stem cell that can be isolated from mesoderm of different organizations. As one of the hottest types of stem cells, MSCs have a number of notable biological characteristics, include a wide range of sources, multi-directional differentiation capacity, strong plasticity, low immunogenicity, paracrine forms of diverse biologically active factors, and low risk of teratogenicity and tumorigenesis [1–3]. On the basis of tissue source type, MSCs can be divided into bone marrow mesenchymal, adipose mesenchymal, umbilical cord mesenchymal, synovial mesenchymal, and nasal mucosa mesenchymal stem cells [4,5]. Among these, umbilical cord mesenchymal stem cells (UCMSCs), isolated from Wharton’s jelly of the umbilical cord, have been demonstrated to have immunomodulatory properties, capacity for self-renewal, and multipotency comparable to those of other MSCs [6]. Moreover, given that the method of UCMSC collection is non-invasive, obtaining this cell has not been hampered by ethical problems [7–9]. Hence, UCMSCs have gradually emerged as the most promising seed cells in tissue engineering and regenerative medicine, and might also serve as an alternative source for stem cell therapy [10].

Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS) [11], are acute progressive respiratory insufficiency or respiratory failure disorders that are commonly encountered in clinical practice, and are generally associated with high mortality rates attributable to trauma, blood transfusion, infection, and other factors [12–15]. Inflammation and lung tissue damage are important pathogenetic markers for ALI, and an attenuation of alveolar inflammation and recovery of barrier function have been established to contribute to an improved prognosis [16–19]. Given their immunosuppressive properties, MSCs have potential utility in ALI therapy, and indeed, the findings of an increasing number of preclinical studies have provided evidence to indicate that UCMSCs can inhibit and attenuate the occurrence and development of ALI [20–23]. Liu et al. [24], for example, found that exosomal miR-451 from UCMSCs inhibited the Toll-like recep-
tor 4 (TLR4)/nuclear factor-κB (NF-κB) pathway, thereby resulting in a reduction in the levels of inflammatory factors and an improvement in burn-induced ALI, whereas Hao et al. [25] have reported that UCMSCs can reduce oxidative stress and inflammatory responses by inhibiting the transforming growth factor (TGF)-β1/Smad2/3 signaling pathway, thereby leading to a reduction in the degree of ALI. Moreover, it has been reported that UCMSCs have the potential to ameliorate ALI by promoting the production of CD4+CD25+ forkhead boxp3 (FOXP3)+ regulatory T cells and balancing anti- and pro-inflammatory factors [26]. Given that numerous studies have confirmed the restorative function of UCMSCs with respect to ALI [27,28], UCMSC-based therapy has been proposed as a promising strategy for ALI treatment. However, the parameters associated with UCMSC transplantation, including infusion routes and doses, initially need to be optimized.

To this end, we compared the effects of UCMSCs on ALI using a lipopolysaccharide (LPS)-induced rat model based on intravenous and intratracheal injection routes. The objective of this study was to provide experimental evidence for evaluating and optimizing the treatment of ALI using UCMSCs.

2. Materials and Methods

2.1 Animals

Healthy male Sprague–Dawley (SD) rats (8 weeks) were provided by Zhejiang Charles River Laboratory Animal Technology Co., Ltd. (Jiaxing, China), which is licensed by the Science Technology Department of Zhejiang Province. All animal experiments were conducted according to the protocols approved by the Institutional Animal Care and Use Committee, Zhejiang Center of Laboratory Animals (ZJCLA-IACUC-20110043), and the acquisition and care of animals followed practices stipulated by the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publications, No. 80–23, revised 1978). The rats were maintained at an ambient temperature of 22–24 °C, relative humidity of 50%–60%, a 12 h/12 h light-dark cycle, and had free access to food and drinking water.

2.2 Experimental Groups

Healthy male SD rats were randomly assigned to one of the following six groups, each containing 10 animals: a normal control group (NC), ALI group (ALI, treated with aerosolized LPS), intravenous injection control group (Vehicle-iv: ALI rats were injected with saline via the tail vein), intratracheal instillation control group (Vehicle-it: ALI rats were injected with saline via the trachea), UCMSC intravenous injection group (UCMSC-iv: ALI rats were injected with UCMSCs via the tail vein), and UCMSC intratracheal instillation group (UCMSC-it: ALI rats were injected with UCMSC via the trachea).

