Effects of Intratracheal Mesenchymal Stromal Cell Therapy during Recovery and Resolution after Ventilator-induced Lung Injury

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ABSTRACT

Background: Mesenchymal stromal cells (MSCs) have been demonstrated to attenuate acute lung injury when delivered by intravenous or intratracheal routes. The authors aimed to determine the efficacy of and mechanism of action of intratracheal MSC therapy and to compare their efficacy in enhancing lung repair after ventilation-induced lung injury with intravenous MSC therapy.

Methods: After induction of anesthesia, rats were orotracheally intubated and subjected to ventilation-induced lung injury (respiratory rate 18 min^{-1}, P_{insp} 35 cm H_2O,) to produce severe lung injury. After recovery, animals were randomized to receive: (1) no therapy, n = 4; (2) intratracheal vehicle (phosphate-buffered saline, 300 μl, n = 8); (3) intratracheal fibroblasts (4×10^6 cells, n = 8); (4) intratracheal MSCs (4×10^6 cells, n = 8); (5) intratracheal conditioned medium (300 μl, n = 8); or (6) intravenous MSCs (4×10^6 cells, n = 4). The extent of recovery after acute lung injury and the inflammatory response was assessed after 48 h.

Results: Intratracheal MSC therapy enhanced repair after ventilation-induced lung injury, improving arterial oxygenation (mean ± SD, 146 ± 3.9 *vs.* 110.8 ± 21.5 mmHg), restoring lung compliance (1.04 ± 0.11 *vs.* 0.83 ± 0.06 ml·cm H_2O^{-1}), reducing total lung water, and decreasing lung inflammation and histologic injury compared with control. Intratracheal MSC therapy attenuated alveolar tumor necrosis factor-α (130 ± 43 *vs.* 488 ± 211 pg·ml^{-1}) and interleukin-6 concentrations (138 ± 18 *vs.* 260 ± 82 pg·ml^{-1}). The efficacy of intratracheal MSCs was comparable with

What We Already Know about This Topic

- There is no recognized therapy for ventilator-induced lung injury, a significant contributor to morbidity and mortality in Acute Respiratory Distress Syndrome

What This Article Tells Us That Is New

- Mesenchymal stromal cell therapy, administered intratracheally or intravenously, enhances recovery and repair following ventilator-induced lung injury

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intravenous MSC therapy. Intratracheal MSCs seemed to act *via* a paracine mechanism, with conditioned MSC medium also enhancing lung repair after injury.

Conclusions: Intratracheal MSC therapy enhanced recovery after ventilation-induced lung injury *via* a paracrine mechanism, and was as effective as intravenous MSC therapy.

MECHANICAL ventilation is essential to support life in acute respiratory failure, but can worsen lung injury severity.[1,2] Repeated cycles of mechanical stretch result in lung inflammation, injury, and ultimately tissue destruction—this is termed ventilator-induced lung injury (VILI).[3,4] The mechanisms by which mechanical ventilation can worsen the severity of acute lung injury (ALI) and Acute Respiratory Distress Syndrome (ARDS) are clear.[5] Conversely, it is clear that conventional low stretch mechanical ventilation strategies save lives.[6,7]

Recent studies have generated considerable interest in human mesenchymal stromal cells (MSCs) as a therapeutic option for patients suffering with ALI/ARDS. MSCs attenuate inflammation and lung injury in preclinical ARDS models.[8,9] An important mechanism of action of human MSCs is restoration of alveolar fluid clearance, which has been demonstrated to occur *via* secretion of keratinocyte growth factor.[10] Our group has recently demonstrated that intravenous MSC therapy enhances epithelial and endothelial repair after ventilation-induced ALI *via* a paracrine mechanism involving keratinocyte growth factor.[11]

The optimal MSC delivery strategy, particularly the delivery route that provides the best balance between therapeutic benefit, invasiveness, and potential for harm, is not known. Intrapulmonary delivery may be more attractive than systemic administration for lung diseases, in that larger numbers of cells may be administered directly to the injury zone. Clinically, local cell delivery can be achieved by direct injection *via* the endotracheal tube in ALI/ARDS patients receiving ventilatory support. Furthermore, the type of lung injury, whether pulmonary or extrapulmonary, may be important, with endothelial injury perhaps treated best by intravenous MSC therapy whereas epithelial injury may be treated optimally *via* the intratracheal route.[12] The safety of intravenous MSC infusion is clear from clinical trials in human disease, such as in myocardial infarction,[13] graft *versus* host disease[14] and stroke,[15] and in preclinical models of ALI/ARDS.[9,10] Moreover, the ability of MSCs to home to injured tissues[16] may obviate the need for local delivery strategies, and may result in localized therapeutic benefit after systemic delivery.[17]

We wished to determine the efficacy of intratracheal MSC therapy, gain insights into the mechanisms underlying these effects, and compare the efficacy of this approach with that of intravenous MSC therapy.[11] We hypothesized that intratracheal delivery of MSCs would (1) enhance functional recovery and lung repair after VILI; (2) that these effects would be mediated *via* a paracrine mechanism; and (3) that the efficacy of intratracheal delivery would be similar to intravenous MSC therapy.

