

Therapeutic Efficacy of Human Mesenchymal Stromal Cells in the Repair of Established Ventilator-induced Lung Injury in the Rat

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ABSTRACT

Background: Rodent mesenchymal stem/stromal cells (MSCs) enhance repair after ventilator-induced lung injury (VILI). We wished to determine the therapeutic potential of human MSCs (hMSCs) in repairing the rodent lung.

Methods: In *series 1*, anesthetized rats underwent VILI (*series 1A*, $n = 8$ to 9 per group) or protective ventilation (*series 1B*, $n = 4$ per group). After VILI, they were randomized to intravenous administration of (1) vehicle (phosphate-buffered saline); (2) fibroblasts (1×10^7 cells/kg); or (3) human MSCs (1×10^7 cells/kg) and the effect on restoration of lung function and structure assessed. In *series 2*, the efficacy of hMSC doses of 1, 2, 5, and 10 million/kg was examined ($n = 8$ per group). *Series 3* compared the efficacy of both intratracheal and intraperitoneal hMSC administration to intravascular delivery ($n = 5$ –10 per group). *Series 4* examined the efficacy of delayed hMSC administration ($n = 8$ per group).

Results: Human MSCs enhanced lung repair, restoring oxygenation (131 ± 19 vs. 103 ± 11 vs. 95 ± 11 mmHg, $P = 0.004$) compared to vehicle or fibroblast therapy, respectively. hMSCs improved lung compliance, reducing alveolar edema, and restoring lung architecture. hMSCs attenuated lung inflammation, decreasing alveolar cellular infiltration, and decreasing cytokine-induced neutrophil chemoattractant-1 and interleukin-6 while increasing keratinocyte growth factor concentrations. The lowest effective hMSC dose was 2×10^6 hMSC/kg. Intraperitoneal hMSC delivery was less effective than intratracheal or intravenous hMSC. hMSCs enhanced lung repair when administered at later time points after VILI.

Conclusions: hMSC therapy demonstrates therapeutic potential in enhancing recovery after VILI. (**ANESTHESIOLOGY 2015; 122:363-73**)

RECENT preclinical studies have generated significant interest in human mesenchymal stem/stromal cells (hMSCs) as a potential therapy for acute respiratory distress syndrome (ARDS). MSCs restore impaired alveolar fluid clearance, decrease alveolar epithelial injury, reduce lung endothelial permeability, and modulate inflammation in preclinical ARDS models.¹⁻⁴ A number of early phase clinical studies of hMSC therapy for ARDS are in progress.⁵

One area of interest is the potential for MSCs to repair the lung after injury induced by mechanical ventilation. While essential to sustain life in severe respiratory failure, mechanical ventilation can cause or worsen lung injury⁶ and can increase the risk of ARDS.⁷ This entity—termed ventilator-induced lung injury (VILI)—may occur even when contemporary low tidal volume strategies are employed because the heterogeneous nature of ARDS means that regional lung overdistention and injury may still occur.⁸

What We Already Know about This Topic

- Mesenchymal stromal cells are being tested in patients with acute respiratory distress syndrome, but their efficacy is not known

What This Article Tells Us That Is New

- Rats with ventilator-induced lung injury who received human mesenchymal stromal cells (MSCs) had enhanced lung repair, improved oxygenation compared to rats who received vehicle or fibroblasts
- MSC treatment was also associated with improved lung compliance, decreased alveolar edema, and restored lung architecture
- The mechanism for improvement appeared to be in part decreased inflammation and decreased alveolar cell neutrophil recruitment

VILI remains a major contributor to morbidity and mortality in patients with ARDS.⁹

Corresponding article on page 238. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org). Data from this manuscript were presented at the 2013 American Thoracic Society Annual Conference, May 22, 2013, Philadelphia, Pennsylvania. Drs. Hayes and Masterson contributed equally to the work in this manuscript.

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We have shown that intravenously or intratracheally administered rat MSCs augment epithelial and endothelial repair after VILI in the rat and encourage restitution in *in vitro* pulmonary epithelial wound assays.^{10,11} Chimenti *et al.*¹² have shown that MSCs may also attenuate the development of VILI when given before high-stretch lung ventilation in a rat model.

The efficacy of hMSCs in repairing the injured lung is not known and is important given the potential mechanistic differences between human and rodent MSCs.¹³ In addition, there remain important deficits in knowledge regarding the best approach to hMSC therapy for ARDS, including optimal dosing and route of hMSC administration, and timing of hMSC dosing in VILI. Clinical trials to date of MSCs in other diseases including rheumatoid arthritis, graft-versus-host disease, and cardiovascular disease have demonstrated safety and modest benefit, but to a large extent have failed to replicate the favorable effects observed in animal models.^{14–16} These issues highlight the need for ongoing preclinical investigation in parallel to the early phase clinical studies of hMSC therapy for ARDS⁵ to optimize the therapeutic approach and address key remaining deficits in our knowledge.

To address these issues, we conducted a number of experimental studies. In the *first series*, we hypothesized that human MSCs would enhance recovery and repair after ventilator induced lung injury in the rat. In the *second series*, we wished to determine the lowest effective therapeutic MSC dose. In the *third series*, we compared the efficacy of hMSC therapy when administered *via* the intravenous, intratracheal, and intraperitoneal routes, in order to determine the optimal route of hMSC administration. We hypothesized that the intratracheal and intraperitoneal routes would be as effective as the intravenous route. *Series 4* examined the efficacy of hMSC administration when given at later time points in the recovery period following VILI. We hypothesized that later administration would be effective, but to a lesser degree than previous hMSC administration.

Materials and Methods

All work was approved by the Animal Ethics Committee of the National University of Ireland, Galway, and conducted under license from the Department of Health, Ireland. Specific-pathogen-free adult male Sprague Dawley rats (Charles River Laboratories, Kent, United Kingdom) weighing between 350 and 450 g were used in all experiments. A full description of the methods is available in the Supplemental Digital Content 1, <http://links.lww.com/ALN/B115>.

