Published in final edited form as:
Crit Care Med. 2010 October ; 38(10 Suppl): S569–S573. doi:10.1097/CCM.0b013e3181f1ff1d.

Mesenchymal stem cells for acute lung injury: Preclinical evidence

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Abstract

Several experimental studies have suggested that mesenchymal stem cells may have value for the treatment of clinical disorders, including myocardial infarction, diabetes, acute renal failure, sepsis, and acute lung injury. In preclinical studies, mesenchymal stem cells have been effective in reducing lung injury from endotoxin, live bacteria, bleomycin, and hyperoxia. In some studies, the cultured medium from mesenchymal stem cells has been as effective as the mesenchymal stem cells themselves. Several paracrine mediators that can mediate the effect of mesenchymal stem cells have been identified, including interleukin-10, interleukin-1ra, keratinocyte growth factor, and prostaglandin E_2. Further preclinical studies are needed, as is planning for clinical trials for acute lung injury.

Keywords

pulmonary edema; acute respiratory distress syndrome; sepsis; acute respiratory failure

Substantial progress has been made in reducing mortality and morbidity from acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) with improved supportive care, specifically lung-protective ventilation and a fluid-conservative strategy (1–3). However, mortality remains unacceptably high (4), and pharmacologic therapies have not been successful (5). Thus, there is a need for innovative therapies to further improve clinical outcomes, especially in patients with severe lung injury.

A promising new approach for treatment of ALI/ARDS has evolved from preclinical studies of mesenchymal stem cells (MSCs). Although initial research on MSCs was focused on the possibility that cell-based therapy with MSCs could provide a mechanism to replace injured lung epithelium (6), subsequent studies in the mature and the immature lung have focused more on the paracrine properties of MSCs, which have value in limiting lung injury and enhancing lung repair (7). This article briefly reviews the preclinical evidence supporting the potential value of cell-based therapy with MSCs for ALI/ ARDS, sepsis, and acute renal failure.

The authors have not disclosed any potential conflicts of interest.

Rationale for MSC therapy

MSCs can be isolated from several sources, most commonly the bone marrow (8). They also can be isolated from adipose tissue, cord blood, and the placenta (7). Although the initial interest focused on engraftment in the injured lung (6), subsequent studies have shown that this is difficult to achieve. However, progress has been made in identifying several paracrine factors that can be effective in reducing inflammation and promoting tissue repair. MSCs release several growth factors that can regulate endothelial and epithelial permeability, as well as enhance repair. MSCs also release anti-inflammatory cytokines that can dampen the severity of inflammation. Furthermore, MSCs can regulate innate and adaptive immunity by effects on T cells, B cells, dendritic cells, monocytes, neutrophils, and macrophages (7–11).

Several experimental studies have indicated that MSCs may have potential therapeutic application in clinical disorders, including myocardial infarction (12, 13), diabetes (14), hepatic failure (15), and acute renal failure (16). Further, one research group (17) found that the intravenous delivery of MSCs decreased mortality in peritoneal sepsis in mice. In all of these studies it appeared that the therapeutic effects of MSCs derived primarily from their capacity to secrete paracrine-soluble factors that could modulate the immune response and reduce barrier injury.

MSC treatment of ALI in animal models

In bleomycin-induced lung injury and fibrosis, Ortiz et al (18) found that MSCs improved survival and lung inflammation when administered intravenously. These beneficial effects were not accounted for by lung engraftment rates, which were less than 5%, but rather through paracrine mechanisms (18, 19). In a subsequent study, the same authors found that there was an important contribution of a subpopulation of mouse MSCs that produced interleukin (IL)-1ra, a paracrine-soluble factor that reduced the severity of bleomycin-induced lung injury. The authors also identified a subpopulation of human MSCs in the same study, approximately 5%, that also produced high levels of IL-1ra (20).