2.3 Establishment of an Animal Model

The ALI model was established based on the inhalation of LPS (Escherichia coli O55:B5; Sigma-Aldrich, St. Louis, MO, USA) according to previous description with certain modifications [29,30]. Briefly, animals were anesthetized by inhalation of isoflurane, fixed on a tilted board (45°), and then intratracheally nebulized with 5 mg/kg LPS on day 1. After 72 h (day 3), the rats were treated with LPS (1 mg/kg, 1000 µL/kg) in the same manner.

2.4 Preparation of UCMSCs

UCMSCs were prepared and characterized as previously described [31]. Umbilical cord tissues were procured from healthy women during labor as waste and UCMSCs were isolated in Institute for Cell-Based Drug Development of Zhejiang Province, S-Evans Biosciences. The collection of umbilical cord was approved by Ethics Committee of S-Evans Biosciences (NO.2020-01). All subjects have signed the informed consent form for umbilical cord donation. In brief, umbilical cord tissues were procured and cut into approximately 1 mm² segments for primary adherent culturing. Having reached 80%–90% confluence, the cells were digested with 0.25% trypsin-EyDTA (Gibco, Carlsbad, CA, USA) for passaging. UCMSCs at passage 5 were characterized based on morphology, surface marker expression, and mesenchymal lineage differentiation (osteogenic, adipogenic, and chondrogenic differentiation), after which the cells were used for further experiments.

2.5 Transplantation of UCMSCs

UCMSC transplantation was performed after 3 h of LPS stimulation on day 1 and day 3 by tail vein injection or intratracheal instillation. For the intravenous administration group, a certain amount of UCMSC suspension (0.4 × 10⁶ cells/kg, in 50 µL of normal saline) was injected through the tail vein, whereas for the intratracheal instillation group, anesthetized rats were placed on a fixator and their glottis was exposed using a small animal laryngoscope to press the tongue. A blunt-needled syringe containing UCMSC suspension (0.4 × 10⁶ cells/kg, in 50 µL of normal saline) was gently inserted into the trachea, and the UCMSC suspension was thereafter slowly instilled into the lungs (within 5–10 s). Following instillation, the head of the rat was rotated to the left and right to evenly distribute the UCMSC suspension in each lung lobe. Rats in the intratracheal instillation and intravenous injection vehicle groups were administered equal volumes of normal saline.

2.6 Blood Gas Analysis

Arterial blood gases were analyzed as previously described [31] on day 4 of treatment. Briefly, having anesthetized rats with intraperitoneal pentobarbital (60 mg/kg), arterial blood samples were collected and immediately analyzed for oxygen partial pressure and saturation using a blood gas analyzer (Radiometer ABL700, Bronshoj, Denmark).
Fig. 1. Characterization of UCMSCs. (A) The morphology of UCMSCs at passage five. (B) Osteogenic, adipogenic, and chondrogenic differentiation was detected after 3 weeks of induction using Alizarin Red, Oil Red O, and Alcian Blue staining, respectively. Scale bar = 50 µm. (C) Phenotypic analysis of surface markers using flow cytometry. Cells were stained with FITC/PE-conjugated primary antibodies targeting selected surface markers (i.e., CD73, CD90, CD105, CD34, CD14, and HLA-DR). As controls, UCMSCs were also stained with fluorochrome-conjugated isotype IgGs to identify positive cells. UCMSCs, umbilical cord mesenchymal stem cells; FITC, fluorescein isothiocyanate; PE, Phycoerythrin.

2.7 Enzyme-Linked Immunosorbent Assay Analysis

The levels of inflammatory factors [TNF-α and IL-1β] in serum were determined using enzyme-linked immunosorbent assays (ELISAs). Briefly, on day 4 of treatment, serum was collected, and the concentrations of TNF-α and IL-1β were detected using respective ELISA kits (RayBiotech Inc., Norcross, GA, USA) according to the manufacturer’s instructions.