Materials and Methods

These experiments were approved by the Animal Ethics Committee at the National University of Ireland, Galway and were performed under license from the Department of Health and Children, Ireland. Specific-pathogen–free adult male Sprague–Dawley rats (Charles River Laboratories, Kent, United Kingdom) weighing between 350 and 450 g were used in these studies. A full description of the methods is available in Supplemental Digital Content 1, http://links.lww.com/ALN/A911.

MSC Isolation and Culture

Bone marrow was aspirated from the tibiae of Sprague–Dawley rats, and plated into tissue culture flasks, as previously described.[18] Adherent cells were grown until 80% confluent and then trypsinized and culture expanded to passage 4, whereupon they were used for experiments. MSCs were characterized according to the international guidelines (see figures, Supplemental Digital Content 2, http://links.lww.com/ALN/A912 and Supplemental Digital Content 3, http://links.lww.com/ALN/A913).[19] Fibroblasts, isolated from the dermis of Sprague–Dawley rats as previously described, were used as control cells.[20]

Conditioned Medium

Allogeneic rat MSCs (4×10^6) were cultured in serum-free media for 24 h. After replacement of the medium, the subsequent serum-free medium was used as the conditioned medium. Fifteen milliliters of this medium was centrifuged through a 3,000 kd filter (Amicon, Billerica, MA) to reduce volume to 300 μl.[20]

Rodent VILI Protocol

We used our established model of repair from VILI.[20] Rats were anesthetized with intraperitoneal ketamine 80 $mg \cdot kg^{-1}$ (Ketalar; Pfizer, Cork, Ireland) and xylazine 8 $mg \cdot kg^{-1}$ (Xylapan; Vétoquinol, Dublin, Ireland). Tail vein intravenous access was obtained, and anesthesia was maintained with Saffan® (Schering Plough, Welwyn Garden City, United Kingdom) and paralysis with *cis*-atracurium besylate 0.5 $mg \cdot kg^{-1}$ (GlaxoSmithKline, Dublin, Ireland). Animals then received high stretch ventilation (P_{insp} 35 cm H_2O, zero positive end-inspiratory pressure; rate 18 min^{-1}). After induction of significant injury, as evidenced by a 50% decrement in static compliance, ventilation was discontinued and the animals allowed to recover[20] (fig. 1).

After recovery, animals were randomized to receive (1) no therapy, (2) intratracheal vehicle (phosphate-buffered saline, 300 μl), (3) intratracheal fibroblasts (4×10^6), (4) intratracheal MSCs (4×10^6), (5) intratracheal conditioned medium (300 μl), or (6) intravenous MSCs (4×10^6). The

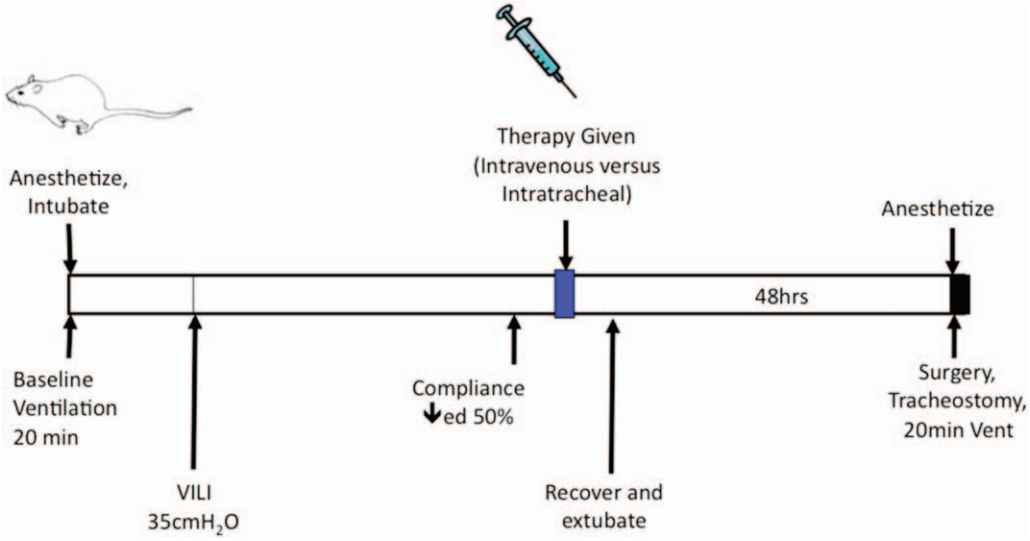

Fig. 1. Flow diagram indicating timelines for experimental interventions. VILI = ventilator-induced lung injury.

