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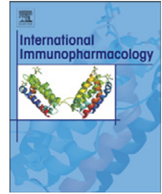

Therapeutic effects of bone marrow-derived mesenchymal stem cells on pulmonary impact injury complicated with endotoxemia in rats

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ABSTRACT

Previous studies have demonstrated that acute lung injury (ALI) is associated with significant mortality and so far no effective pharmacotherapy is shown to reverse the natural progression. This study aims to investigate whether the transplantation of bone marrow derived mesenchymal stem cells (MSCs) might restore damaged pulmonary function and tissue structure in ALI. By using a sublethal chest impact injury plus endotoxemia model, MSCs were intravenously transplanted into the injured rats 2 h after trauma. The blood samples were obtained for determination of blood gas. Bronchoalveolar lavage fluid (BALF) was collected for analysis of inflammatory cell count and cytokine (TNF-α, IL-1β, IL-6 and IL-10) levels. The left lower lobes of the lungs, nearby the impact zone, were collected for SRY gene analysis and histological examination and scoring. After engraftment of MSCs, the number of inflammatory cells in BALF was decreased. Meanwhile, the secretions of pro-inflammatory TNF-α and IL-6 were alleviated, while the secretion of anti-inflammatory IL-10 was elevated. Engraftment of MSCs improved the pulmonary gas exchanges, alleviated the lung injury, and reduced the rats' mortality. These results suggested that the MSCs based measures might be a promising strategy in trauma and endotoxemia induced ALI treatment.

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1. Introduction

Chest impact injury is a common pattern of injury during road traffic trauma (RTT), accounting for 45% of total injuries and is a leading cause of mortality among this cohort [1,2]. Acute lung injury (ALI) is most commonly seen after chest impact injury and may develop into acute respiratory distress syndrome (ARDS), particularly when there is associated sepsis [3–5]. Despite advanced supportive therapies for patients with ALI, it remains a life-threatening disease with an increasing mortality in these years [6,7]. Pathological findings of ALI/ARDS include diffuse epithelial and endothelial damage with a large number of inflammatory cells, protein-rich edema fluid filling the alveolar spaces, and deposition of intra-alveolar fibrin [8,9]. The clinical consequence of this pathological process is impaired gaseous exchange and respiratory failure. The principal management of ALI/ARDS is largely supportive which includes treatment of underlying diseases, adequate hemodynamic support, and mechanical ventilation with lung protective strategies [10,11]. Hastening the repair process of damaged pulmonary tissues has been thought to be the most effective treatment strategies for ALI/ARDS.

Recent studies have shown that bone marrow-derived mesenchymal stem cells (MSCs) might play an important role in repair processes of injured tissues. Several studies have shown that both ectogenic and endogenic MSCs can migrate into the lung and differentiate into the phenotype of lung cells in places where the injured tissue needs repair [12,13]. Moreover, MSCs reveal strong immunosuppressive effects, and are therapeutically effective in immunocompetent rodent models of diseases, such as multiple sclerosis and stroke [14,15]. In addition, MSCs produce large quantities of bioactive factors, inducing anti-scarring, angiogenic, antiapoptotic and regenerative courses [16,17]. It has been shown that MSCs given intravenously could localize to the lung and thereby provide a local source of trophic factors in the pulmonary environment [18,19]. In ALI/ARDS, growing evidence has suggested that MSCs contribute to lung repair, decreasing mortality rate, lung edema, alveolar epithelial cell permeability, and systemic and local inflammatory response following ventilator-induced lung injury in the rat [20]. The demonstration that human MSCs exert benefit in the endotoxin-injured human lung is also persuasive [21]. Therefore, cell-based therapy seems to be a promising treatment for ALI/ARDS. However, little is known about the therapeutic effect of MSCs in ALI/ARDS induced by pulmonary contusion associated with endotoxemia.

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The current study was aimed to investigate whether the transplantation of MSCs improves pulmonary function and gas exchange in rats with trauma and endotoxemia. Understanding the role of MSCs in the ALI will contribute to further disclosing the repair mechanism in traumatic endotoxemia. Hence, we chose a blunt trauma plus lipopolysaccharide (LPS) challenge model to observe the effects of MSCs on inflammatory parameters, and their relationship with the repair of injured lung tissues. It is necessary to further elucidate the protective mechanism of MSCs in uncontrolled inflammation and provide insights on its effective prophylaxis and therapy.

2. Materials and methods

2.1. Animals

Adult Sprague–Dawley (SD) rats (6 weeks old, 250–300 g, n = 102) were used in this study. The animals were housed in hanging wire mesh cages in the accredited animal facilities of the Research Institute of Surgery. The animal room was specific pathogen-free and was maintained with controlled temperature, humidity and lighting (12-hour light–dark cycles). All animals were given rat chow and tap water ad libitum. The experimental procedures were approved by the Institutional Animal Care and Use Committee of the Third Military Medical University that follows the Ethical principles and guidelines for scientific experiments on animals' of the Swiss Academy of Medical Sciences. This includes minimizing the number of animals for the experiment and taking measures to minimize their suffering.