2.8 Preparation and Analysis of BALF

Bronchoalveolar lavage fluid (BALF) was prepared using a previously described method [31]. Briefly, on day 4 of treatment, the tracheas of anesthetized animals were surgically exposed, and a cannula was inserted in the left bronchus, following which, the left lung bronchus was separated and ligated. Phosphate-buffered saline (PBS: 3 mL) was injected into the lung using a syringe attached to the tracheal cannula, retained within the lung for 10 s, and then retrieved. This step was repeated three times. The collected BALF was thereafter centrifuged at 2000 rpm for 10 min at 4 °C, with the total protein content of the resulting supernatant being determined using a BCA total protein detection kit (Beyotime Institute of Biotechnology, Shanghai, China). The sedimented pellet was resuspended in 1 mL of PBS, and the neutrophils, total white blood cells, and lymphocytes in the suspension were counted using an automatic blood cell analyzer (IC1000; Countstar, Shanghai, China).

2.9 Lung Tissue Histological Analysis

Lung tissues were fixed in 4% paraformaldehyde solution (Sangon Biotech, Shanghai, China), embedded in paraffin blocks, and cut into 5-µm-thick sections. The sec-
Fig. 2. Blood gas parameters and survival rate analysis after UCMSC treatment via two delivery routes. (A) Oxygen pressure and oxygen saturation were measured using arterial blood gas analysis. (B) Analysis of survival rate. Normal: normal mice group; ALI: LPS-induced ALI model; vehicle-iv: ALI model with intravenous injection of saline; vehicle-it: ALI model with intratracheal injection of saline; MSC-iv: ALI model with intravenous injection of UCMSCs; MSC-it: ALI model with intratracheal injection of UCMSCs. *p < 0.05; **p < 0.01. ALI, acute lung injury; LPS, lipopolysaccharide; MSC, Mesenchymal stem cell.

3. Results

3.1 Isolation and Characterization of UCMSCs

UCMSCs were isolated from umbilical cord tissues, and having cultured the cells until passage 5, they were characterized based on morphology, mesenchymal lineage differentiation, and surface markers. The UCMSCs had a typical spindle-shaped morphology (Fig. 1A), and following in vitro induction, were successfully differentiated into mesenchymal derivatives, including osteoblasts, adipocytes, and chondrocytes (Fig. 1B). Furthermore, as determined using flow cytometry, cells were positive for CD73, CD90, and CD105, although negative for the hematopoietic markers CD34, CD14, and HLA-DR (Fig. 1C). These findings indicated that the isolated UCMSCs fulfilled the standard criteria for typical MSCs, as outlined in the International Society for Cellular Therapy guidelines [32].

3.2 Establishment of an LPS-Induced ALI Rat Model

We initially constructed an LPS-induced ALI rat model, as confirmed by observations indicating that the LPS aerosol-treated mice had developed a series of pathologies consistent with ALI, including alveolar cell infiltration, epithelial cell proliferation, wall thickening, and hemorrhage, and fibrinoid exudation. Moreover, we detected a significant upregulation of the levels of total white blood cells, lymphocytes, total proteins, and neutrophils in BALF, and significant increases in levels of the inflammatory cytokines TNF-α and IL-1β in the serum of ALI model rats. In addition, we recorded significant reductions in oxygen pressure and saturation in response to LPS treatment (Figs. 2,3,4,5). These findings accordingly indicated the successful construction of an LPS-induced ALI rat model which could be used for subsequent experiments.