Xu et al (21) reported that intravenous administration of MSCs after intraperitoneal endotoxin decreased the influx of neutrophils into the air spaces of the lung and also reduced the quantity of pulmonary edema. Our research group has studied the effects of bone marrow-derived MSCs in a mouse model of severe lung injury. We administered endotoxin by the intrapulmonary route (5 mg/kg), which was followed by MSCs 4 hours later (750,000 cells), also by the intratracheal route. Controls included saline, apoptotic MSCs, and mouse fibroblasts. MSCs reduced the quantity of lung edema and protein in the air spaces of the lung (22). The histologic appearance of lung injury was reduced in the MSC-treated mice. Furthermore, survival was increased by MSC treatment (80% vs. 42%; $p < .01$). The beneficial effects were associated with a reduction in proinflammatory cytokines (tumor necrosis factor-α and macrophage inflammatory protein-2) and an increase in anti-inflammatory cytokines (IL-1ra, IL-10, IL-13). Another group has also demonstrated that MSCs can be transfected with human angiopoietin-1, which further reduced the severity of *Escherichia coli* endotoxin-induced lung injury (23).

MSC therapy for sepsis

Sepsis is responsible for up to 200,000 deaths per year in the United States. In a recent study, Nemeth et al (17) tested the hypothesis that bone marrow stromal stem cells or MSCs could attenuate sepsis-induced inflammation by modulating the immune response. They used the cecal ligation and puncture model in mice, a well-established preclinical model. Systemic administration of MSCs through the tail vein (24 hrs before or 1 hr after cecal ligation and puncture) improved survival at day 4, an effect that was associated with less

organ dysfunction as indicated by lower serum creatinine levels, decreased serum amylase concentrations, lower hepatic enzyme concentrations, and decreased peritoneal, liver, and renal permeability. The reduced organ injury seemed to be attributable to less neutrophil-mediated oxidative injury, as suggested by lower myeloperoxidase levels in the liver and kidney. An increased number of circulating neutrophils in MSC-treated mice may have contributed to lower the bacterial counts in the blood.

Serum levels of IL-6 and tumor necrosis factor-α were decreased in MSC-treated mice compared to controls, and the concentration of the anti-inflammatory cytokine IL-10 nearly doubled within the first 6 hrs, a finding that prompted the authors to determine whether MSCs acted on subpopulations of immune cells. An involvement of T cells, B cells, and natural killer cells was ruled out, but monocyte and macrophage depletion of mice using clodronate-filled liposomes resulted in the loss of the beneficial effects of MSCs, suggesting a central role for these cells.

Mice treated with an IL-10 neutralizing antibody or an IL-10 receptor antibody also abrogated the beneficial effect of MSCs, revealing a critical role for IL-10. Because MSCs isolated from IL-10 knockout mice were able to induce a similar improvement in survival, macrophages and monocytes were suspected to be the source of IL-10. Therefore, the authors tested the hypothesis that MSCs were able to reprogram monocytes or macrophages, leading to an increased IL-10 secretion. They found that $CD11b^+$ (monocytes/macrophages) isolated from MSC-treated mice, as well as macrophages cultured in direct contact with MSCs, produced more IL-10 on lipopolysaccharide stimulation compared to control macrophages. In addition, the authors found that lipopolysaccharide-induced IL-10 secretion by macrophages was dependent on the production of prostaglandin E_2 by MSCs. Prostaglandin receptors E_2 and E_4 expressed on macrophages were required for prostaglandin E_2-mediated induction of IL-10. The authors showed that prostaglandin E_2 production by MSCs required upstream endotoxin signaling consisting of the toll-like receptor-4/nuclear factor κB/COX2 cascade.

Thus, in this mouse model of peritoneal sepsis, systemically administered MSCs reduced multi-organ damage and improved survival by controlling the inflammatory process by increasing IL-10 levels, decreasing tissue leukocyte invasion, and maximizing bacterial killing in the circulation. Aside from uncovering a new mechanism for the effects of MSCs, this study suggests that MSCs may be useful for the treatment of severe sepsis in humans.

