Study of mesenchymal stem cells derived from lung-resident, bone marrow and chorion for treatment of LPS-induced acute lung injury

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ABSTRACT

Background: Mesenchymal stem cells (MSCs) have been shown to improve acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). However, the optimal source of MSCs for cell-based therapy remains unknown. To determine which kind of MSCs are more effective, we compared the effects of rat lung resident MSC (LRMSC), human chorion-derived MSC (HMSC-C) and human bone marrow derived MSC (HMSC-BM) in LPS-induced ALI in mice.

Methods: LPS (Pseudomonas aeruginosa) was used to induce ALI model. All three kinds of MSCs were administered via tail vein 4 h after LPS instillation. The mice were sacrificed 48 h after LPS instillation. HE staining of lung tissue, wet-to-dry weight ratio of lung tissue, ratio of regulatory T cells (Tregs) and Th17 cells, and total protein concentration, leukocytes counting and cytokines in bronchoalveolar lavage fluid (BALF) were evaluated.

Results: The data showed that compared with LRMSC and HMSC-BM, HMSC-C more significantly attenuated lung injury, upregulated the Tregs/Th17 cells ratio, and inhibited release of inflammatory cytokines (IL-1β, IL-6 and TNF-α) and recruitment of neutrophils and macrophages into alveolus.

Conclusions: Although all three kinds of LRMSC, HMSC-C and HMSC-BM are protective against LPS-induced lung injury, HMSC-C was more effective than LRMSC and HMSC-BM to treat LPS-induced lung injury.

1. Introduction

ARDS is characterized by hypoxemia and increased lung permeability, would result in acute respiratory failure and with high mortality (Ware and Matthay, 2000; Rubenfeld et al., 2005). Although mechanical ventilation with small tidal volume and prone position ventilation can reduce the mortality rate of ALI/ARDS, the mortality still up to 40% due to no effective pharmacological treatment options (Matthay et al., 2012; Calfee and Matthay, 2007; Lee et al., 2009).

In recent years, the strategy of mesenchymal stem cells (MSCs) to treat lung injury has become a research hotspot, and it is playing a great therapeutic potential. MSCs are important members of the stem cell family, derived from mesoderm and ectoderm. Due to its multidirectional differentiation potential, proliferative ability and self-replication, it has attracted increasing attention.

Numerous studies have confirmed that MSCs can reduce the acute lung injury caused by lipopolysaccharide (LPS) derived from Escherichia coli or Pseudomonas aeruginosa, effectively inhibit the inflammatory response, reduce bacterial colonization and regulate the immune response (Feng et al., 2020; Park et al., 2019; Morrison et al., 2017; Krasnodembskaya et al., 2012, 2010; Lee et al., 2013; Wang et al., 2018). Park’s team found that microvesicles released by MSCs can

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increase alveolar fluid clearance and significantly reduced bacterial colonization in human isolated lung E. coli infection models (Park et al., 2019). Morrison et al. reported that human bone marrow MSC transduce their mitochondria to macrophages by generating extracellular vesicles, thereby enhancing the ability of macrophages to phagocytose and inhibit the release of inflammatory cytokines in bronchial alveolar lavage fluid (BALF) of patients with ARDS (Morrison et al., 2017). Early study by our group found that MSC can enhance monocyte-macrophage phagocytic capacity (Krasnodembskaya et al., 2012), secrete antimicrobial peptide LL-37 (Krasnodembskaya et al., 2010) and keratinocyte growth factor (KGF) (Lee et al., 2013) to improve lung injury. We recently found that there is an imbalance of regulatory T cells (Treg) and Th17 cells in the ALI model induced by intratracheal LPS instillation, and administration of MSCs can upregulate Treg cells and downregulate Th17 cells to correct the imbalance of Treg cells and Th17 cells and reduce lung injury (Wang et al., 2018). These studies have confirmed that MSCs have positive effects on lung injury.