Preparation of hMSCs and Fibroblasts

The human MSCs used in these studies were provided by Orbsen Therapeutics Ltd (Galway, Ireland). Briefly, bone marrow was harvested from volunteers, filtered, centrifuged, and the cell pellets cultured as previously described.¹⁷ Adherent cells

were further expanded until 80% confluent, and then trypsinized and culture expanded to passage 4, whereupon they were used for experiments. hMSCs were characterized according to international guidelines¹⁸ (Supplemental Digital Content 1, <http://links.lww.com/ALN/B115>). Primary human lung fibroblasts used in these experiments were purchased from American Type Culture Collection (ATCC®, Manassas, VA).

Rodent Ventilator-induced Injury Protocol

We used our established model of repair after VILI.¹⁹ Adult male Sprague Dawley rats were anaesthetized with intraperitoneal ketamine 80 mg·kg⁻¹ (Ketalar, Pfizer, Cork, Ireland) and xylazine 8 mg kg⁻¹ (Xylapan, Vétroquinol, Dublin, Ireland). After confirmation of depth of anesthesia by paw clamp, intravenous access was obtained *via* tail vein, laryngoscopy was performed and the trachea was intubated with a 14G catheter (BD Insyte®, Becton Dickinson Ltd., Oxford, United Kingdom). The lungs were ventilated using a small animal ventilator (CWE SAR 830 AP, CWE Inc., Ardmore, PA). Anesthesia was maintained with repeated boli of Saffan® (Schering Plough, Welwyn Garden City, United Kingdom) and paralysis with cisatracurium besylate 0.5 mg kg⁻¹ (Glaxo-SmithKline, Dublin, Ireland).

The following ventilator settings were used for injurious mechanical ventilation (*series 1A*, and 2–4): Fio₂ of 0.3, P_{insp} 35 cm H₂O, respiratory rate 18 min⁻¹, and PEEP 0 cm H₂O. When respiratory static compliance had decreased by 50%, the animals were allowed to recover.¹⁹ A protective ventilation protocol was used in *series 1B*, which comprised of mechanical ventilation for 90 min with the following settings: Fio₂ of 0.3, respiratory rate 80 min⁻¹, tidal volume 6 ml kg⁻¹ and positive end-expiratory pressure of 2 cm H₂O.¹⁹

Experimental Series

Series 1A examined the efficacy of hMSC therapy in VILI. Animals were subjected to injurious ventilation, and after recovery (approximately 15–30 min after establishment of VILI), were randomly allocated to intravenous administration of (1) vehicle (phosphate-buffered saline, 300 µl); (2) human lung fibroblasts (1 × 10⁷ cells/kg); or (3) hMSCs (1 × 10⁷ cells/kg) and the extent of recovery from VILI assessed at 24 h. *Series 1B* examined the effects of hMSC therapy in the setting of noninjurious ventilation. Animals underwent protective ventilation, and after recovery, were randomly allocated to intravenous administration of vehicle, human lung fibroblasts, or hMSC therapy as for *series 1A*. *Series 2* examined the efficacy of lower hMSC doses in animals after VILI. After induction of VILI, animals were randomized to intravenous administration of (1) vehicle (phosphate-buffered saline, 300 µl); (2) 1 × 10⁶ cells/kg hMSCs; (3) 2 × 10⁶ cells/kg hMSCs; (4) 5 × 10⁶ cells/kg hMSCs; or (5) 1 × 10⁷ cells/kg hMSCs; and the extent of recovery from VILI assessed at 24 h. *Series 3* compared the efficacy of intratracheal and intraperitoneal administration of 1 × 10⁷ cells/kg hMSC to the intravascular route. *Series 4*

examined the efficacy of delayed hMSC administration when given at 0.25, 6, and 24 h after VILI. The recovery period was extended to 48 h in this series, and the later treatment points represented 12.5 and 50% of the recovery period, respectively.

Assessment of Injury and Repair

At 24 or 48 h after VILI induction (depending on the experimental series), animals were reanesthetized. A tracheostomy was performed, and arterial blood gases and static inflation lung compliance were measured as previously described.^{20,21} After 20 min, the inspired gas was altered to a F_{iO_2} of 1.0 for 15 min, and a final arterial blood sample was taken. After heparinization (400 IU·kg⁻¹, CP Pharmaceuticals, Wrexham, United Kingdom), animals were euthanized by exsanguination, the heart–lung block was dissected, and bronchoalveolar lavage (BAL) collection was performed.^{22,23} BAL differential cell counts were obtained. Protein concentration was determined using a Micro BCA™ Protein assay kit (Pierce, Rockford, IL).²⁴ BAL concentrations of cytokine-induced neutrophil chemoattractant-1 (CINC-1), interleukin (IL)-6, IL-10, and keratinocyte growth factor (KGF) were determined using quantitative sandwich enzyme-linked immunosorbent assays (R&D Systems, Abingdon, United Kingdom).²⁵ Wet/dry lung weights were determined using the lowest lobe of the right lung.²⁶ The left lung was isolated and fixed for morphometric examination, and the extent of histologic lung damage was determined using quantitative stereological techniques.^{22,26} The physiologic assessment of lung function (oxygenation, lung compliance) was performed by unblinded investigators. All *ex vivo* analyses (BAL analyses, wet:dry ratios, histologic analyses) were performed by blinded investigators.

Statistical Analysis

Data were analyzed using Sigma Stat (SYSTAT® Software, Richmond, CA). Sample sizes were guided by our previous experience with this model.¹⁹ The distribution of all data was tested for normality using Kolmogorov-Smirnov tests. In *series 1, 2* and *4*, Data were analyzed by one-way ANOVA, with *post hoc* testing using Dunnett's test, with the vehicle group as the comparison group. In *series 3*, which examined the efficacy of different routes of hMSC administration, a two-way ANOVA was used, with treatment (hMSC *vs.* vehicle) and route of administration as the two factors. Subsequent between group analyses, where indicated, were restricted to comparisons of hMSC *versus* vehicle for each route of administration. Underlying model assumptions were deemed appropriate on the basis of suitable residual plots. A two-tailed *P* value of <0.05 was considered significant.

Results

Series 1—Efficacy of hMSCs in Enhancing VILI Repair

Series 1A. Thirty animals were entered into the experimental protocol, with 10 allocated to each of the VILI groups.

