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Therapeutic Potential of Mesenchymal Stromal Cells for Acute Respiratory Distress Syndrome

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Abstract

Based on preclinical data, cell-based therapy with bone marrow–derived mesenchymal stem (stromal) cells (MSCs) is a potentially attractive new therapeutic option for treating patients with the acute respiratory distress syndrome. Small and large animal models of acute lung injury from endotoxin, live bacteria, and sepsis have shown that MSCs can decrease lung injury and increase survival. The mechanisms for benefit are mediated in part by paracrine release of several antiinflammatory cytokines, keratinocyte

growth factor, angiopoietin-1, as well as the release of antimicrobial peptides. There is also evidence that MSCs can transfer mitochondria and restore normal bioenergetics to injured alveolar epithelium. Some of the beneficial effects are mediated by microvesicles. A phase 1 safety and dose-escalation trial was completed and a randomized, double-blind clinical trial is currently underway.

Keywords: pulmonary edema; acute lung injury; mesenchymal stem cells; acute respiratory failure

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Cell-based therapy with bone marrow–derived mesenchymal stem (stromal) cells (MSCs) is a potentially attractive option for treating patients with acute respiratory distress syndrome (ARDS) because the mechanisms of efficacy are mediated by several pathways that may reduce the magnitude of lung injury and enhance recovery from lung injury (1, 2). Previous publications have indicated that bone marrow–derived MSCs have the potential for therapeutic benefit in several clinical disorders, including sepsis (3–6), acute renal failure (7), acute myocardial infarction (8), and graft versus host disease (2). The potential therapeutic value of MSCs for ARDS is based on preclinical studies that have been published in the past 7 years. This article reviews some of these data as well as the design of current clinical trials to test MSCs for treatment of ARDS.

Preclinical Studies

The beneficial effect of MSCs seems to derive from their ability to home to injured

organs and secrete several paracrine-soluble factors that can decrease inflammation and increase repair of the injured endothelium or epithelium. MSCs have a low immunogenicity, based in part on low expression of the major histocompatibility complex (MHC) I and II proteins. Thus, therapy with allogeneic human mesenchymal stem cells does not engender a host response and does not lead to graft rejection. More than 2,000 patients have been treated with human MSCs without apparent major adverse events (2).

In a mouse model of bleomycin-induced lung injury, MSCs decreased mortality and inflammation in the lung in part by secreting IL-1ra (9). Our research group found that MSCs were effective in reducing lung injury and improving mortality in mice given high-dose intratracheal endotoxin (10). In follow-up studies from our laboratory, MSCs were effective in reducing mortality in a model of severe *Escherichia coli* pneumonia (4) as well as in a model of gram-negative

peritonitis (5). Furthermore, these studies demonstrated an unexpected finding, namely that MSCs exert a potent antimicrobial effect, in part by releasing LL-37, a well-described antimicrobial peptide, and also by enhancing monocyte phagocytosis (5, 11). The ability of MSCs to stimulate bacterial clearance was also reported by another group studying abdominal sepsis (12).

Because of the promising results with studies in mice, we also tested the potential efficacy and mechanisms by which MSCs could reduce lung injury in our *ex vivo* perfused human lung preparation. In the initial studies, in which lungs were warmed with a physiologic perfusate supplemented with 125 ml of fresh blood, the lung was injured with high-dose intraalveolar endotoxin. Treatment with intrabronchial MSCs 1 hour later markedly reduced lung edema and restored normal lung endothelial and epithelial permeability and also restored alveolar fluid clearance to a normal level. Interestingly, most of the

beneficial effect was replicated in these experiments by using conditioned media from cultured MSCs (13). Using an siRNA knockdown strategy, keratinocyte growth factor (KGF) appeared to be responsible for approximately 70% of the beneficial effect. Follow-up studies in the perfused human lung with severe lung injury induced by *E. coli* demonstrated that delivery of MSCs by the intravenous route (perfusate) or directly into the airspaces (intra-bronchial) reduced edema, restored alveolar fluid clearance, and also had a similar antimicrobial effect as in the mouse studies (14). Interestingly, enhanced monocyte phagocytosis was demonstrated in the perfused human lung studies as a mechanism to account for the antimicrobial effect. Although treatment with antibiotics markedly reduced the number of bacteria, antibiotics alone did not result in restoration of normal alveolar fluid clearance, indicating that MSCs were necessary to improve barrier function and transport capacity of the alveolar epithelium. As in the prior study, KGF appeared to be responsible for some of the beneficial effects. The beneficial effects of the MSC therapy in restoring alveolar fluid clearance could be blocked by amiloride, an inhibitor of apical epithelial sodium uptake (13). Thus, the beneficial effects of KGF may be mediated by favorable effects on apical sodium absorption as well as potentially cytoprotective effects on the alveolar epithelial barrier. In one recent human lung study, we also found that intravenous delivery of MSCs restored alveolar fluid clearance in human lungs that we received in our laboratory that had a baseline markedly impaired alveolar fluid clearance (<10% per hour). The MSCs restored alveolar fluid clearance in the injured lungs to a normal level, whereas the control perfusion had no effect. Some of the beneficial effect of the MSCs was reduced by intrabronchial administration of a neutralizing antibody to KGF (15).