MSC therapy for acute renal failure in an animal model

Bruno et al (24) demonstrated that cultured medium from MSCs contains small membrane-bound vesicles that mediate improved recovery from experimentally induced acute renal failure in mice, primarily by increasing proliferation of renal tubular epithelial cells. Vesicles were isolated from growth medium conditioned by human MSCs through differential centrifugation. After removal of large-particulate material, a high-speed spin recovered a small membrane-bound vesicle fraction with an average diameter of 134 nm, identified as microvesicles. Based on cell sorting-based technology, the human MSC-derived microvesicles contained a subset of integral membrane proteins found on the cell surface of human MSCs, including the beta integrin CD45 but not major histocompatibility class I. There was visual and biochemical evidence that human MSC microvesicles were taken up by mouse renal tubular epithelial cells. Pretreatment with blocking antibodies to CD45 or with soluble cognate ligand to CD45 prevented the therapeutic effects on mouse renal tubular epithelial cells both *in vitro* and *in vivo*, suggesting regulated targeting and internalization of microvesicles. Interestingly, messenger RNA (mRNA) could be isolated from microvesicles, and pretreatment with RNAse impaired the therapeutic effects *in vivo*,

but not the presence of key surface proteins or uptake of microvesicles. The mRNA derived from human MSCs was demonstrated in mouse renal tubular cells after incubation with microvesicles, as were expression of their protein products and proper subcellular localization over time. Taken together, the data from this study indicate that mRNA packaged into CD45-dependent microvesicles can target to, incorporate into, and promote more rapid recovery of injured renal tubular epithelial cells.

MSC therapy for perinatal lung injury in animal models

Despite the success of surfactant replacement in attenuating mortality and morbidity in infant premature lung disease, bronchopulmonary dysplasia remains an ongoing unsolved clinical problem in premature neonates. The pathogenesis of bronchopulmonary dysplasia includes oxidative and inflammation-related lung injury secondary to factors such as hyperoxia, infection, and ventilator-induced lung injury.

Two important and complementary studies reported that MSCs or their secreted products can reduce lung inflammation and improve lung structure in experimental rodent models of hyperoxia-induced bronchopulmonary dysplasia. In one study, van Haaften et al (25) found that intratracheal MSC administration reduced rat pup hyperoxia-induced lung injury. In this study, physiologic and morphologic analyses demonstrated protection of lung vascular and alveolar structures as well as an improved exercise capacity and prevention of pulmonary hypertension. The number of engrafted MSCs in this model was low, making it more likely that paracrine effects were responsible for the beneficial effects.

In a separate report, Aslam et al (26) reported protection against pulmonary hypertension and cellular mediators of inflammation with MSC therapy. In this study, MSCs or their conditioned medium were administered intravenously soon after birth to neonatal mice subjected to hyperoxia-induced lung injury. This lung injury model resembles bronchopulmonary disease because there are fewer alveoli that are less well-developed, thickened alveolar septa, and evidence of pulmonary hypertension through tunica media thickening of pulmonary arterioles and right ventricular hypertrophy. Neonatal mice approximate the lung development of a human preterm neonate between 24 and 28 weeks' gestation, representing the saccular and alveolar stages of lung development. Intravenous delivery of MSCs decreased pulmonary hypertension and pulmonary arteriolar wall muscularization without evidence of local engraftment of the MSCs. Interestingly, the same degree of protection against medial hypertrophy was achieved by intravenous injection of MSC-conditioned medium alone, as well as normalization of the number of alveoli and their architecture. These results support the interpretation that paracrine factors derived from MSCs may be of value in protecting against perinatal lung injury and normalizing lung development.

MSC therapy for endotoxin-induced lung injury in the ex vivo perfused human lung

Our research group has developed an *ex vivo* perfused human lung model to provide more clinically relevant and mechanistic information regarding potential new therapies for human ALI/ ARDS. We found that the perfused human lung was stable for up to 6 hrs; furthermore, basal alveolar fluid clearance proceeded at a level of 18% to 20% per hour, which was much faster than clearance in the nonperfused human lung (27). In addition, we found that it was possible to stimulate alveolar fluid clearance with β-agonist therapy to a level of approximately 35% to 40% (27). Subsequently, we adapted this preparation to study the potential therapeutic mechanistic effects of allogeneic MSC therapy for *E. coli* endotoxin-induced lung injury.