Four animals died during induction of VILI, while one fibroblast and one vehicle treated animal died during the recovery period. There were no baseline group differences and no difference in the duration of high-stretch ventilation required to induce VILI across the groups.

hMSC therapy enhanced repair after VILI compared to vehicle or fibroblast therapy. hMSCs restored arterial oxygenation and lung static compliance (fig. 1, A and B) and decreased lung microvascular permeability (fig. 1, C and D). hMSC therapy decreased overall alveolar inflammatory cell infiltration, substantially decreasing lung neutrophil accumulation while increasing the proportion of macrophages in the alveolar fluid ($P < 0.001$) (fig. 1, E and F). Alveolar concentrations of CINC-1 and IL-6 were decreased, and KGF was increased (fig. 2, A–C) after hMSC treatment. In contrast, alveolar IL-10 concentration was not significantly increased by hMSC treatment (fig. 2D).

hMSCs decreased alveolar thickening and increased recovery of airspace volume, as evidenced by reduced alveolar tissue volume fraction and increased alveolar air-space volume fraction, respectively (fig. 3A). Representative histologic sections of lung demonstrate the greater degree of resolution of injury and alveolar infiltrates in the hMSC treated animals (fig. 3, B–E).

Series 1B. Twelve animals were entered into the experimental protocol, with four allocated to each of the groups. There was no evidence of lung injury or inflammation after low-stretch ventilation and no effect of hMSC or fibroblast therapy in these animals (figs. 1–3).

Series 2—Determination of Lowest Effective hMSC Dose that Enhances Repair

Of 53 animals studied, 8 died during induction of VILI, while 5 did not recover post-VILI, leaving 8 animals in each group. There were no significant between group differences in baseline data, duration of high-stretch ventilation required to induce VILI or in the duration of survival post-VILI.

There appeared to be a threshold dose effect of hMSC therapy on restoration of lung function after VILI. hMSC doses of 2 million hMSC/kg and higher demonstrating similar efficacy, resulting in improved arterial oxygenation (fig. 4A) and lung compliance (fig. 4B), enhanced lung barrier function (fig. 4, C and D), reduced alveolar cellular and neutrophilic accumulation (fig. 4, E and F), and restored lung structure, reducing alveolar thickening, and increasing recovery of airspace volume (fig. 1, Supplemental Digital Content 2, <http://links.lww.com/ALN/B116>). In contrast, the 1 million hMSC dose/kg was ineffective in restoring lung function or structure after VILI.

Series 3—Determination of Optimal Route of hMSC Delivery

Forty-five animals were entered into the experimental protocol, with 10 allocated to each of the 3 hMSC treatment groups (intravenous *vs.* intratracheal *vs.* intraperitoneal delivery), and 5 allocated to each of the 3 route control groups.