timing of MSC delivery was 15–30 min after cessation of high stretch ventilation, *i.e.*, approximately 2.5–3 h after the initiation of VILI.

Lung Injury and Repair Assessment

Animals were reanesthetized 48 h after VILI induction as described in rodent ventilator-induced injury protocol. A tracheostomy was performed, and static lung compliance assessment and arterial blood gas analysis performed as previously described.[21,22] After 20 min, Fi_{O2} was increased to 1.0 for 15 min, and arterial PO_2 measured. After heparin (400 $U \cdot kg^{-1}$; CP Pharmaceuticals, Wrexham, United Kingdom) administration, animals were euthanized by exsanguination under anesthesia. Immediately postmortem, the heart–lung block was removed and bronchoalveolar lavage (BAL) carried out.[23,24] BAL differential cell counts were performed. BAL protein was measured using a Micro BCA™ Protein assay kit (Pierce, Rockford, IL).[25] BAL tumor necrosis factor-α, interleukin-1β, interleukin-6, and interleukin-10 concentrations were determined using enzyme-linked immunosorbent assays (R&D Systems, Abingdon, United Kingdom).[26] Wet:dry lung weight ratios were measured using the right lower lung lobe.[27] The left lung was fixed using paraformaldehyde, and histologic lung injury assessed using quantitative stereology.[23,27] The investigator performing the physiologic assessment was not blinded to group allocation; however, the investigators were blinded for all other assessments and assays.

Analysis of Lung MSC Distribution Patterns

Cell tracking studies were performed in the intravenous and intratracheal MSC groups. MSCs were labeled with red fluorescent marker (PKH26; Sigma, St. Louis, MO; see Supplemental Digital Content 1, http://links.lww.com/ALN/A911 and were then administered intravenously or intratracheally after VILI. At 1, 4, and 24 h post-MSC administration, lungs from rats were removed and digested to generate single-cell suspensions for subsequent flow-cytometry (see Supplemental Digital Content 1, http://links.lww.com/ALN/A911). Flow cytometry was performed using FACScan and CellQuest-Pro software (Becton Dickinson, Franklin Lakes, NJ).

Statistical Analysis

Sigmastat 3.1 (Systat Software, San Jose, CA) was used for all statistical analyses. Sample size was determined based on our previous studies.[11] Data distribution was assessed using Kolmogorov–Smirnov tests. Data were analyzed by one-way ANOVA, followed by Student–Newman–Keuls, or by Kruskalis–Wallis followed by Mann–Whitney U test with the Bonferroni correction for multiple comparisons, as appropriate. Residual plots were used to validate underlying model assumptions. A P value of less than 0.05 (two-tailed) was considered to be significant.

Results

Forty animals were entered into the experimental protocol. All survived the injury and subsequent treatment allocation. Eight animals were each entered into the fibroblast, vehicle control, intratracheal MSC, and intratracheal conditioned medium groups; four animals were each entered into the intravenous MSC and no therapy groups. There were no baseline between-group differences in terms of preinjury variables, the duration of injurious ventilation, or the extent of the lung injury produced (table 1). All animals in each group survived the VILI recovery protocol. There were no differences in arterial pH, PCO_2, bicarbonate, lactate, or mean arterial pressure among the groups at the end of the recovery period (table 2).

Table 1. Baseline Data Regarding Animals Subjected to Ventilation-induced Lung Injury

Variable	No Therapy	Vehicle	Intratracheal Fibroblasts	Intratracheal MSCs	Intratracheal CM	Intravenous MSCs
Number of animals	4	8	8	8	8	4
Animal weight (g)	451 ± 33	478 ± 12	449 ± 11	445 ± 9	448 ± 12	407 ± 9
Ventilation injury time (min)	184 ± 18	189 ± 30	191 ± 20	189 ± 18	188 ± 15	184 ± 17
Lung compliance preinjury (ml/cmH_2O)	1.03 ± 0.02	1.11 ± 0.07	1.06 ± 0.07	1.16 ± 0.11	1.11 ± 0.08	0.99 ± 0.11
Lung compliance post-VILI	0.57 ± 0.01	0.59 ± 0.02	0.57 ± 0.02	0.60 ± 0.02	0.59 ± 0.02	0.55 ± 0.04

Data are expressed as mean ± SD.

