Mesenchymal stem cells in the treatment of acute respiratory distress syndrome

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ABSTRACT. Acute respiratory distress syndrome is a potentially lethal condition that arises after direct or indirect injury of the lung. Supportive care is the mainstay of treatment with lung protective ventilation and fluid conserving strategies. Cell therapy, and particularly mesenchymal stem cells therapy, has emerged as a promising therapeutic alternative. Multiple preclinical studies have shown that mesenchymal stem cells express their protective effect in the lungs through various mechanisms, including modulation of immune response, repair of endothelial and epithelial injury and enhanced bacterial clearance. This review focuses on the experimental data regarding the use of mesenchymal stem cells and acute respiratory distress syndrome, and the potential mechanism underlying their therapeutic effects. Pneumon 2015, 28(1):70-79.

INTRODUCTION

Stem cells are undifferentiated progenitor cells with a broad developmental potential, that exhibit the capacity to give rise to cells identical to itself or to cells of multiple lineages. Depending on their residency they can be classified as embryonic stem cells and adult stem cells, while according to their potency they are divided in totipotent, multipotent, and unipotent. Adult stem cells are multipotent and include mesenchymal stem cells (MSCs), hematopoietic stem cells, endothelial progenitor cells and organ-specific stem cells, including endogenous lung stem cells. Recent studies showed that stem cell therapy may have application in various disorders including sepsis1, hepatic2 and renal failure3, diabetes4 and myocardial infarction5. Furthermore, an increasing body of evidence from preclinical studies suggests stem cells as alternative treatment for pulmonary fibrosis, chronic obstructive pulmonary disease, asthma and pulmonary hypertension6,7.

Acute respiratory distress syndrome (ARDS) is a leading cause of death in the critical care with approximately 200,000 new cases every year in the US and mortality rates up to 40%8,9. It has now become apparent that ARDS is not simply a form of pulmonary edema caused by increased microvascular permeability, but rather represents a manifestation of direct
or indirect injuries to the alveolar-capillary membrane of the lungs. To date, there are no specific pharmacologic interventions of proven efficacy for the treatment of ARDS. Various therapeutic options proposed in the past including corticosteroids, nitric oxide, exogenous surfactants, anti-oxidant agents, and prone position ventilation in case of severe hypoxemia. Thus, the need for new specific and efficient treatment options is imperative. Preclinical data from cell-based therapies open new perspectives in the management of ARDS. The aim of this review is to summarize current knowledge in the field of stem cells therapy for ARDS. Our search focused on the following databases: PubMed, EMBASE, and Google Scholar using combinations of the following keywords: ALI, ARDS, stem cells and cell based therapy. The search included all types of articles written in English up to November 2014.

MESENCHYMAL STEM CELLS (MSCS)

MSCs are a class of adult stem cells, possess the ability of self-renewal and can differentiate into muscle, bone, fat, fibroblasts, and cartilage. They were discovered on 1968 by Friedenstein et al who first identified bone marrow cells that were adherent, clonogenic and presented with a fibroblast-like morphology. Currently, they can be also isolated from a variety of human tissue including adipose tissue, muscle, lung and placenta. Although MSCs represent approximately 0.1% of total bone marrow cells, they can be easily isolated from whole bone marrow aspirates due to their ability to adhere to plastic and form colonies. Given the lack of a specific surface cell marker, the International Society of Cellular Therapy used the three following criteria in order to define MSCs: 1) plastic adherent cells under standard tissue culture conditions 2) cells with capacity of in vitro duplication and differentiation into mesenchymal lineages, such as osteoblasts, adipocytes and chondroblasts, and 3) cells expressing certain surface markers, like CD73, CD90 and CD105, but lack of expression of other markers including CD45, CD34, CD14, or CD11b.