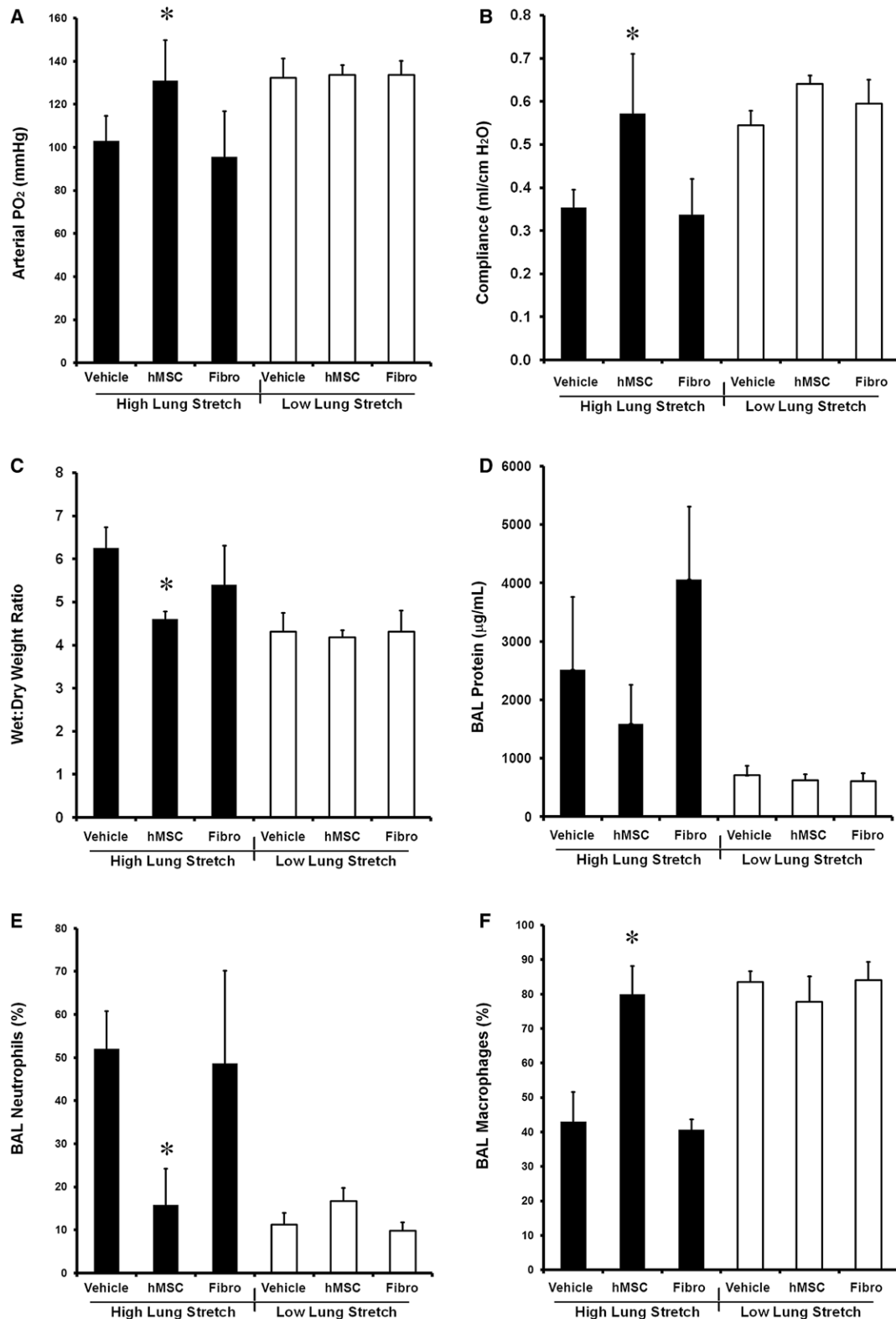


Fig. 1. hMSCs enhance lung repair after VILI. hMSC therapy restored arterial oxygenation (A), increased static lung compliance (B), reduced lung wet:dry weight ratios (C), decreased BAL protein concentrations (D), decreased the proportion of BAL neutrophils (E), and increased the BAL percentage of macrophages (F) 24 h after VILI, compared to either vehicle or fibroblast therapy. There was no effect of hMSC therapy in lungs subject to protective ventilation. $n = 8$ to 9 animals per high-stretch group and $n = 4$ animals per low-stretch group. Error bars represent standard deviation. BAL = bronchoalveolar lavage; Fibro = human fibroblast; hMSC = human mesenchymal stromal cell; Po₂ = partial pressure oxygen; Vehicle = treatment with vehicle alone; VILI = ventilator-induced lung injury. *Significantly ($P < 0.05$) different from Vehicle high-stretch group.

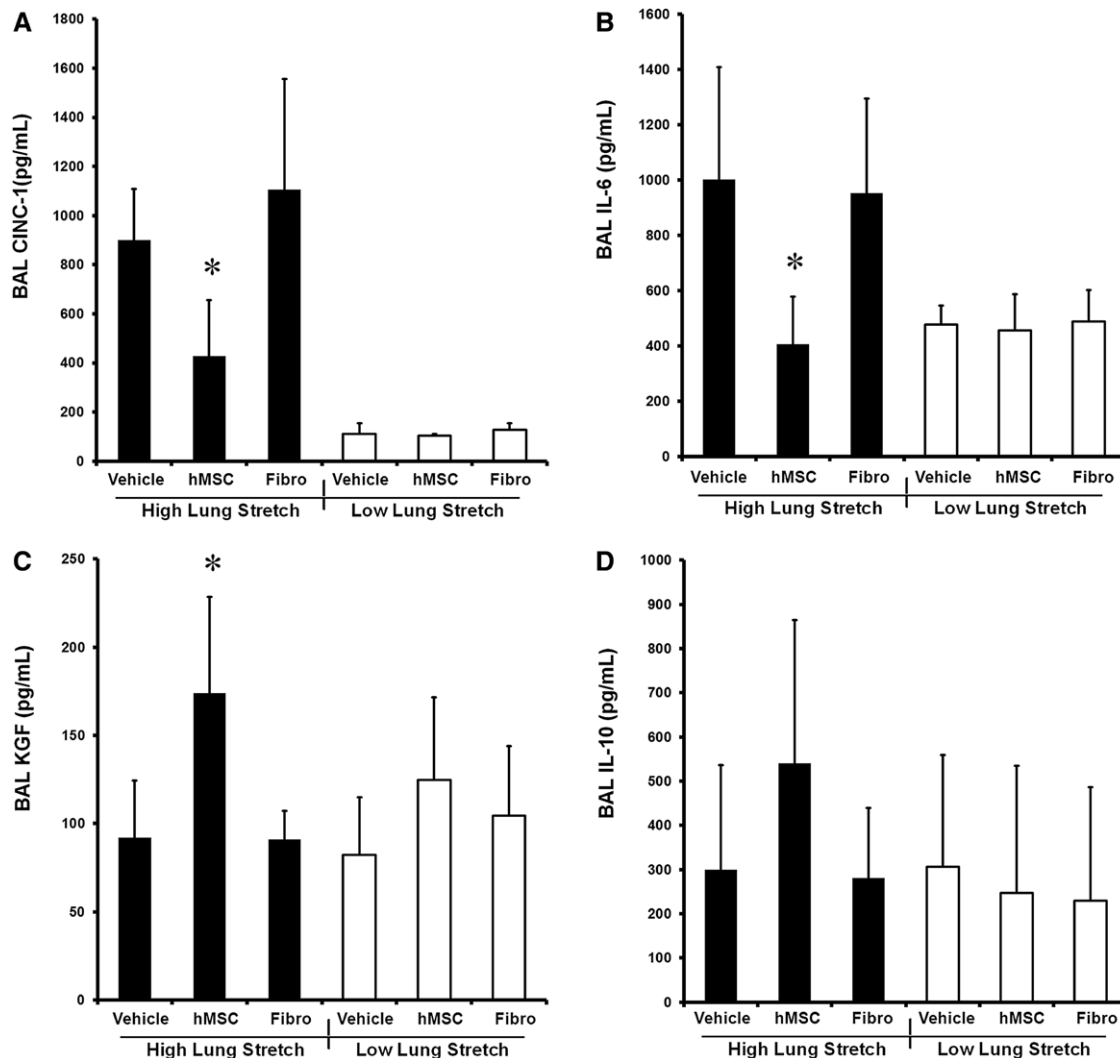


Fig. 2. hMSCs modulate the cytokine response after ventilator-induced lung injury. hMSC therapy decreased BAL CINC-1 (A), decreased BAL IL-6 (B), and increased BAL KGF (C) concentrations. In contrast, there was no significant effect of hMSC therapy on BAL IL-10 (D) compared to either vehicle or fibroblast therapy. Error bars represent standard deviation. $n = 8$ to 9 animals per high-stretch group and $n = 4$ animals per low-stretch group. BAL = bronchoalveolar lavage; CINC-1 = cytokine-induced neutrophil chemoattractant-1; Fibro = human fibroblast; hMSC = human mesenchymal stromal cell; IL = interleukin; KGF = keratinocyte growth factor; Vehicle = treatment with vehicle alone. *Significantly ($P < 0.05$) different from Vehicle high-stretch group.

All animals survived the VILI protocol, and there were no differences among groups at baseline.

hMSC therapy enhanced repair irrespective of the route of administration used. Both intravenous and intratracheal hMSC administration more effectively enhanced recovery of arterial oxygenation (fig. 5A), improved lung compliance (fig. 5B), did not alter lung wet:dry weight ratios (fig. 5C), reduced alveolar protein concentrations (fig. 5D), reduced the alveolar total white cell and neutrophil (fig. 5, E and F) infiltration, and restored lung structure (fig. 2, Supplemental Digital Content 2, <http://links.lww.com/ALN/B116>) compared to their vehicle controls. While intraperitoneal hMSC delivery was less effective, with no significant restoration of lung function or structure, intraperitoneal hMSCs did decrease alveolar neutrophil infiltration.