CM = conditioned medium; MSC = mesenchymal stem/stromal cell; No Therapy = no treatment given, Vehicle = treatment with vehicle alone; VILI = ventilation-induced lung injury.

Intratracheal MSCs Restored Lung Function

Intratracheal MSC administration facilitated restoration of arterial oxygenation, reducing alveolar-arterial oxygen gradient ($P < 0.001$) (fig. 2A) and increasing arterial oxygenation ($P < 0.001$) (table 2) compared with vehicle. Further functional recovery in lung physiology in response to intratracheal MSC therapy was demonstrated by significant improvements ($P < 0.001$) in respiratory system static compliance in comparison with vehicle (fig. 2B). Intratracheal MSCs improved lung microvascular permeability, decreasing lung wet:dry weights ($P < 0.001$) (fig. 2C) and reducing BAL protein concentrations ($P = 0.006$) (fig. 2D).

Intratracheal MSCs Modulated Inflammation

Intratracheal MSCs decreased total inflammatory cell counts in BAL fluid ($P < 0.001$) (fig. 3A), and substantially attenuated ($P < 0.001$) lung neutrophil and macrophages accumulation (fig. 3, B and C). MSC therapy altered the proportions of inflammatory cells recruited to the ventilator-injured lung. Interestingly, intratracheal and intravenous MSCs increased the proportion of alveolar lymphocytes, whereas conditioned medium increased the proportion of alveolar macrophages, although this latter effect was modest (fig. 3, C–F). Intratracheal MSC therapy decreased alveolar concentrations of tumor necrosis factor-α ($P < 0.001$) (fig. 4A) and interleukin-6 ($P < 0.001$) (fig. 4B). In contrast, intratracheal MSC therapy did not alter BAL interleukin-10 concentrations ($P = 0.873$) (fig. 4C). Alveolar concentrations of keratinocyte growth factor were modestly increased by MSC therapy, being significantly ($P = 0.018$) increased only with intratracheal MSC therapy (fig. 4D).

Intratracheal MSCs Repair the Injured Lung

Intratracheal MSCs reduced alveolar thickening, as evidenced by decreased fractional alveolar tissue volume ($P < 0.001$), and enhanced the restoration of airspace volume, as evidenced by enhanced fractional alveolar air-space volume ($P < 0.001$) (fig. 5, A and B). Representative histologic sections of lung demonstrate the enhanced repair of the injured lung in the MSC-treated animals (fig. 5, C and D).

Mechanism of Action

The efficacy of intratracheal MSC therapy in enhancing lung repair after injury was also seen with MSC conditioned medium (fig. 2A–D). Intratracheal MSC conditioned medium also resulted in a similar pattern of reduction in lung inflammatory cells and altered cytokine profile in response to VILI (figs. 3 and 4). These findings suggest a paracrine mechanism of action for these cells. Importantly, nonstem/stromal cells, *i.e.*, fibroblasts, did not have any therapeutic effect.

Table 2. Data Regarding Extent of Resolution 48 h after Ventilation-induced Acute Lung Injury

Variable	No Therapy	Vehicle	Intratracheal Fibroblasts	Intratracheal MSCs	Intratracheal CM	Intravenous MSCs
Arterial oxygen tension (mmHg)	114.8 ± 7.1	110.8 ± 21.5	118.5 ± 11.9	146.2 ± 3.9*	142.6 ± 3.8*	141.6 ± 2.9*
Arterial pH	7.33 ± 0.02	7.37 ± 0.02	7.41 ± 0.03	7.46 ± 0.03	7.46 ± 0.03	7.40 ± 0.01
Arterial PCO_2 (mmHg)	32.1 ± 3.2	31.6 ± 3.1	29.6 ± 2.2	30.4 ± 3.1	30.2 ± 1.8	31.7 ± 2.0
Arterial bicarbonate (mM)	17.1 ± 0.3	18.9 ± 0.7	21.8 ± 0.8	23.9 ± 0.9	24.1 ± 0.7	21.4 ± 0.3
Arterial lactate (mM)	1.7 ± 0.2	1.2 ± 0.4	1.8 ± 0.6	1.8 ± 0.6	1.8 ± 0.5	2.0 ± 0.4
Mean arterial pressure (mmHg)	107.8 ± 12.1	90.4 ± 10.4	71.9 ± 9.0	75.8 ± 10.1	87.5 ± 21.7	82.0 ± 13.9

Data are expressed as mean ± SD. Final data are data collected after completion of the experimental protocol.