2.2. Isolation and culturing of MSCs

Bone marrow cells were collected by a modified method [22]. Briefly, 6 week-old male SD rats were individually euthanized by injection with i.p. pentobarbital sodium and sacrificed by cervical dislocation. The femurs and tibiae were dissected out and placed in complete culture media (CCM) [Iscove's modified Dulbecco's medium (IMDM, Hyclone), 20% fetal bovine serum, 100 U/mL penicillin, 100 mg/mL streptomycin and 12 mM L-glutamine (Gibco, MD, USA)]. The ends of each tibia and femur were clipped to expose the marrow. The bones were flushed and marrow cells were collected, centrifuged, resuspended in CCM using a 21-gauge syringe, and followed by filtration through a 70 μm nylon mesh filter. The nucleated cells were isolated by density gradient centrifugation with Ficoll/Paque (Amersham Pharmacia) and resuspended in CCM. The non-adherent cells were discarded after 48 h of culture. After 2 weeks, the cells were washed and detached with trypsin (0.25%)–EDTA. The cells that did not detach within 2 min were discarded. Collected cells from passage 1 (p1) were replated in flasks with CCM for further culture. The MSCs in passages 3–4 (p3–p4) were used for experiments.

2.3. Characterization of MSCs

Differentiation of MSCs was evaluated using a Mesenchymal Stem Cell Functional Identification Kit (R&D Systems, http://www. rndsystems.com/). Chondrogenic and osteogenic differentiation assays were performed in six-well Primaria plates (BD Biosciences, http://www.bdbiosciences.com/). The adipogenic assay was performed in eight-well Lab-Tek II Chamber Slide System (NUNC, http://www.nuncbrand.com/). MSCs were labeled with anti-rat antibodies, CD34, CD45, CD73 and CD90 and corresponding isotype controls purchased from BD Biosciences, and analyzed by flow cytometer (Cytomics FC500, Beckman Coulter, http://www.beckmancoulter.com/).

2.4. Flow cytometric analyses

The isolated cells were suspended in staining buffer (2% heat-inactivated fetal calf serum, 0.09% sodium azide in Dulbecco's PBS; Invitrogen) and blocked using purified rat anti-rats Fc block (BD Pharmingen, San Jose, CA). Cells were stained with phycoerythrin-conjugated anti-CD34 (0.2 mg/mL), anti-CD45 (0.2 mg/mL), anti-CD73 (0.2 mg/mL) or anti-CD90 (0.2 mg/mL) respectively, with streptavidin-APC Cy7 as the secondary stain (0.2 mg/mL; BD Pharmingen). Isotype controls were used to determine background staining. Flow cytometry was performed using the Beckman Coulter Cytomics FC500 (BD Biosciences, USA).

2.5. Acute lung injury

Acute lung injury was reproduced through impacting rat chest using our-own made small impact machine [23] (Fig. 1) in combination with intravenous administration of bacterial LPS (*Escherichia coli* serotype *E. coli* O55:B5, Difco Laboratories, Detroit, MI). After anesthetization with i.p. pentobarbital sodium (35 mg/kg), rats were fixed on the left lateral position. The impact region was the cross point between anterior line axillary and the fourth intercostal space on the right side. The driving pressure was 250 kPa. The weight of the impact pendulum was 7.65 g. The impact velocity was about 9 m/s. Fifteen mg/kg of LPS was intravenously given in 0.9% saline via a tail vein immediately after chest impact.

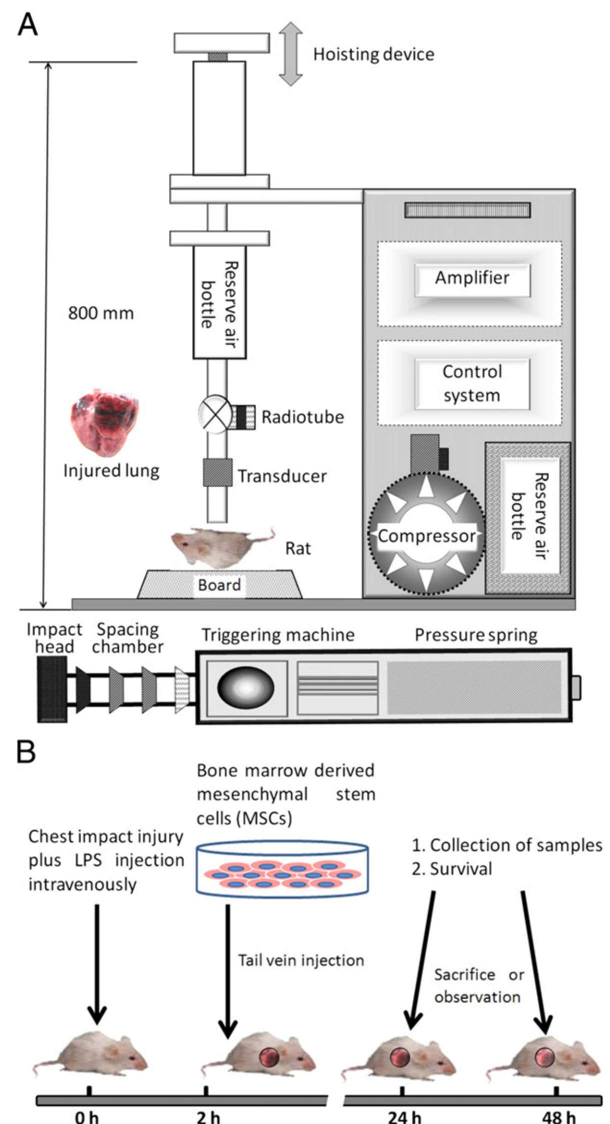

Fig. 1. Schematic illustration of the therapeutic experimental procedure of bone marrow-derived mesenchymal stem cells (MSCs) for the acute lung injury in rats. (A) A small impact machine for the acute lung injury. (B) Therapeutic experimental procedure of MSCs.

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