Series 4—Determination of Therapeutic Window for hMSC Therapy

Thirty-two animals were entered into the experimental protocol, with eight allocated to each of the groups. All animals survived induction of VILI, while two animals in the vehicle group died during the 48-h recovery period.

hMSC therapy restored lung function when given at 0.25, 6, and 24 h after VILI. Previous MSC administration, that is, at 0.25 and 6 h post-VILI, was more effective than later MSC administration at 24 h after VILI. MSC administration at 0.25 and 6 h, but not 24 h, post-VILI significantly improved arterial oxygenation (fig. 6A). MSC administration at each time point post-VILI restored respiratory static compliance (fig. 6B). There was no effect of MSC therapy on lung wet:dry ratios (fig. 6C). hMSC therapy at 0.25 and 6 h decreased alveolar

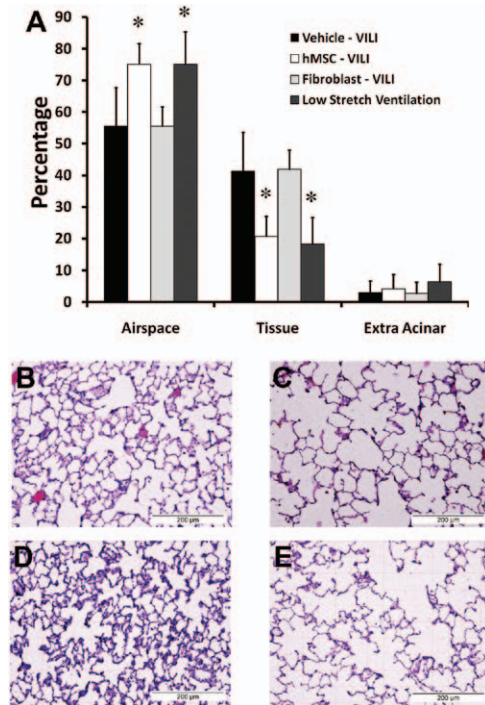


Fig. 3. hMSC therapy enhances the resolution of structural lung injury after VILI. hMSC therapy enhanced resolution of histologic injury as evidenced by decreased alveolar lung tissue and increased alveolar airspace fractions (A). Representative photomicrographs of lung from a vehicle-treated (B), a hMSC-treated (C), a fibroblast-treated (D), and a low-stretch ventilation (E) animal demonstrate greater resolution of lung injury with MSCs at 24 h ($n = 8$ to 9 animals per VILI group). Scale bar is 200 μm . Error bars represent standard deviation. hMSC = human mesenchymal stromal cell; VILI = ventilator-induced lung injury. *Significantly ($P < 0.05$) different from Vehicle VILI group.

protein leak (fig. 6D). MSC administration at each time point post-VILI decreased alveolar inflammatory cellular (fig. 6E) and neutrophil (fig. 6F) infiltration, and was effective in restoring lung structure (fig. 3, Supplemental Digital Content 2, <http://links.lww.com/ALN/B116>).

Discussion

In these studies, we report that human MSCs, transplanted xenogeneically into the rat, augment physiological, biochemical, and structural recovery of the lung after injury induced by mechanical ventilation. hMSCs were similarly efficacious in enhancing repair after VILI over a wide dose range, with a lower threshold effective dose of 2×10^6 hMSC/kg. Consistent with our previous work, we found that intratracheal hMSC administration was as effective as intravenous delivery. In contrast, the intraperitoneal route, while demonstrating some benefit, was less effective in repairing the injured lung. Of relevance to clinical translation, hMSCs were effective in repairing the injured lung even when administered at later time points during the repair process after VILI.

hMSC's Restored Lung Function after VILI

hMSC therapy enhanced resolution of lung injury after VILI, as evidenced by a reduced alveolar-arterial oxygen gradient, improvements in lung compliance and lung permeability, decrease in lung wet:dry weight ratios, and a decrease in alveolar fluid protein concentrations. hMSC therapy also facilitated restoration of lung structure after VILI. Of importance, hMSCs did not demonstrate any adverse effects in protectively ventilated animals, suggesting that they were well tolerated. The finding that fibroblasts did not have any therapeutic effect suggests the reparative effects are specific to MSCs. Whilst xenogeneic transplantation of MSC has been demonstrated to attenuate sepsis-induced ALI in mice,²⁷ this is, to our knowledge the first study investigating the role of hMSC in immunocompetent rats with ALI induced by mechanical ventilation.

Mechanisms of Action of hMSCs

The immunomodulatory effects of hMSC therapy appear important in mediating their effects in restoring the injured lung after VILI. hMSC therapy reduced alveolar neutrophil infiltration while increasing the proportion of macrophages in the lung. hMSC therapy also decreased alveolar concentrations of the proinflammatory cytokines CINC-1 and IL-6. IL-6 contributes to neutrophil accumulation and alveolar barrier disruption.^{28,29} In the ARDSnet tidal volume study, plasma levels of IL-6 in patients with ARDS were positively correlated with mortality,⁸ while lower tidal volume ventilation, which improved patient survival, decreased IL-6 concentrations.³⁰ While alveolar IL-10 concentrations were higher in hMSC-treated animals, this difference was not statistically significant, contrasting with previous work using rodent MSCs.^{1,11} Taken together, these findings suggest that hMSC therapy modulates the immune response to the injurious stimulus, reducing inflammation-induced injury while facilitating repair in the lung.

In keeping with previous studies from our group and others,^{11,17} we found that hMSC therapy increased alveolar concentrations of the growth factor KGF. The ability of MSCs to enhance pulmonary epithelial wound healing,¹¹ and to restore alveolar fluid clearance in human lung explants,¹⁷ is KGF dependent, suggesting that this MSC-secreted chemokine is central to the reparative effects of MSCs.