*Significantly different from vehicle group, $P < 0.05$.

CM = conditioned medium; MSC = mesenchymal stromal cell; No Therapy = no treatment given; Vehicle = treatment with vehicle alone.

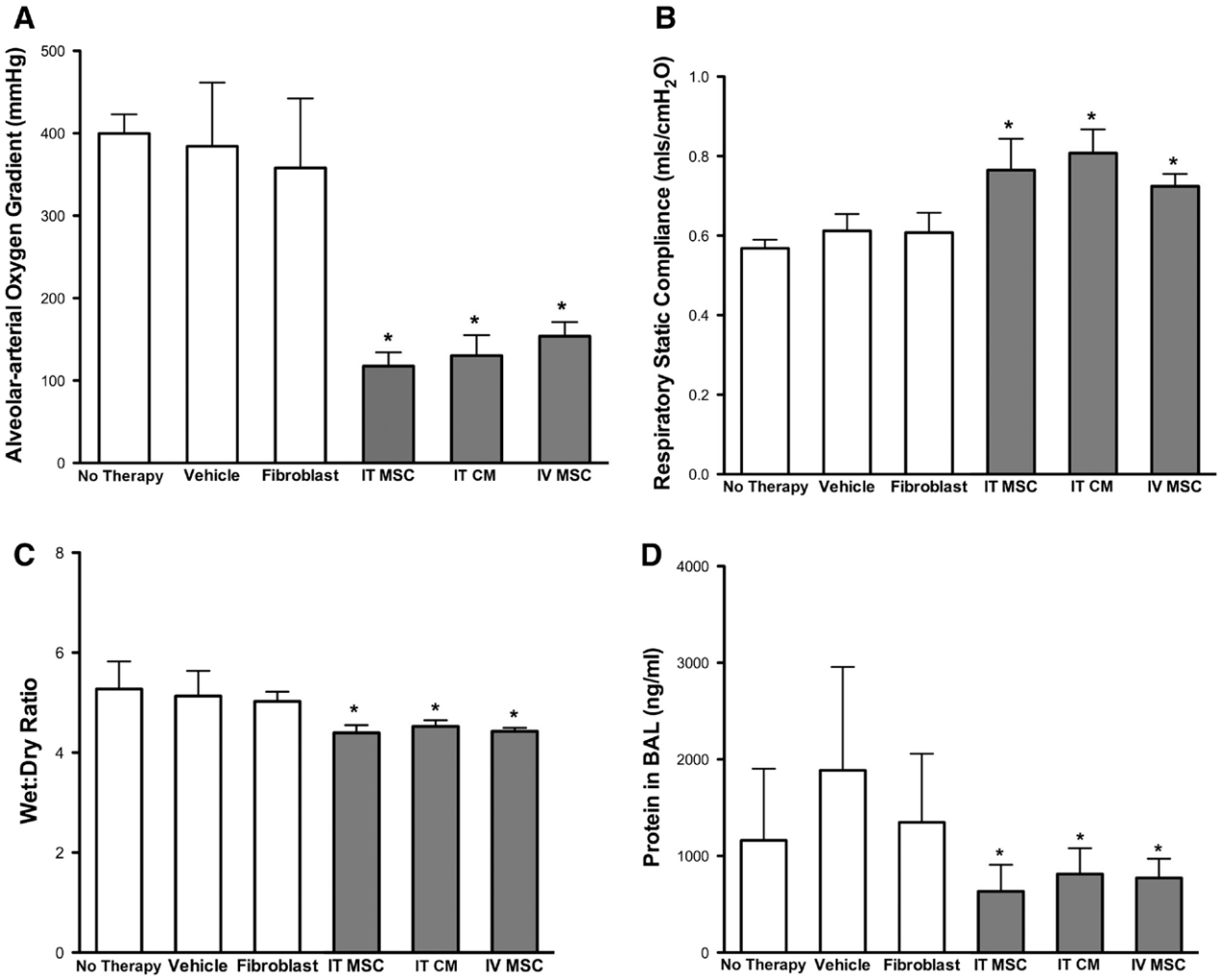

Fig. 2. MSCs and MSC conditioned medium enhance lung repair. IT and IV MSC therapy and IT MSC conditioned medium each decreased ($P < 0.001$) alveolar-arterial oxygen gradient (*A*), increased ($P < 0.001$) static lung compliance (*B*), reduced ($P < 0.001$) lung wet:dry weight ratios (*C*), and decreased ($P = 0.007$) BAL protein concentrations (*D*), 48 h after induction of severe stretch induced lung injury, compared with the other groups. BAL = bronchoalveolar lavage; CM = conditioned medium; IV = intravenous; IT = intratracheal; MSC = mesenchymal stromal cell; No Therapy = no treatment given; Vehicle = treatment with vehicle alone. *Significantly ($P < 0.05$) different from Vehicle, fibroblast, and No therapy groups.