Now, extensive clinical studies are designed to test the effect of MSCs in patients with moderate to severe ARDS. However, the optimal source of MSCs for the treatment ALI/ARDS is still unknown. MSCs are adult nonhematopoietic precursor cells, which can be derived from a variety of tissues, such as bone marrow, placenta, adipose tissue and lung. MSCs residing in the tissue represent a reservoir of endogenous organ-specific adult progenitor cells and has a potential role in local tissue homeostasis and repair. Our team found that FGF-10 pretreatment can also be used to isolate MSCs from the lower respiratory tract of adult healthy rats, and the administration of LR-MSC into the lungs after LPS injury reduced the inflammatory response (Tong et al., 2016). To determine which kind of MSCs is more efficacious, we compared LR-MSC, human chorion-derived MSCs (HMSC-C) and human bone marrow derived MSCs (HMSC-BM) in LPS-induced ALI in mice.

2. Methods

2.1. Isolation and culture of LRMSC, HMSC-C and HMSC-BM

LRMSC were isolated from BALF of rats as previously described (Lee et al., 2013; Wang et al., 2018). Briefly, BALF was obtained from rats pretreated with FGF-10, centrifuged, resuspended, seeded in T25 cell culture flasks. A homogeneous population of LRMSC was obtained after 3–5 passages. And the 4th and 5th passages of LRMSC were used for the experiments.

Human chorion-derived MSCs were a kind gift from Shunxi regenerative medicine company (China, Yunnan). Bone marrow derived MSCs purchased from Sciencell corporation. The 4th and 5th passages of these three kinds of MSCs were used for the experiments, and met all MSC criteria defined by the International Society for Cell Therapy.

2.2. Animals

Eight weeks old male Sprague-Dawley rats, and eight weeks old male C57BL/6 mice weighing 20–25 g were purchased from JSJ Lab (Shanghai, China), raised carefully in specific pathogen-free cages and maintained at temperatures between 20 °C and 25 °C and relative humidity of 50–70% in the Medical School of Fudan University. The animal protocol in this work was in accordance with the ethical guidelines of the National Institutes of Health on Animal Care and the Animal Care and Use Committee of Fudan University, Shanghai.

2.3. Animal treatment

For the LPS-induced ALI model, the procedures as previously described [14]. 4 h after LPS instilled into trachea, PBS or 5 × 10^5 LRMSC, HMSC-C or HMSC-BM (dissolved in 0.2 mL PBS) were administrated into mice via the tail vein. After 48 h when LPS instillation, the mice were sacrificed with an intraperitoneal injection of chloral hydrate.

2.4. Total and differential cell count

The procedures to process BALF as previously described in our previous study (Wang et al., 2019). Briefly, BALF was centrifuged, and the supernatant was stored at −80 °C for further analysis. The total number of nucleated cells was counted with a hemocytometer, stained with the Wright–Giemsa stain.

2.5. Wet-to-dry ratio (W/D)

The right lower lobe of the lung was isolated, wiped clean with tin foil paper. Weight was determined immediately after isolation and after being oven dried at 60 °C for 72 h.

2.6. Protein concentration and cytokines in BALF

Bicinchoninic acid (BCA) protein assay kit (Beyotime, China) was used to test BALF protein concentration. Enzyme-linked immunosorbent assays (ELISA) were used to measure levels of IL-1β, IL-6, TNF-α, IL-17, IL-22, IL-10, and TGF-β (eBioscience, San Diego, CA, USA) in BALF supernatant.

2.7. H&E staining

After the mice were sacrificed, the right upper lobe of the lung was fixed in 4% parafomaldehyde overnight, embedded in paraffin, sectioned, stained with hematoxylin and eosin (H&E). The histological score of lung injury was determined by two investigators in a blind manner.

2.8. Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TRIZol reagent (Invitrogen), reverse-transcribed into cDNA using the cDNA kit (TOYOBO, Japan), amplified by SYBR-Green I Real-Time PCR kit (TOYOBO). The required primers were designed and synthesized by Shenggong (Shanghai, China). Each reaction was run in triplicate and normalized to the housekeeping gene β-actin transcripts.

2.9. Flow cytometry analysis

The single cell suspensions of lungs were obtained as previously described (Wang et al., 2019). Briefly, lung was dissected into single lobes, digested with an enzyme mix (buffer S, enzyme D, and enzyme A) (Miltenyi, Bergisch Gladbach, Germany) into single cells suspensions, subjected to density gradient centrifugation using Ficoll-Paque to isolate lymphocytes. Collected cells were used to test Tregs and Th17 cells as previously described (Wang et al., 2019). Briefly, for analysis of Tregs in lung, the collected cells incubated with surface markers antibodies FITC-anti-CD4 and APC-anti-CD25 and intracellular staining with PE-anti-Foxp3 (eBioscience).