Several characteristics owned by MSCs placed them in a privileged position among cell based therapy candidates for ARDS. From an immunologic point of view, allogeneic MSCs present low immunogenicity and can evade immune destruction by the host immune system. This property could permit transplantation that is not restricted by the major histocompatibility complex (MHC). The abovementioned tolerance is achieved through a variety of mechanisms including decreased expression of MHC I and MHC II proteins and lack of T-cell co-stimulatory molecules CD80 and CD86. However, recent evidence suggested that allogeneic MSCs may trigger donor specific cellular and humoral immune responses with consequences ranging from reduced in vivo survival and accelerated rejection, to tolerance promotion. Additional benefits refer to their immunomodulatory effects that may shift the balance from an inflammatory to an anti-inflammatory response, their ability to produce a large number of soluble factors, their ease of isolation and propagation and absence of ethical issues regarding their use.

PRECLINICAL STUDIES

A variety of animal models and different routes of delivery were used by the investigators in order to evaluate the effect of MSCs on experimental acute lung injury (Table 1). Systemic administration of MSCs conferred a survival benefit in animal models of bleomycin-induced lung injury. Bleomycin induced loss of endogenous lung MSCs and produced fibrosis and inflammation in mice. Intravenous administration of isolated lung MSCs attenuated the bleomycin-associated pathology through regulation of effector T-cell proliferation. In the study of Ortiz et al intravenous administration of MSCs in mice significantly reduced the effect of bleomycin-induced inflammation and collagen deposition within lung tissue. This effect was independent from engraftment. The same authors later identified interleukin 1 (IL-1) receptor antagonist as the principal mediator of this protective effect. Zhao et al detected 4',6-diamidino-2-phenylindole (DAPI) -labeled MSCs in the lungs of rats after two weeks of bleomycin-induced injury. A number of MSCs were positive for pan-cytokeratin staining, an indicator of alveolar epithelial cells. Additionally, MSCs administration reduced markers of fibrosis and lung injury content in both pulmonary tissue and bronchoalveolar lavage fluid (BALF) and decreased mRNA levels of transforming growth factor-β1 (TGF-β1), platelet derived growth factor- A (PDGF-A), platelet derived growth factor- B (PDGF-B), and insulin growth factor-1 (IGF-1). In two studies where MSCs were transduced by a lentiviral vector respectively expressing 7ND, a dominant-negative inhibitor of the monocyte chemotactic protein 1 (MCP1/CCL2), and keratinocyte...
Lipopolysaccharide (LPS) instillation is a common tool for inducing lung injury. In a murine model where MSCs were administered intratracheally four hours post-injury, a decrease in inflammation by reducing TNF-α and MIP-2 and an increase in IL-10 were observed. Similarly, in a model of LPS-induced lung injury, intratracheal injection of MSCs reduced TNF-α and MIP-2 and increased IL-10 levels.

**Abbreviations:** IFN-γ: interferon-gamma; MMIF: macrophage migration inhibitory factor; TNF-α: tumor necrosis factor-alpha; TGF-β: transforming growth factor-beta; MMP-2: matrix metalloproteinase-2; IL-1: interleukin-1; BAL: bronchoalveolar lavage; TGF-β1: transforming growth factor-beta 1; PDGF-A: platelet derived growth factor –A; IGF-I: insulin-like growth factor I; MIP-2: macrophage inflammatory protein 2; IL-10: interleukin-10; KGF: keratinocyte growth factor; AFC: alveolar fluid clearance; LPS: lipopolysaccharide; IL-1β: interleukin 1beta; IL-6: interleukin-6; SP-C: surfactant protein-C; α-SMA: alpha-smooth muscle actin.

growth factor (KGF) histological damage was attenuated after treatment. Lipopolysaccharide (LPS) instillation is a common tool for inducing lung injury. In a murine model where MSCs...
were delivered directly in the air-space following intrapulmonary administration of Escherichia coli endotoxin, survival was significantly increased in the treated group at 48 hours compared with controls (80% versus 42% respectively, p < 0.01). Protective effects included decrease in extravascular lung water and bronchoalveolar lavage protein, as well as an attenuation of endotoxin induced inflammation expressed as reduced levels of TNF-α and MIP-2 in the bronchoalveolar lavage and plasma and increased levels of IL-10. Similar results were observed by Xu and colleagues. In their study, ex vivo cultures of MSCs and lung cells from endotoxemic animals showed a bilateral conversation in which lung cells stimulated proliferation and migration of stem cells and stem cells caused decreased inflammatory response of lung cells.