Intratracheal versus *Intravenous MSC Therapy*

The magnitude of the therapeutic effect of intratracheal MSCs was similar to that seen with intravenous MSC therapy (figs. 2–4). However, animals that received intravenous MSC therapy demonstrated an increase in BAL interleukin-10 concentrations ($P < 0.001$) (fig. 4C). This was not seen in animals that received intratracheal MSCs or intratracheal conditioned medium. Higher alveolar concentrations of keratinocyte growth factor were demonstrated in the intratracheal MSC group alone ($P = 0.018$) (fig. 4D). In addition, a greater number of administered MSCs were retained in the lung after intratracheal than after intravenous delivery at 1, 4, and 24 h post-MSC administration (table, Supplemental Digital Content 4, http://links.lww.com/ALN/A914).

Discussion

MSCs exhibit considerable therapeutic promise for patients with ALI/ARDS. MSC therapy attenuated endotoxin-induced ALI when given during the injury phase in mice.[8,9] Cells derived from the bone marrow, including MSCs, have improved survival and reduced injury in preclinical models of systemic polymicrobial sepsis.[28,29] Human MSCs attenuate the decrement in alveolar epithelial fluid clearance *via* secretion of keratinocyte growth factor in *ex vivo* perfused human lung after endotoxin injury.[10] Recently, we have demonstrated that intravenous MSC therapy enhances epithelial and endothelial repair after ventilation-induced ALI by a keratinocyte growth factor-dependent paracrine mechanism.[11] Although studies suggest that both the intratracheal and systemic MSC delivery routes may