These beneficial effects of hMSCs appear to be mediated, at least in part, *via* a paracrine mechanism, as suggested by the fact that intraperitoneal hMSC administration demonstrated efficacy, albeit reduced compared to intravenous or intratracheal administration. Furthermore, the observation that hMSCs attenuate inflammation after xenogeneic transplantation to immunocompetent rats supports previous work²⁷ and suggests that these beneficial effects of hMSCs are not major histocompatibility complex restricted. This is a reassuring finding for the future use of allogeneic hMSCs in humans.

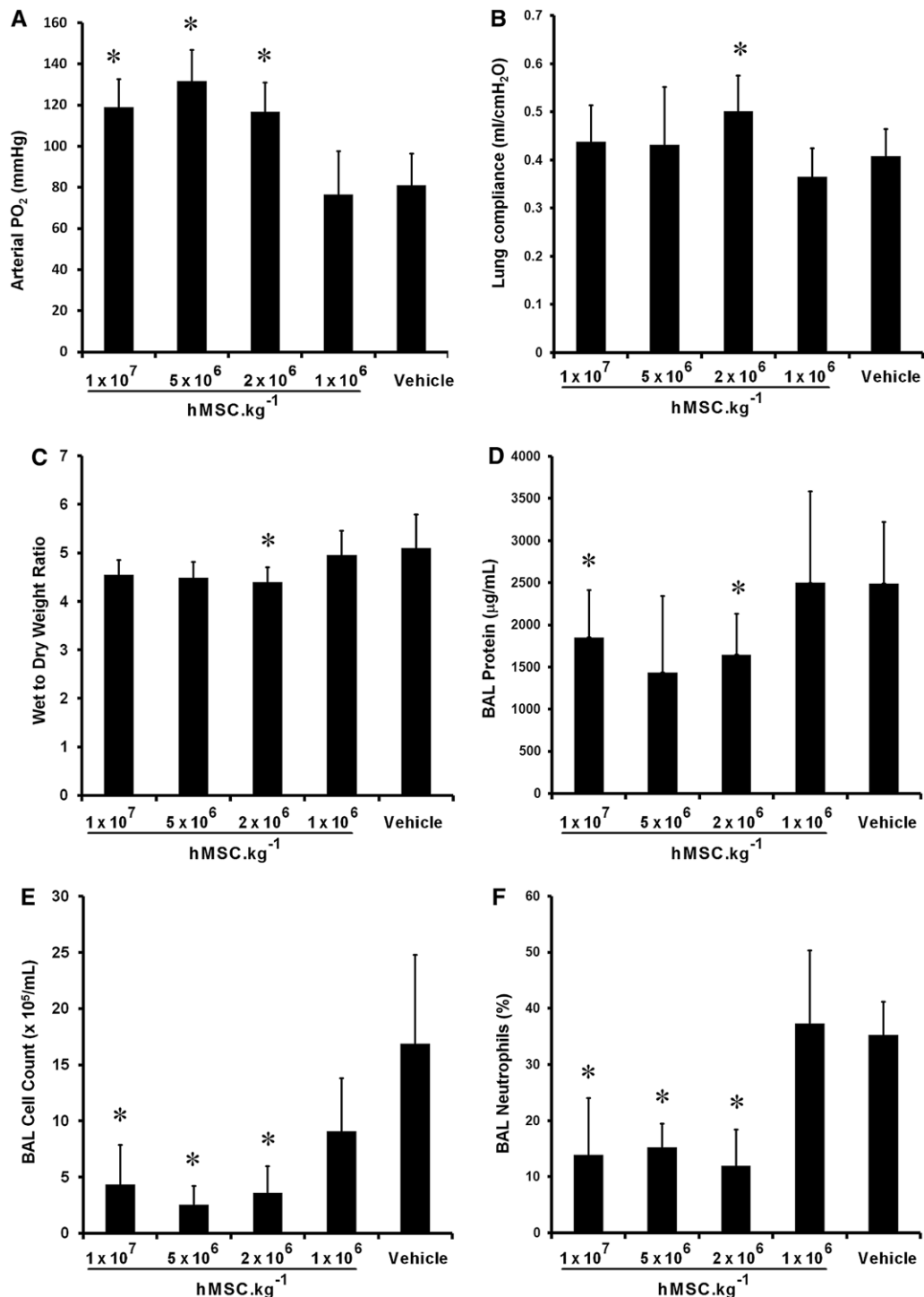


Fig. 4. Dose–response effects of hMSC therapy to enhance lung repair. Doses of hMSCs as low as 2×10^6 per kg restored arterial oxygenation (A), increased static lung compliance (B), reduced lung wet:dry weight ratios (C), decreased BAL protein concentrations (D), reduced BAL total cell counts (E), and decreased the proportion of BAL neutrophils (F) 24 h after ventilator-induced lung injury. There was no effect of 1×10^6 per kg hMSC, suggesting this dose is ineffective in repairing the injured lung ($n = 8$ animals per group). Error bars represent standard deviation. BAL = bronchoalveolar lavage; hMSC = human mesenchymal stromal cell; Vehicle = treatment with vehicle alone. *Significantly ($P < 0.05$) different from Vehicle group.

hMSC Dose–Response

The lowest effective dose of hMSCs was 2×10^6 MSCs/kg, with this dose constituting a threshold above which greater efficacy

was not seen. In contrast, the 1×10^6 cells/kg MSC dose fell below the critical cell dosage threshold and failed to show efficacy. While these findings represent proof-of-concept for a