In another study, LPS induced lung injury was attenuated after intrapulmonary administration of umbilical cord MSCs through augmenting alveolar CD4⁺CD25⁺Foxp3⁺Treg levels and balancing anti- and pro-inflammatory mediators in mice. Instillation of human allogeneic MSCs in an ex vivo perfused human lung preparation injured by endotoxin, reduced extravascular lung water, improved lung endothelial barrier permeability and restored alveolar fluid clearance. Authors identified KGF as the fundamental mediator for this restorative effect.

Qin and colleagues compared lung injury and histopathology between controls and rats with endotoxin-induced lung injury after intrapleural injection of MSCs. Tissue samples and BALF were collected on days 1, 3 and 7 after instillation and MSCs were marked with DAPI. Treatment group demonstrated improved histopathology at day 3 and decreased total cell counts and protein concentration in BALF at day 7. Additionally, TNF-α levels at day 3 were significantly reduced in both BALF and lung tissue in the MSCs group in comparison to controls, while IL-10 levels in lung tissue were significantly higher in day 1. In another LPS-induced lung injury animal model, adrenaline enhanced proliferation of bone marrow MSCs, promoted migration towards injured areas, modulated inflammation by shifting to an anti-inflammatory response and limited the extent of injury. Adipose derived MSCs overexpressing soluble IL-1 receptor-like-1 applied in a murine lung injury model preserved alveolar architecture, abolished apoptosis and minimized inflammatory cell infiltration. Li et al. studied the effect of human umbilical cord MSCs injection in LPS-induced lung injury in a rat model. Treated animals demonstrated improved survival rates with decreased serum concentrations of pro-inflammatory mediators TNF-α, IL-1β, and IL-6 and stable levels of anti-inflammatory cytokine IL-10. Lung tissue exhibited improved myeloperoxidase activity, decreased malondialdehyde production and increased heme oxygenase-1 protein production and activity.

Zhang et al. assessed the effects of bone marrow derived MSCs in a hyperoxia induced lung injury murine model. Intraperitoneal injection of cells improved pulmonary architecture, attenuated pulmonary fibrosis and increased the survival rates in treated animals. MSCs homed to the affected lung and expressed surfactant protein-C, a specific marker of type II alveolar epithelial cells. Plasma expression levels of IL-1 and TNFα were also decreased. Chang et al. examined the effects of intratracheal and intraperitoneal transplantation of human umbilical cord-derived MSCs. Hyperoxia-induced increase in the number of TUNEL positive cells, myeloperoxidase activity and IL-6 mRNA levels were attenuated independently from delivery route. On the other hand, impaired alveolarization and increases in level of TNF-α, TGF-β mRNA, α-SMA protein and collagen were significantly attenuated only with the intratracheal treatment. The significance of appropriate dosing was investigated in a study that used increasing doses of human umbilical cord MSCs in neonatal rats. Results showed attenuation of hyperoxia-induced lung injuries and inflammatory markers in a dose-dependent manner. Treatment was proved more effective when applied in the early, postnatal day 3, but not in the late phase, postnatal day 10, of inflammation. The long-term effects and safety of intratracheal delivery of human umbilical cord blood-derived MSCs were evaluated in a hyperoxic lung injury rat model. Indices of impaired alveolar and vascular growth (increased mean linear intercept and decreased amount of von Willebrand factor) and inflammatory responses were both attenuated after treatment. Protective effects were sustained until postnatal day 70 without any long-term adverse effects.