First, a mixture of PMA, ionomycin, and brefeldin A was used to stimulate cytokine expression in Th17 cells for 6 h. Then, the surface marker antibodies FITC-anti-CD3, APC-anti-CD8 and intracellular staining with PE-anti-IL-17A were to detect Th17 cells.

2.10. Statistics

SPSS v17.0 statistical software for Windows was used for all statistical analyses. The results are expressed as mean ± standard deviation (SD). P < 0.05 was defined as a statistical significance.
3. Results

3.1. Therapeutic effects of MSCs on lung injury and inflammatory response

Intratracheal instillation of LPS induced a robust inflammatory response in lung (Figs. 1A and 1B). Administration of LRMSC, chorion-derived MSCs and bone marrow derived MSCs reduced inflammatory cells infiltration and interstitial thickening induced by LPS (Fig. 1C-E). We also found that administration of LRMSC, chorion-derived MSCs and bone marrow derived MSCs significantly decreased the lung injury score, and the effect of chorion-derived MSCs and bone marrow derived MSCs were stronger than that of LRMSC (Fig. 1F). Moreover, all these three kinds of MSCs reduced the influx of inflammatory cells in BALF (Fig. 2) and decreased the secretion of the inflammatory cytokines IL-1β, IL-6, and TNF-α in the injured alveolus, and the effect of chorion-derived MSCs is

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Fig. 1. Histological evaluation of lung injury. Lung sections were stained with hematoxylin and eosin (40 × magnification). A: PBS+PBS group; B: LPS+PBS group; C: LPS+LRMSC group; D: LPS+HMSC-C; E: LPS+HMSC-BM; F: Bar graph of lung injury score. * vs. PBS+PBS, # vs. LPS+PBS.

Fig. 2. Measurement of inflammatory cells in BALF. (A) total cell count; (B) percentage and absolute count of neutrophils in BALF; (C) percentage and absolute count of neutrophils in BALF. * vs. PBS+PBS, # vs. LPS+PBS.
3.2. Therapeutic effects of MSCs on lung vascular permeability

Lung W/D ratio and protein concentration in BALF are markers of lung vascular permeability. Lung W/D ratio and total protein concentration in BALF were increased in LPS ALI model. Administration of LRMSC, chorion-derived MSCs and bone marrow derived MSCs significantly reduced lung W/D ratio (Fig. 3B) and total protein concentration (Fig. 3C) in BALF. The effect of these kinds of MSCs on lung W/D ratio was similar, and chorion-derived MSCs was the strongest to decrease total protein concentration in BALF.

3.3. Therapeutic effects of MSCs on balance of Tregs and Th17 cells

Studies included our previous study indicated that it is crucial to maintain the balance of Tregs and Th17 cells in ALI, and LRMSC administration could regulate the balance [12,14]. In this our study, we compared the effect of LRMSC, chorion-derived MSCs and bone marrow derived MSCs on the balance of Tregs and Th17 cells. We found that LPS instilled through the trachea significantly increased Tregs in lung (Fig. 4B) and Tregs-related cytokine IL-10, but not TGF-β (Fig. 5A). LPS instillation also elevated the percentage of Th17 cells in lung (Fig. 4A) and the levels of the Th17-related cytokines IL-17 and IL-22 (Fig. 5B). These data are consistent with our previous studies (Wang et al., 2019). Administration of LRMSC, chorion-derived MSCs and bone marrow derived MSCs increased Tregs and IL-10 levels (Figs. 4B and 5A), reduced Th17 cells and levels of IL-17 and IL-22 (Figs. 4A and 5B).

We further found the ratio of Tregs and Th17 cells in lung decreased after LPS instillation. Treatment with LRMSC, chorion-derived MSCs and bone marrow derived MSCs also increased the ratio of Tregs and Th17 cells compared with LPS-induced mice, and the therapy effect of chorion-derived MSCs were the best (Fig. 4C).