3.3 Arterial Blood Gas Analyses and Survival

ALI is characterized by acute respiratory insufficiency, and oxygen deficiency in arterial blood is the main criterion assessed for the diagnosis of ALI [33]. Accordingly, arterial blood gas analysis is considered to be important in assessing lung function and monitoring the respiratory status of ALI/ARDS patients [34]. In the present study, having collected the arterial blood of ALI rats subjected to different treatments, we measured oxygen pressure and saturation using a blood gas detection instrument, and found that compared with rats in the ALI group, there were significant elevations in both the pressure and oxygen content of arterial blood in the UCMSCS-iv and UCMSCS-it groups, thereby indicating that there had been a certain improvement in the lung function of these latter rats, and that there was no significant difference between the two UCMSC-treated groups in this regard (Fig. 2A). Moreover, compared with the ALI rats, those in the two UCMSCs-treated groups were characterized by increased survival (Fig. 2B). These findings thus indicated that transplantation of UCMSCs via intravenous and intratracheal routes has similar effects with respect lung function improvement and survival.
Fig. 3. Histopathology analysis of lung tissue. (A) Representative images of histopathologic changes in different groups by H&E staining. Scale bar = 50 µm. Black arrows indicated neutrophil infiltration; blue arrows indicated thickened alveolar walls. (B) Pathological scores of alveolar wall thickness. (C) Pathological scores of neutrophil infiltrations. Normal: normal mice group; ALI: LPS-induced ALI model; vehicle-iv: ALI model with intravenous injection of saline; vehicle-it: ALI model with intratracheal injection of saline; MSC-iv: ALI model with intravenous injection of UCMSCs; MSC-it: ALI model with intratracheal injection of UCMSCs. *p < 0.05; **p < 0.01.

3.4 Pathological Evaluation

ALI causes extensive lung tissue damage, and in severe cases, irreversible pulmonary impairment, which may lead to death [35]. Our assessment of the pathological features of ALI in rats revealed that the degree of pathological changes induced by LPS, including multifocal, interstitial, inflammatory cell infiltration, accumulation of foamy macrophages in alveoli, and other structural destruction, was reduced in rats in the UCMSCs-iv and UCMSCs-it groups (Fig. 3). This amelioration of lung injury in response to UCMSC treatment was further confirmed by evaluations of alveolar wall thickness and neutrophil infiltration, and we detected no significant difference between rats in the two UCMSC-treated groups in this regard.

3.5 Effects on BALF Constituents

Increases in the number of cells and protein concentrations in BALF are considered to be indicative of augmented endothelial and epithelial permeability [36]. In the present study, we collected BALF on day 4 of treatment, using which, we performed cell counts and determined pro-
protein concentrations. The results revealed that, compared with the control group, UCMSC transplantation resulted in reductions in the numbers of total white blood cells, lymphocytes, and neutrophils, and in the concentration of total proteins in BALF. Moreover, compared with rats that had received UCMSCs intravenously, intratracheal administration was found to result in more pronounced reductions in the numbers of total white blood cell and lymphocytes, as well as total protein concentrations (Fig. 4).

3.6 Effects on Inflammatory Cytokines

ALI is characterized by the release of multiple inflammatory factors, including TNF-α, IL-1, IL-6, PAF, IFN-1, and PLA2, among which, TNF-α and IL-1β have the potential to trigger further inflammatory responses [37]. Our ELISA analyses of the concentrations of TNF-α and IL-1β in rat sera revealed significant increases in the levels of these cytokines in the sera of rats treated with LPS, whereas levels were reduced in the rats administered UCMSCs. Moreover, compared with rats receiving UCMSCs via the intratracheal route, those administered these cells intravenously were found to be characterized by more pronounced reductions in serum IL-1β levels (Fig. 5).

4. Discussion

Given the associated high mortalities and the limited treatment options, ALI and its more severe form ARDS present considerable challenges in critical care medicine [38–40]. In this respect, UCMSCs have emerged as a promising therapeutic avenue for the treatment of ALI, of-
Fig. 5. Detection of inflammatory factors in serum. (A) The expression levels of TNF-α in serum. (B) The expression levels of IL-1β in serum. Normal: normal mice group; ALI: LPS-induced ALI model; vehicle-iv: ALI model with intravenous injection of saline; vehicle-it: ALI model with intratracheal injection of saline; MSC-iv: ALI model with intravenous injection of UCMSCs; MSC-it: ALI model with intratracheal injection of UCMSCs. *p < 0.05; **p < 0.01. TNF-α, tumor necrosis factor α; IL-1β, interleukin 1β.