MECHANISMS OF ACTION

Cell engraftment

Early studies suggested that engraftment played a fundamental role in the treatment of ARDS with MSCs. Stem cells could differentiate into lung epithelial cells and replace damaged cells. Krause et al. reported that a single bone marrow-derived haematopoietic stem cell could give rise to a variety of cells from multiple organs, including the lung. They noted 20% engraftment of bone marrow-derived cells in the lung, including epithelial
cells. Intravenously injected bone marrow derived MSCs engrafted in the injured lung parenchyma as cells expressing morphological and molecular characteristics of type I pneumocytes of the alveolar epithelium. Additionally, in vitro cultures of fresh aspirates of bone marrow MSCs expressed type I pneumocyte markers, such as T1α and aquaporin-5. Fluorescence in situ hybridization, after systemic administration of MSCs purified by immunodepletion from male bleomycin-resistant into female bleomycin-sensitive mice, showed that engrafted male cells homed to areas of bleomycin-induced injury lung and exhibited an epithelium-like morphology. Yamada et al treated mice, with LPS-induced lung injury, with bone marrow-derived cells from a fluorescent protein donor. Results demonstrated accumulation of bone marrow-derived cells within the inflammatory site and differentiation into epithelial and endothelial cells that expressed cytokeratin and CD34. Animals receiving MSCs transplantation after bleomycin-induced lung injury presented substantial numbers of donor-derived cells in their lungs 14 days after bleomycin. These cells showed phenotypic characteristics of several lung cell types, including type I and II pneumocytes, fibroblasts and endothelial cells.

However, a number of studies questioned the above-mentioned results. Wagers et al generated chimeric animals by transplantation of a single bone marrow hematopoietic stem cells green fluorescent protein–marked into lethally irradiated non-transgenic recipients. Although hematopoietic stem cells contributed to the generation of mature hematopoietic cells detected in all tissues, no green fluorescent protein positive cells were found in lung, kidney, gut or muscle. In another study investigators used a lineage-specific reporter system based on transgenic mice that expressed the green fluorescent protein reporter gene only in lung epithelial cells to assay for engrafted cells. They concluded that once autofluorescence, dead cells, and contaminating blood cells were excluded from analysis, there was no detectable reconstitution of lung alveolar epithelial cells by unfractionated bone marrow cells or purified hematopoietic stem cells. Low engraftment rates were also described by other laboratories.

Mechanisms by which MSCs might be recruited to the lungs remain poorly understood. A hypothesis suggested that lung injury could induce release of a variety of signaling factors, which may trigger the expansion of endogenous pool of MSCs or serve as local chemokine-attractants for migration of exogenous MSCs. It was also proposed that MSCs may express chemokine receptors in response to the chemokine-attractive gradient generated by the injured lung tissue and the interaction between chemokines and their receptors could induce MSCs to home various tissues. Overall, results from different studies are often contradicting. Even if engraftment and differentiation into lung epithelial cells does not seem to play a significant role in mediating the effects of MSCs in ARDS, further research is necessary.