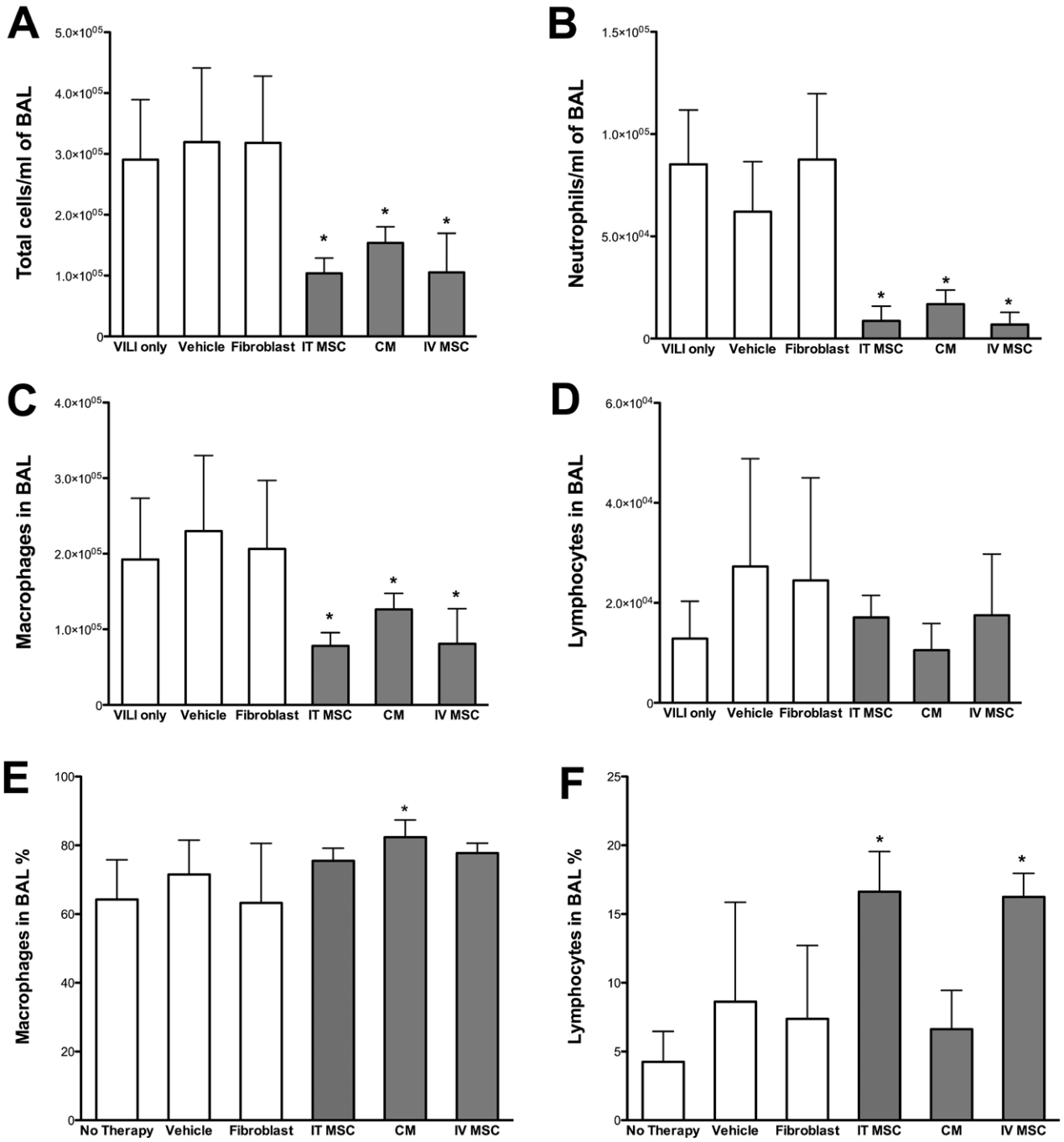

Fig. 3. MSCs and conditioned medium modulates the cellular inflammatory response to VILI. IT and IV MSC therapy and IT MSC conditioned medium each decreased ($P < 0.001$) BAL total cell counts (*A*), and decreased ($P < 0.001$) BAL neutrophil (*B*) and ($P < 0.01$) macrophage (*C*), but not lymphocyte (*D*) counts. IT MSC conditioned medium increased the percentage of macrophages (*E*), whereas both IT and IV MSCs increased the percentage of lymphocytes (*F*) in the alveolar infiltrate. All assays were performed 48 h after induction of severe stretch induced lung injury, compared with the other groups. BAL = bronchoalveolar lavage; CM = conditioned medium; IV = intravenous; IT = intratracheal; MSC = mesenchymal stromal cell; No therapy = no treatment given; Vehicle = treatment with vehicle alone; VILI = ventilation induced lung injury. *Significantly ($P < 0.05$) different from Vehicle, fibroblast, and No therapy groups.

be effective in attenuating ALI,[8,30] the optimal delivery method remains unclear, and there are no data comparing these routes in the setting of repair after injury. In these studies, we report that intratracheal administration of MSCs restored lung function after ventilation-induced injury, that they appear to act *via* a paracrine mechanism, and that they are as effective in restoring lung function as intravenous MSC therapy.