Finally, based on recommendations from the Federal Drug Administration, we tested the potential efficacy of human MSCs in a large animal model of ARDS. For these studies, we used adult sheep who underwent acute lung injury with cottonwood smoke insufflation followed by instillation of live *Pseudomonas aeruginosa* (2.5×10^{11} cfu) into both lungs under isoflurane anesthesia. After the injury, the sheep were ventilated, resuscitated with

Table 1. Mesenchymal stem cell improved arterial oxygenation at 24 hours in sheep with acute lung injury

Parameter	Group	24 h
Pa _{O₂} /F _I O ₂	Control	97 ± 15 mm Hg
	Lower dose	288 ± 55 mm Hg*
	Higher dose	327 ± 2 mm Hg*

Data are expressed as mean ± SEM. Number of sheep in each group: control (n = 6), lower dose (n = 6), higher dose (n = 3). Data from Reference 16.

*P < 0.003 compared with the control group.

lactated ringer solution, and studied for 24 hours. The sheep required positive pressure ventilation, and we measured systemic and pulmonary hemodynamics. We compared the effects of Plasmalyte A alone as a control to a lower dose of human MSCs (5×10^6) and a higher dose of human MSCs (10×10^6). There were no adverse effects from the MSC treatment on systemic blood pressure, pulmonary arterial pressure, pulmonary vascular resistance, renal function, metabolic acidosis, or the level of lactate. The severity of arterial hypoxemia was significantly improved at 24 hours in both of the MSC-treated groups (Table 1). In addition, the degree of pulmonary edema was lower in the sheep treated with the higher dose of MSCs compared with the control group (Table 2). These studies provided reassurance in a clinically relevant large animal model that the MSCs were likely to be safe and also reinforced the possibility that they might be effective in the clinical setting of ARDS (16).

Although paracrine pathways may explain much of the beneficial effect of MSCs in treating experimental acute lung injury, there are also data that MSCs can transfer their mitochondria to injured alveolar epithelium and restore ATP to normal levels (17). In addition, there is

considerable evidence that microvesicles released from MSCs can have therapeutic value. The mitochondrial transfer depends on connexin-43 bridges between the MSC and the epithelial cell. Figure 1 summarizes many of the known cell contact-dependent and -independent pathways that seem to explain the beneficial effect of MSCs in preclinical models of ARDS.

Design of Phase 1 and 2 Clinical Trials in ARDS

Because this is a first-in-humans application of human MSCs in patients with moderate to severe ARDS, the primary end points for both phase 1 and 2 were focused on the safety and tolerability of the human MSC product. In phase 1, this analysis examined the incidence of prespecified infusion-associated events and unexpected severe adverse events in patients with ARDS treated with the human MSCs. For phase 2, the primary end point has continued to be the incidence of prespecified infusion-associated events and the rate of unexpected severe adverse events observed in patients with ARDS treated with human MSCs (n = 40) compared with patients treated with placebo (n = 20) (18).

Table 2. The higher dose of human mesenchymal stem cells reduced the degree of pulmonary edema by gravimetric measurements in sheep

Parameter	Group	Post Mortem	IQR
Gravimetric lung water	Control	6.7 g wet/dry weight	6.4–7.5
	Lower dose	6.5 g wet/dry weight	5.8–8.4
	Higher dose	5.0 g wet/dry weight*	4.9–5.8

Definition of abbreviation: IQR = interquartile range.

Data are expressed as ± SEM. The number of sheep in each group are control (n = 8), lower dose (n = 7), higher dose (n = 4). Data from Reference 16.

*Lung water was significantly lower in the sheep with the higher dose of MSCs compared to the controls (P = 0.01).