Immunomodulation

MSCs possess the ability to modulate immune response, including both innate and adaptive responses, probably by inhibiting the activation and proliferation of T lymphocytes and altering the function of natural killer cells, B cells and dendritic cells. The immunomodulation is expressed through cell contact-dependent and paracrine mechanisms. A variety of soluble factors are involved, such as transforming growth factor-β, tumour necrosis factor-α-induced protein-6, IL-10 and IL-1-receptor antagonist. Indoleamine 2,3-dioxygenase and prostaglandin E2. In the study of Bustos et al bone marrow derived human MSCs, with or without activation with serum from ARDS patients, were injected to endotoxemic mice. When compared with control mice, treated with saline, bronchoalveolar lavage of MSCs injected mice presented low number of inflammatory cells, decreased levels of IL-1 and TNF-α and increased levels of IL-10. From another study showed that production of IL-1-receptor antagonist by MSCs protected mice from bleomycin-induced lung injury by blocking the production and activity of TNF-α and IL-1α, the predominant proinflammatory cytokines in lung tissue. Nemeth et al administered bone marrow MSCs in mice with sepsis induced after cecal ligation and puncture. Serum concentrations of IL-6 and TNF-α were significantly decreased in treated versus untreated mice after 24 hours, while IL-10 levels were increased. When MSCs were pretreated with antibodies against IL-10 or IL-10 receptor the beneficiary effects of treatment were eliminated. In the same study, a significant increase in the expression and activity of cyclooxygenase-2 and prostaglandin E2 was noted in MSCs after LPS stimulation. This increase was abolished when MSCs were incubated with antibody to TNF-α or lacked toll-like receptor 4. In a model of ARDS, after intrabronchial administration of E.coli endotoxin, treatment with MSCs reduced bronchoalveolar lavage and plasma levels of proinflammatory cytokines TNF-α and MIP-2 and increased anti-inflammatory levels of IL-10.
may possess an additional role as an immunostimulatory cell. MSCs could function as antigen presenting cells through MHC II expression at low levels of interferon-γ. In vitro experiments showed that MSCs can stimulate B-cell antibody secretion and can protect neutrophils by inhibiting apoptosis and reactive oxygen species production without impairing phagocytosis and chemotaxis.

The immunomodulatory and paracrine properties of MSCs make them an attractive choice for ARDS therapy. However, their action on the immune system seems to be complex and further studies in order to better understand the immunosuppressing and immunostimulatory action of MSCs are mandatory.

**Alveolar fluid clearance (AFC)**

In the early phases, ARDS is characterized by alveolar epithelial and endothelial damage causing alveolar feeling. AFC refers to resolution of pulmonary edema and involves a variety of mechanisms, implicating sodium channels, aquaporin and sodium–potassium adenosine triphosphatase. AFC is impaired in the majority of patients with ARDS and the grade of impairment is associated with morbidity and mortality.

MSCs can produce several epithelial growth factors, such as epidermal growth factor, transforming growth factor, keratinocyte growth factor (KGF), that play important role in the epithelial repair mechanisms in the lung. In the study of Chen et al, KGF-engineered MSCs were intravenously administered in mice with LPS-induced lung injury. This MSCs-mediated administration of KGF improved pulmonary microvascular permeability. Authors suggested that the protective effect induced by KGF could be attributed to the promotion of type II lung epithelial cell proliferation and the enhancement of surfactant synthesis. In an ex vivo perfused human lung preparation injured by E. coli endotoxin, treatment with allogeneic human MSCs or its conditioned medium reduced extravascular lung water, improved lung endothelial barrier permeability and restored alveolar fluid clearance. In the same study, the abolition of potential paracrine soluble factors proved that the secretion of keratinocyte growth factor was essential for the restorative effect of MSCs. Curley et al studied the effect of intravenous injection of MSCs in rats with ALI caused by injurious mechanical ventilation. Treatment with MSCs restored systemic oxygenation and lung compliance, reduced total lung water and decreased lung inflammation. The beneficial effect of the MSC secretome on repair of pulmonary epithelial wounds was attenuated after depletion of keratinocyte growth factor. In a recent study conducted by Rojas et al, endotoxin was administered intravenously in 14 sheep from which 6 were subsequently treated with different doses of adult stem cells using the intrabronchial route of delivery, while the rest were treated with saline solution. After administration of endotoxin, moderate-to-severe ARDS with hypoxemia and impaired carbon dioxide clearance was developed. The treated group presented significantly higher PaO2/FiO2 levels compared to control group and reached baseline levels less than 3 hours after endotoxin infusion. Similarly, the experimental group reached baseline values of partial pressure of carbon dioxide at half an hour after maximal lung injury. Lung edema was evaluated using wet to dry ratio of lower lobe lung biopsies. Analysis showed that treatment with stem cells prevented the increase in edema in either lung. Similarly, oxygenation index was improved and lung water content was lower in a sheep model of bacterial pneumonia and ALI after intravenous administration of MSCs when compared with a non-treated control group. AFC is a central aspect on ARDS development and the use of MSCs seems to represent a valid therapeutic alternative.