For all of these studies, human lungs were donated by the Northern California Transplant Donor Network (Oakland, CA). We used lungs that were not considered suitable or appropriate for lung transplantation. The mean age of the donor lungs was 48 ± 13 yrs, and the ischemia time was 21 ± 13 hrs (mean ± sd). Perfusion was established with a physiologic solution with 5% albumin. Then, approximately 100 mL whole blood was removed from healthy donors and added to the perfusate (with a final hematocrit of 4%). The whole blood was not cross-matched or type-specific with the donor's human blood type because the human lungs contained almost no residual blood and the lung was flushed initially during reperfusion with a perfusate solution. There were no adverse hemodynamic or pulmonary effects associated with the addition of the whole blood to the perfusate. The preparation was stable and suitable for experimental studies for a period up to 7 hrs with an appropriate baseline (28).

Lung injury was induced by the instillation of 0.1 mL/kg of *E. coli* endotoxin into either the right middle lobe or the left lower lobe. The preparation was performed with perfusion of one lung. We compared the effects of instilling MSCs or the cultured medium of the MSCs or control normal human lung fibroblasts in this preparation. The results showed that endotoxin caused an acute severe neutrophilic inflammation in the right middle lobe with a three-fold increase in lung vascular permeability and a marked increase in lung water. Furthermore, alveolar fluid clearance was reduced to zero with endotoxin compared to normal levels of nearly 18% to 20% per hour. In addition, endotoxin exposure produced a marked increase in the alveolar compartment of proinflammatory cytokines, including IL-1β, tumor necrosis factor-α, and IL-8.

Treatment with MSCs was administered 1 hr after the endotoxin instillation, with instillation of the MSCs into the same region of the right middle lobe where the endotoxin had been instilled. The MSCs completely restored both lung endothelial permeability and lung water to a normal level (Fig. 1). Histologic analysis showed that the inflammatory infiltrate was markedly decreased. In addition, alveolar fluid clearance was restored to a normal level by the MSCs.

Interestingly, the cultured medium of the MSCs was equally effective in reversing the effects of ALI/ARDS from endotoxin. The cultured medium normalized lung endothelial permeability, lung water, and alveolar fluid clearance, and it decreased the histologic injury. There was no effect on cytokine levels with the exception of a small decrease in IL-1β levels with the cultured milieu.

In searching for the mechanisms that might account for these interesting effects, we speculated that the release of a growth factor could be important because we and other investigators (29) have found that keratinocyte growth factor (KGF) was capable of preventing a wide variety of acute lung injury. We found that the production of KGF by the allogeneic human MSCs was substantial. Therefore, we performed a small interfering RNA (siRNA) inhibition strategy to eliminate the secretion of KGF in the MSCs. We then instilled the cultured medium from the MSCs and found that approximately 80% of the benefit was lost when KGF release was inhibited. To be certain that KGF was the critical factor, we also added recombinant KGF (100 ng) as rescue therapy to the cultured medium that had no KGF present (because of the KGF siRNA) and found that the recombinant KGF restored alveolar fluid clearance to a normal level under these conditions (29). Additional *in vitro* studies suggested that KGF might work, in part, by upregulating the total quantity of epithelial sodium channel in alveolar type 2 cells and potentially increasing the transport of epithelial sodium channel to the apical cell membrane. It is well-known that KGF has cytoprotective effects, which may help to explain the beneficial effects on lung endothelial and epithelial permeability.

Although the results of these studies are interesting, there are important limitations. These experiments were performed in the perfused human lung, so the preparation is restricted to the lungs themselves. The duration of the experiments was short-term (4 hrs of injury). The absence of immunomodulatory organs such as the liver, spleen, and lymph nodes makes it difficult to know what the net effect might be in the intact human with ALI/ARDS. Based on animal studies, one could speculate that the anti-inflammatory properties might have been more substantial. However, although the perfusate contained white blood cells and platelets, the quantity of cells in the perfusate was below normal. Nevertheless, the results indicated that MSCs themselves and their paracrine products may be effective in reducing the severity of lung injury.