By providing valuable insights regarding the comparative therapeutic effects of UCMSCs administered via intravenous injection and intratracheal instillation in an LPS-induced ALI model, our findings in this study reaffirm the therapeutic potential of UCMSCs for the treatment of ALI and shed light on the differential outcomes associated with distinct delivery routes. Both intravenous injection and intratracheal instillation of UCMSCs were found to be effective in alleviating the characteristic features of ALI, as evidenced by improvements in arterial blood gas parameters, lung histopathology, BALF composition, and the serum concentrations of inflammatory factors. Importantly, the levels of arterial blood gas can serve as valuable prognostic indicators in ALI/ARDS patients, reflecting disease severity and the response to treatment [43,44]. These findings accordingly highlight the multiple benefits of UCMSC therapy in alleviating ALI-induced lung injury and inflammation.

Notably, our findings revealed certain differences between the two UCMSC delivery routes regarding their effects on specific ALI-related parameters. Intratracheal instillation of UCMSCs was found to be associated with lower levels of lymphocytes, total proteins and inflammatory cytokines (TNF-α and IL-1β) in BALF (Supplementary Fig. 1), thereby tending to indicate a more pronounced effect on local lung inflammation and alveolar wall repair. In contrast, UCMSCs administered via intravenous injection were observed to promote a significant reduction in the serum levels of the key pro-inflammatory cytokine IL-1β, thus highlighting the systemic anti-inflammatory effects of UCMSCs delivered via this route. These differential delivery-route-associated responses would tend to imply that the therapeutic effects of UCMSCs administered through different routes may be mediated via distinct mechanisms. For example, it is conceivable that intratracheally instilled UCMSCs may interact directly with resident lung cells or infiltrating neutrophils [45], thereby influencing local inflammatory processes and promoting tissue repair, whereas systemically delivered UCMSCs may modulate systemic inflammatory responses and promote distant effects on target organs or various immune cells in peripheral blood [23,46] beyond the lungs. These findings thus provide a valuable basis for further investigations with a view toward elucidating the mechanisms underlying the distinct responses to UCMSCs administered via different delivery routes. This is considered to be of particular importance given that an understanding of the specific cellular targets and molecular pathways modulated by the intravenous injection and intratracheal instillation of UCMSC will be essential for optimizing treatment strategies and enhancing therapeutic outcomes.

The observed robust efficacy of UCMSC therapy highlights the potential of stem cell-based interventions in ALI management. Further clinical analyses are warranted to validate and expand upon the findings of this study. Determining the long-term effects, optimal dosing regimens, and potential synergistic effects of combining multiple delivery routes for UCMSC transplantation could further enhance the efficacy of this promising therapeutic approach. Moreover, characterizing the immunomodulatory proper-
ties and regenerative potential of UCMSCs in diverse preclinical models of ALI and ARDS will ultimately pave the way for the translation of UCMSC therapy into clinical practice.

5. Conclusions

Our findings in this study provide compelling evidence to indicate the potential therapeutic efficacy of UCMSC transplantation in ALI treatment and highlight the significance of considering delivery route-dependent differences in therapeutic outcomes. By elucidating the distinct mechanisms underlying the effects of intravenous injection and intratracheal instillation of UCMSCs, these findings lay a solid foundation for further studies that will seek to optimize stem cell-based therapies for acute lung injury and related respiratory conditions, thereby offering hope for improved outcomes in critical care settings.

Availability of Data and Materials

The data of this study are available from the corresponding author upon reasonable request.

Author Contributions

XLX, FFW, QZ designed and performed the experiments; SSX, XXY analyzed and interpreted the data; XHF collected literatures and drafted manuscript; RLP, YJ proposed the concept, revised and reviewed the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The experimental protocol for animal study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), Zhejiang Center of Laboratory Animals (ZJCLA), NO. ZJCLA-IACUC-20110043. The collection of umbilical cord was approved by Ethics Committee of S-Evans Biosciences (NO.2020-01). All subjects have signed the informed consent form for umbilical cord donation.

Acknowledgment

Not applicable.

Funding

This work was supported by the Zhejiang Province Traditional Chinese Medicine Science and Technology Project (grant number 2021ZB2906217); and the Key Technologies R&D Program of Zhejiang Province (grant number 2019C03041, 2021C03077).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2906217.

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