**Bacterial clearance**

A large body of evidence demonstrates that MSCs express antibacterial effects by enhancing bacterial clearance. In an E. coli pneumonia model in an ex vivo perfused human lung, instillation of human MSCs increased bacterial killing and reduced bacteremia. This effect was attributed to increased alveolar macrophage phagocytosis and secretion of antimicrobial factors, mainly KGF. Additionally, KGF decreased apoptosis of human monocytes through protein kinase B phosphorylation, an effect that increased bacterial clearance. MSCs and their conditioned medium showed marked inhibition of bacterial growth in comparison with control medium or normal human lung fibroblasts regarding Gram-negative and Gram-positive bacteria. Analysis of expression of major antimicrobial peptides indicated that one of the factors responsible for the antimicrobial activity against Gram-negative bacteria was the human cathelicidin antimicrobial peptide. In an E. coli murine pneumonia model, intratracheal instillation of MSCs enhanced bacterial clearance from the alveolar space as early as 4 h after administration. The antibacterial effect with MSCs was conferred by up-regulation of the antibacterial protein lipocalin. In a murine polymicrobial model of sepsis, treatment with MSCs significantly reduced bacterial burden. Microarray analysis demonstrated overall down-regulation of inflammation.
and inflammation-related genes, such as IL-10 and IL-6, and shift toward up-regulation of genes involved in promoting phagocytosis and bacterial killing\textsuperscript{82}. Results from these studies imply that enhanced bacterial clearance induced by MSCs could contribute to the improvement of lung injury caused by bacterial infection, one of the principal causes of ARDS.

**CLINICAL TRIALS**

The use of MSCs in the treatment of ARDS appears close to clinical translation, given the evidence from preclinical studies. In the clinical setting, they are recently explored in clinical trials. Chang et al\textsuperscript{83} reported the case of an ARDS patient who was treated with umbilical cord blood-derived MSCs using intratracheal route of delivery. After cell infusion, the patient presented improved mental status, increased lung compliance, increased PaO\textsubscript{2}/FiO\textsubscript{2} ratio and ameliorated chest radiography. In a randomized, placebo controlled pilot study conducted by Zheng et al\textsuperscript{84} the efficacy and safety of intravenous administration of allogeneic adipose-derived MSCs was evaluated in 12 patients meeting the Berlin definition criteria for ARDS. Patients were randomized to receive placebo or MSCs in a 1:1 fashion. No serious adverse events were recorded. Significant improvements in oxygenation index was recorded in the treated group, while there was no statistically significant difference regarding length of hospital stay, ventilator-free days and ICU-free days between two groups. When serum ARDS biomarkers were considered, no statistically significant differences were noted among groups regarding IL-6 and IL-8 levels, but SP-D levels were significantly lower 5 days after treatment with MSCs. Even though the first clinical trials bring encouraging results, further research is warranted in order to assess the safety and efficacy of MSCs in the therapy of ARDS.

**CONCLUSIONS**

Treatment with MSCs is proposed as a promising therapy for ARDS. A large amount of data from preclinical studies illustrates the mechanisms for the therapeutic potential of MSCs. Evidence suggests that MSCs therapy favourably modulates the immune response to reduce lung injury, facilitates lung regeneration and repair and reduces bacterial burden. However there are still unanswered issues regarding optimal routes of administration, dosage regimens, possible mechanisms of action, definition and characterization of MSCs. It is also questionable how far MSCs can be applied to treat critically ill patients and which steps are necessary to translate from preclinical studies to clinical therapies. Even though the first passes are made, further research in this field should continue in order to surpass limitations and develop a novel and safe therapy for ARDS.

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