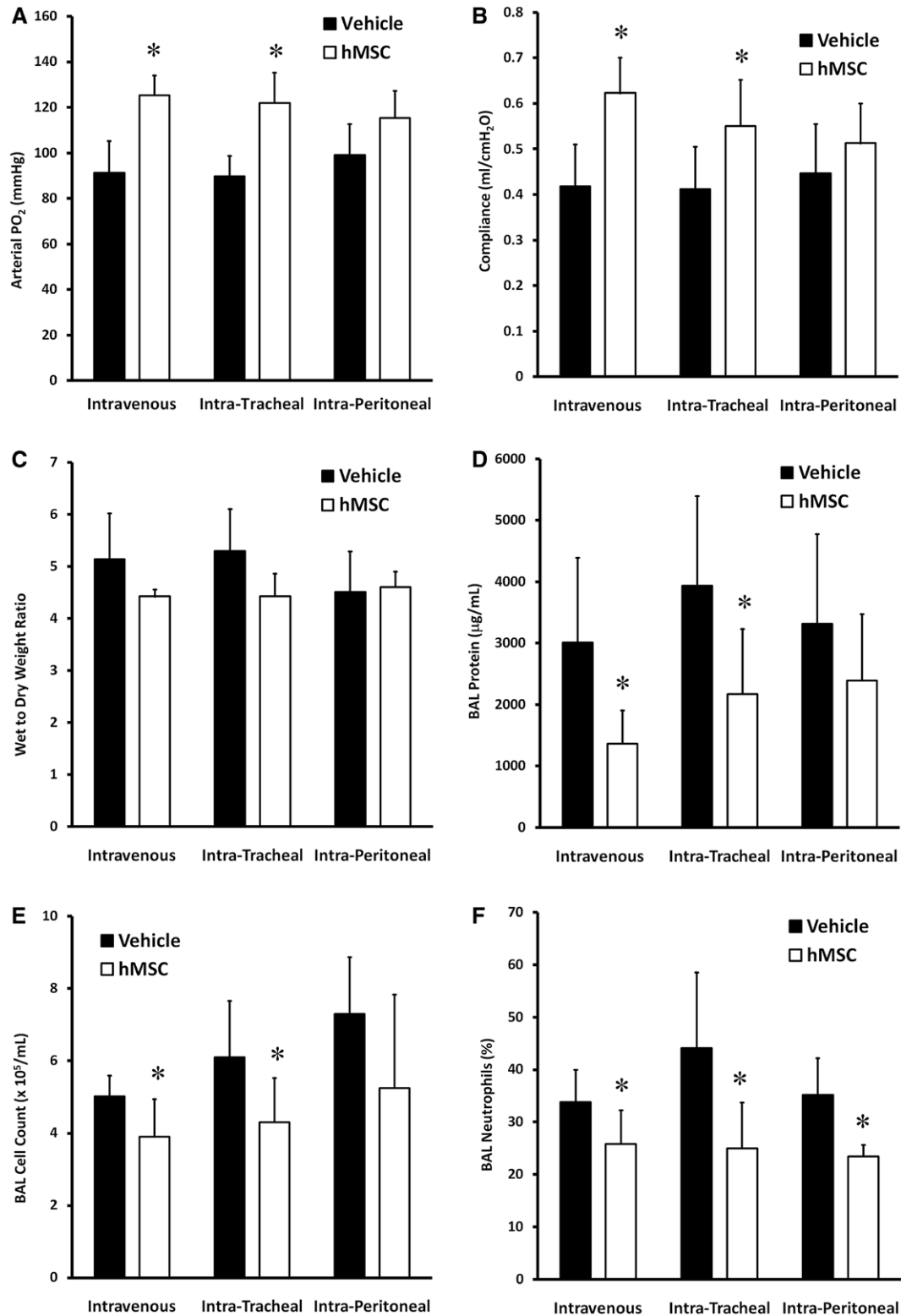


Fig. 5. Effect of route of administration on hMSC efficacy. Delivery of hMSCs by the intravenous and intratracheal routes restored arterial oxygenation (A), increased static lung compliance (B), had no effect on lung wet:dry weight ratios (C), decreased BAL protein concentrations (D), reduced BAL total cell counts (E), and decreased the proportion of BAL neutrophils (F) 24 h after ventilator-induced lung injury. The intraperitoneal administration route was less effective. *Error bars* represent standard deviation. $n = 10$ animals per hMSC group and $n = 5$ animals per Vehicle group. BAL = bronchoalveolar lavage; hMSC = human mesenchymal stromal cell; IP = intraperitoneal; IT = intratracheal; IV = intravenous; Vehicle = treatment with vehicle alone. *Significantly ($P < 0.05$) different from Vehicle group for each administration route.