Future directions

The publication of several promising preclinical studies of animal models of ALI/ARDS, both in the newborn and the mature lung, indicate that further preclinical studies are warranted to evaluate MSCs as a potential therapy for ALI/ ARDS. The results of these studies can point the way, potentially, to specific paracrine factors that might be useful in the treatment of lung injury. KGF, for example, has long been known to be effective in preventing lung injury in animal models. The data in the recent *ex vivo* perfused human lung studies suggest that KGF may have value as a treatment. Considerable work will need to be performed to test the safety of MSC therapy in humans with lung injury, whether they are adults or pediatric patients. Nevertheless, there is a need for innovative therapy. The recent outbreak of severe viral pneumonia from H1N1 resulting in deaths in susceptible populations, including children, pregnant women, and immunocompromised patients, illustrates the urgent need for therapies that can reverse lung injury.

Considerable progress has been made in the treatment of ALI/ARDS in the past 10 yrs. Mortality has declined substantially with the use of lung-protective ventilation (1), and there is evidence that ventilator-free days can be increased with a fluid-conservative strategy (2, 3). Nevertheless, new therapies are needed to further reduce morbidity and mortality from this syndrome. Cell-based therapy with MSCs or their products need to be studied and tested in further preclinical studies and clinical trials.

Acknowledgments

This work was supported by NHLBI HL51856 and HL51854 (MAM), NHLBI HL093026 (JWL), Pediatric Scientist NIH (JPH), the European Respiratory Society/ Marie Curie Joint Research Fellowship MC 1219-2009, the European Respiratory Society, and the European Community's Seventh Framework Programme FP7/ 2007-2013 Marie Curie Actions under grant agreement RESPIRE, PCOFUND-GA-2008-229571 (AG).

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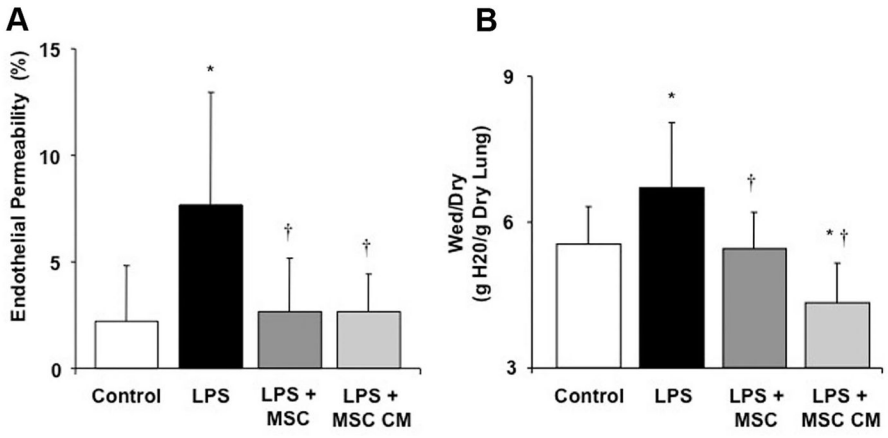

Figure 1.

Effect of human mesenchymal stem cells or their conditioned medium on lung endothelial permeability to protein and wet-to-dry (W/D) ratio in the *ex vivo* perfused human lung injured with *Escherichia coli* endotoxin. Instillation of mesenchymal stem cells (*MSCs*) or their conditioned medium (*CM*) into the *E. coli* endotoxin (0.1 mg/kg) injured right middle lobe 1 hr after injury restored lung endothelial permeability to protein (*A*) and W/D ratio (*B*) to control values. Data are expressed as mean % endothelial permeability or W/D ratio ± SD; n = 4 to 5 lungs. (*A*) $*p < .0001$ vs. control lobe. $\dagger p < .0011$ vs. lipopolysaccharide (*LPS*) (0.1 mg/kg) injured lobe for lung endothelial permeability. (*B*) $*p < .0014$ vs. control lobe. $\dagger p < .005$ vs. LPS (0.1 mg/kg) injured lobe for the W/D ratio by analysis of variance (Bonferroni). Reprinted with permission from Aslam et al (26).