Fig. 3. Lung inflammatory cytokines and total protein concentration in BALF and lung W/D weight ratio. (A) IL-1 β, IL-6 and TNF-alpha in BALF; (B) total protein concentration in BALF; (C) lung W/D weight ratio. * vs. PBS+PBS, # vs. LPS+PBS.

Fig. 4. Percentage of CD4⁺CD25⁺Foxp3⁺ Tregs and Th17 cells of lung. (A) Percentage of Th17 cells in lung was determined using flow cytometry. (B) Percentage of CD4⁺CD25⁺Foxp3⁺ Tregs in lung was determined using flow cytometry. (C) Ratio of Tregs and Th17 cells is shown in bar graphs. * vs. PBS+PBS, # vs. LPS+PBS.
3.4. Therapeutic effects of MSCs on expression of KGF-2 and SPC in lung tissue

Instillation of LPS decreased KGF-2 and SPC mRNA levels in mice, whereas administration of LRMSC, chorion-derived MSCs and bone marrow derived MSCs significantly restored its levels (Fig. 6A and B). Moreover, we found caspase 3 and PCNA mRNA increased after instillation of LPS, and administration these three kinds of MSCs significantly decreased its levels (Fig. 6C and D). And the effect of chorion-derived MSCs is the most obvious to upregulate SPC mRNA.

4. Discussion

So far, many ALI/ARDS preclinical studies indicated MSCs could improve both survival and lung inflammation (Xu et al., 2007; Ortiz et al., 2003; Rojas et al., 2005). To determine the optimal source of MSCs for ALI/ARDS, we compared the effect of bone marrow (BM), chorion derived human MSC and lung resident MSCs to treat ALI. In this study, we found that HMSC-BM, HMSC-C and LRMSC had a profound anti-inflammatory effect in LPS-induced ALI in mice by preventing the influx of inflammatory cells, decreasing the level of chemokines/cytokines in the injured alveolus and the lung W/D ratio, and attenuated the observed histopathological impairments. These results demonstrate that the potential advantages of MSCs of the restoration of alveolar-capillary membrane function and lung injury repair.

Further work is needed to investigate the underlying mechanism.

IL-10 is an anti-inflammatory cytokine secreted by monocytes and plays an important role in downregulating the expression of Th1 cytokines, costimulatory molecules on macrophages and MHC class II antigens. In this study, we found that the expression of cytokine IL-10 in BALF was significantly increased after MSCs transplantation, and the increase was most obvious after HMSC-C transplantation. We hypothesized that MSCs may inhibit inflammation in lung injury mainly by paracrine function. Further work is needed to confirm this hypothesis.

KGF-2 is an important growth factor in the repair of damaged lung. The benefit of KGF-2 in the injured lung has been confirmed (She et al., 2012; Fang et al., 2014; Bi et al., 2014). It could promote the proliferation of alveolar type 2 cells and inhibit the inflammatory response in the injured lung (Tong et al., 2014; Feng et al., 2016). The higher expression of KGF-2 in the all MSCs groups demonstrated the effect of MSCs.

The current study has several limitations. First, the dose of LPS, the quantity and sources of MSCs, and the timing and route of MSCs administration remains controversial which related to the different results. Studies have confirmed that systemic intravenous MSC delivery may have an efficacy equal to that of direct intratracheal delivery. Second, better beneficial effects of the HMSC-C administration were observed, but the precise mechanisms remain unclear.

In conclusion, our findings indicate that all these kinds of LR-MSC, HMSC-C and HMSC-BM administration improve lung injury and inhibits the inflammatory response by upregulating the Tregs/Th17 cells ratio and IL-10, decreasing inflammatory cells recruited into alveolus, and the therapeutic effect of HMSC-C was the best.
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CRediT authorship contribution statement

Linlin Wang performed the experiments, analyzed the data, and wrote the manuscript. Maosen Dou, Yun Feng, Jian Wang, Donghui Zhang, Dongni Hou, Jing Bi and Cuicui Chen analyzed the data. Yuanlin Song, Lin Tong, Jian Zhou and Chunxue Bai designed the study, and wrote and revised the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors have stated explicitly that there are no conflicts of interest regarding this article.

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