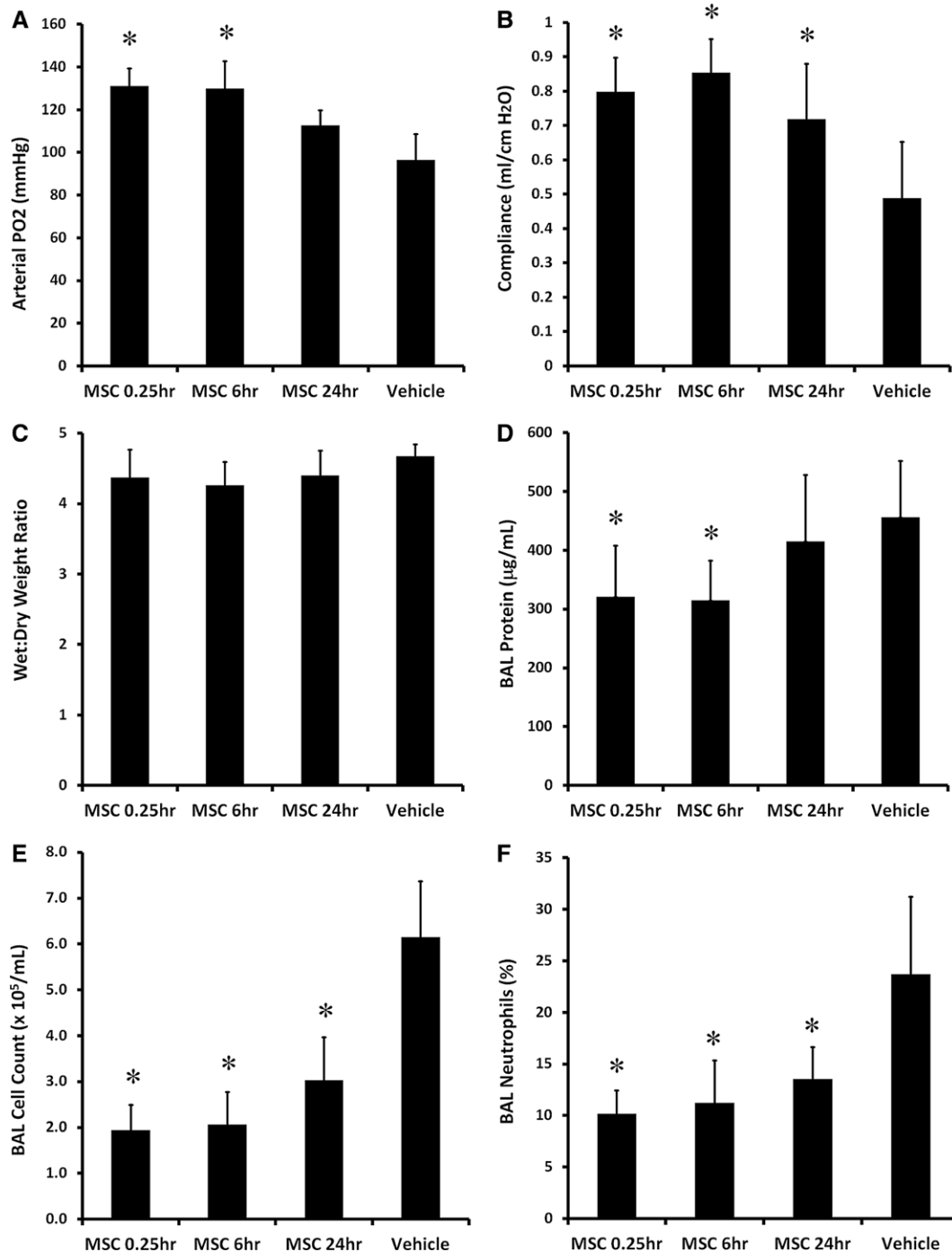


Fig. 6. Effect of delayed hMSC administration on resolution of lung injury after VILI. Administration of hMSCs at 0.25 and 6 h after VILI restored arterial oxygenation (A), increased static lung compliance (B), had no effect on lung wet:dry weight ratios (C), decreased BAL protein concentrations (D), reduced BAL total cell counts (E), and decreased the proportion of BAL neutrophils (F). hMSC administration at 24 h after VILI did demonstrate reduced efficacy, restoring lung compliance and decreasing alveolar infiltration ($n = 8$ animals per group). Error bars represent standard deviation. BAL = bronchoalveolar lavage; hMSC = human mesenchymal stromal cell; Vehicle = treatment with vehicle alone; VILI = ventilator-induced lung injury. *Significantly ($P < 0.05$) different from Vehicle group.

nonlinear dose–response curve for hMSC therapy, and should not be directly extrapolated to clinical studies, they have important implications for clinical translational studies. Our findings suggest that the dose–response curve for hMSC therapy may

not be linear. In the absence of clear evidence for benefit for higher doses of hMSCs, the potential for higher cell doses to precipitate embolic phenomena and increased right ventricular strain, which may be very poorly tolerated in the ARDS patient,

should be borne in mind. These concerns reinforce the need for careful dose titration studies in the early phase clinical trials

Optimal Route for hMSC Therapy

Systemic intravenous hMSC delivery demonstrated equal efficacy to direct intratracheal delivery, which confirms our previous findings with rodent MSC therapy.¹⁰ Given the demonstrated importance of MSC paracrine effects, in studies from our group¹¹ and others,³¹ we investigated the therapeutic potential of remote (*i.e.*, intraperitoneal) hMSC delivery. The option to administer MSCs remote to the injured lung is attractive clinically as allogeneic MSCs could be packaged and implanted using minimally invasive techniques and then removed after disease resolution. Intraperitoneal delivery did demonstrate some therapeutic effect, albeit diminished in comparison to the intravenous or intratracheal delivery routes. It is possible that a higher dose of hMSCs would further enhance lung repair when the intraperitoneal delivery route is used. In addition, these studies do not rule out the possibility that the intraperitoneal delivered hMSCs may have migrated to the lung to exert their effects. Nevertheless, our findings support previous studies suggesting that the protective effect of MSCs may be enhanced through cell–cell contact,^{32,33} in the injury environment. In addition, systemic delivery may have a greater impact on modulation of inflammation.¹⁰

Delayed hMSC Therapy Is Effective

For successful clinical translation, any treatment must be effective even when started well after the onset of the injury process. However, most preclinical ARDS studies to date administered MSC therapy within 6h of induction of injury.³⁴ In our preclinical model of VILI, the most severe injury and inflammation is seen at 6h, with significant histologic injury persisting up to 96h, and with full resolution of the injury at later time points.¹⁹ In the current studies, hMSCs significantly enhanced repair after VILI even when administered at 24h after injury, that is, after the cytokine response to the injury has peaked. This suggests that the efficacy of hMSC therapy in the VILI-injured lung is not solely antiinflammatory. The finding that MSCs are effective at later time points enhances their therapeutic potential, in that it is rarely feasible to administer a therapy in the clinical setting in the earliest stages after the injurious event has occurred

Limitations

There are a number of limitations to these studies. First, our studies were carried out in a rodent model and caution must be exercised in extrapolating to the clinical situation. These studies provide important proofs of concept in regard to hMSC efficacy, dose–response, and dose timing characteristics rather than providing direct information on how to perform hMSC therapy in humans. Second, we did not provide baseline data on these animals. However, we provide data on protectively ventilated animals that were also exposed to these therapeutic

strategies. This allows the reader to assess the magnitude of effects of injurious ventilation on the parameters measured. Finally, we have not examined the fate of injected cells or performed detailed mechanism of action studies. Previously, we found that systemically or intratracheally injected cells accumulate in lung in the first 24h, and thereafter are distributed to other organs or the reticuloendothelial system.¹⁰

Conclusions

Xenogeneic transplantation of hMSCs enhances restoration of lung function and structure in immunocompetent rats after lung injury induced by mechanical ventilation. These studies demonstrate the presence of a threshold hMSC dose, suggesting a nonlinear dose–response curve, and show that the intraperitoneal hMSC administration route is less effective than the intravenous or intratracheal administration, while MSCs may demonstrate efficacy at later time points in the recovery process after VILI. When taken together with other studies examining the effects of MSCs during the injury phase of ARDS, these findings strongly suggest that MSCs may have therapeutic potential for ARDS, particularly in that subgroup of patients with or at high risk for VILI.

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Competing Interests

Dr. Elliman is a senior research scientist at Orbsen Therapeutics Ltd., Galway, Ireland, a company which is developing mesenchymal stromal cells for therapeutic purposes. The remaining authors declare no competing interests.

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