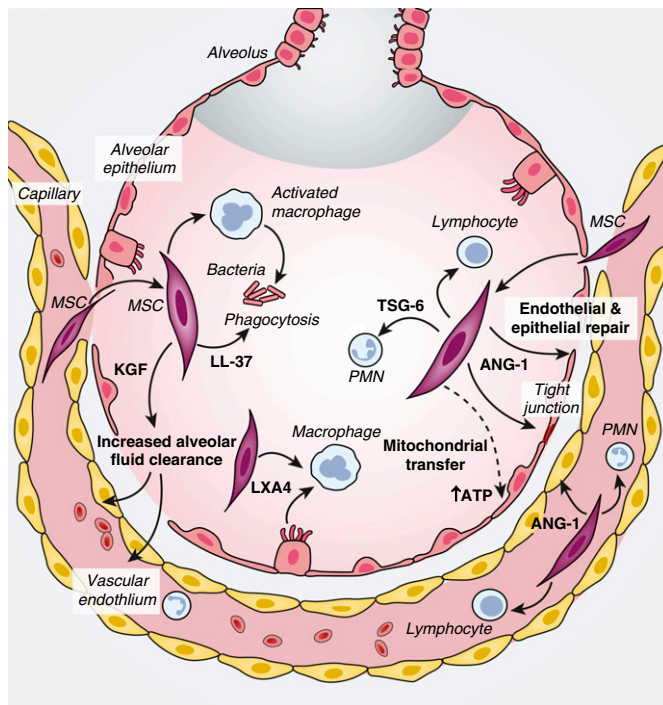


Figure 1. The figure identifies several pathways that may explain the beneficial effects of mesenchymal stromal cells in preclinical models of ARDS. Ang-1 = angiopoietin 1; ATP = adenosine triphosphate; KGF = keratinocyte growth factor; LL-37 = cathelicidin LL-37 antimicrobial peptide; LXA4 = lipoxin A4; MSC = mesenchymal stromal cells; PMN = polymorphonuclear cells; TSG-6 = TNF- α -stimulated gene/protein. (Design by Diana Lim.)

The inclusion criteria for the phase 1 and 2 trials were patients with moderate to severe ARDS with a PaO₂/FiO₂ ratio of less than 200 mm Hg while being ventilated with lung protective positive pressure ventilation with at least 8 cm H₂O of positive end-expiratory pressure within 96 hours of the diagnosis of ARDS. The primary exclusions included history of treatment for cancer within the past two years, grade 3 or 4 pulmonary hypertension, trauma as the main etiology of ARDS, lung transplantation, and/or not expected to survive 6 months.

For the phase 1 trial, the three treatment groups were 1 × 10⁶ MSCs per kg (n = 3), 5 × 10⁶ MSCs per kg (n = 3), and the target dose of 10 × 10⁶ MSCs per kg

(n = 3). The phase 1 trial was completed in January 2014 without adverse events. The phase I trial is registered under ClinicalTrials.gov (NCT01775774).

The phase 2 trial of 60 patients was begun in April 2014. The trial was registered under ClinicalTrials.gov (NCT02097641). The phase II trial was designed as a randomized, double-blind trial with a 2:1 randomization of MSCs to placebo (Plasmalyte), with the MSCs being given over 1 hour with the same inclusion and exclusion criteria as described above. The primary end point of the phase 2 trial was still focused on safety, based on predetermined hemodynamic and respiratory variables. In each patient, a 2-hour baseline period of

hemodynamic and respiratory stability must be documented before the MSCs are administered over 1 hour, and then there is a subsequent 4-hour period of close observation to document hemodynamic and respiratory stability. The secondary end points for the phase 2 trial include the oxygenation index, acute lung injury score, and ventilator-free days. Systemic effects will be measured by Sequential Organ Failure Assessment scores as well as vasopressor-free and organ failure-free days, and 60 days without cause mortality. Biologic end points will include measurements of plasma and bronchoalveolar lavage for markers of lung epithelial injury (receptor for advanced glycation end products, RAGE), pro- and antiinflammatory markers (IL-6, IL-8, IL-10, and IL-1ra), markers of endothelial injury (vWF, ANG-2), and biomarkers that may reflect the paracrine activity of administered human MSCs (ANG-1, and KGF).

Conclusions

The preclinical data suggest the potential efficacy of bone marrow-derived human MSCs for the treatment of moderate to severe ARDS. The mechanisms of benefit may depend on both paracrine release of soluble molecules plus transfer of mitochondria and histologically active microvesicles. Thus, the encouraging preclinical data suggesting possible efficacy for ARDS has stimulated testing of human MSCs for safety and tolerability and limited efficacy in a phase 1/2 clinical trial supported by the National Heart, Lung, and Blood Institute. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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