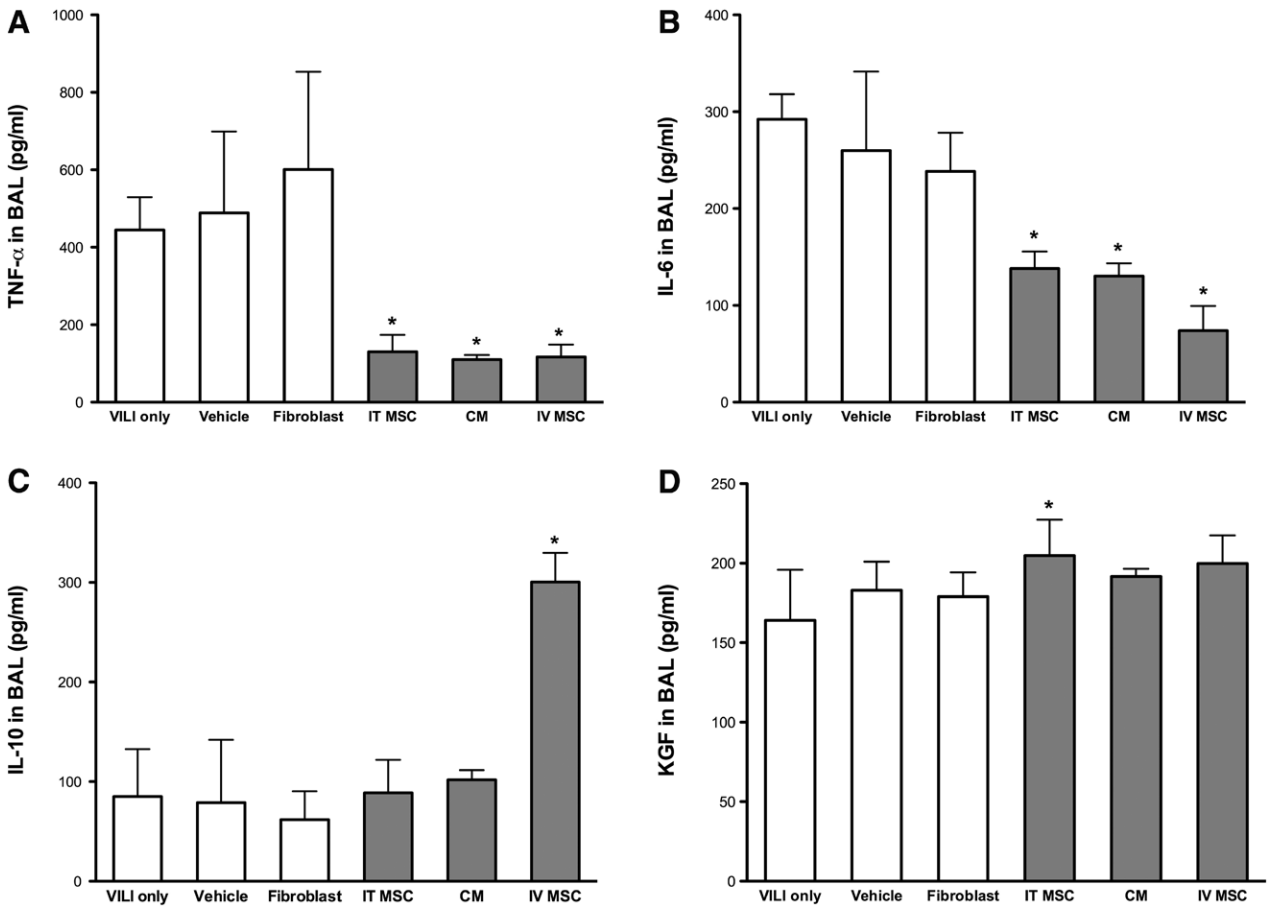

Fig. 4. MSCs and MSC conditioned medium modulates the cytokine response to VILI. IT and IV MSC therapy and IT MSC conditioned medium each decreased ($P < 0.001$) BAL TNF-α concentrations (*A*), and decreased ($P < 0.001$) BAL IL-6 concentrations (*B*). IV MSCs, but not IT MSCs or IT conditioned medium, increased ($P < 0.001$) BAL IL-10 (*C*) concentrations. IT MSCs increased ($P < 0.001$) BAL KGF (*D*) concentrations. All assays were performed 48 h after induction of severe stretch induced lung injury, compared with the other groups. BAL = bronchoalveolar lavage; CM = conditioned medium; IV = intravenous; IL = interleukin; IT = intratracheal; KGF = keratinocyte growth factor; MSC = mesenchymal stromal cell; No Therapy = no treatment given; TNF-α = tumor necrosis factor-α; Vehicle = treatment with vehicle alone; VILI = ventilation-induced lung injury. * Significantly ($P < 0.05$) different from Vehicle, fibroblast, and No therapy groups.

Intratracheal MSC Therapy Enhances Resolution of Lung Damage

Intratracheal MSC therapy enhanced lung repair after VILI, as demonstrated by a reduced alveolar-arterial oxygen gradient, improvements in lung compliance and alveolar-capillary permeability. Intratracheal MSC therapy also modulated the inflammatory response to injury, decreasing alveolar white cell and neutrophil counts, and decreasing alveolar tumor necrosis factor-α and interleukin-6 concentrations. Intratracheal MSC therapy also facilitated restoration of lung structure after stretch injury.

In our studies, both MSC and conditioned medium therapy decreased overall alveolar inflammatory cell infiltration. In contrast, intravenous and intratracheal MSC therapy increased the percentage of lymphocytes in the alveolar fluid. MSCs have well-described effects on T- and B-lymphocytes, decreasing the proliferation and activation of these cells, while enhancing the production of T-regulatory cells, a T-cell population that plays a role in down-regulation of inflammation and tissue repair.[31] Although the composition of the T-cell population was not examined here, MSC/T-cell interactions seem to require cell contact.[31] Our finding that this increase in the proportion of alveolar lymphocytes was not seen with MSC conditioned medium seems to support this. The significance of these findings is unclear, given that both MSCs and conditioned medium augmented lung repair in these studies.

Mechanism of Action of Intratracheal MSC Therapy

Intratracheal MSC therapy seems to enhance repair after VILI by a mechanism that is paracrine dependent. Conditioned medium from MSCs was as effective as intratracheal MSC therapy in repairing the injured lung. MSC-conditioned medium also resulted in a similar pattern of reduction in lung inflammatory cells and altered cytokine profile in response to VILI. The finding that intravenous

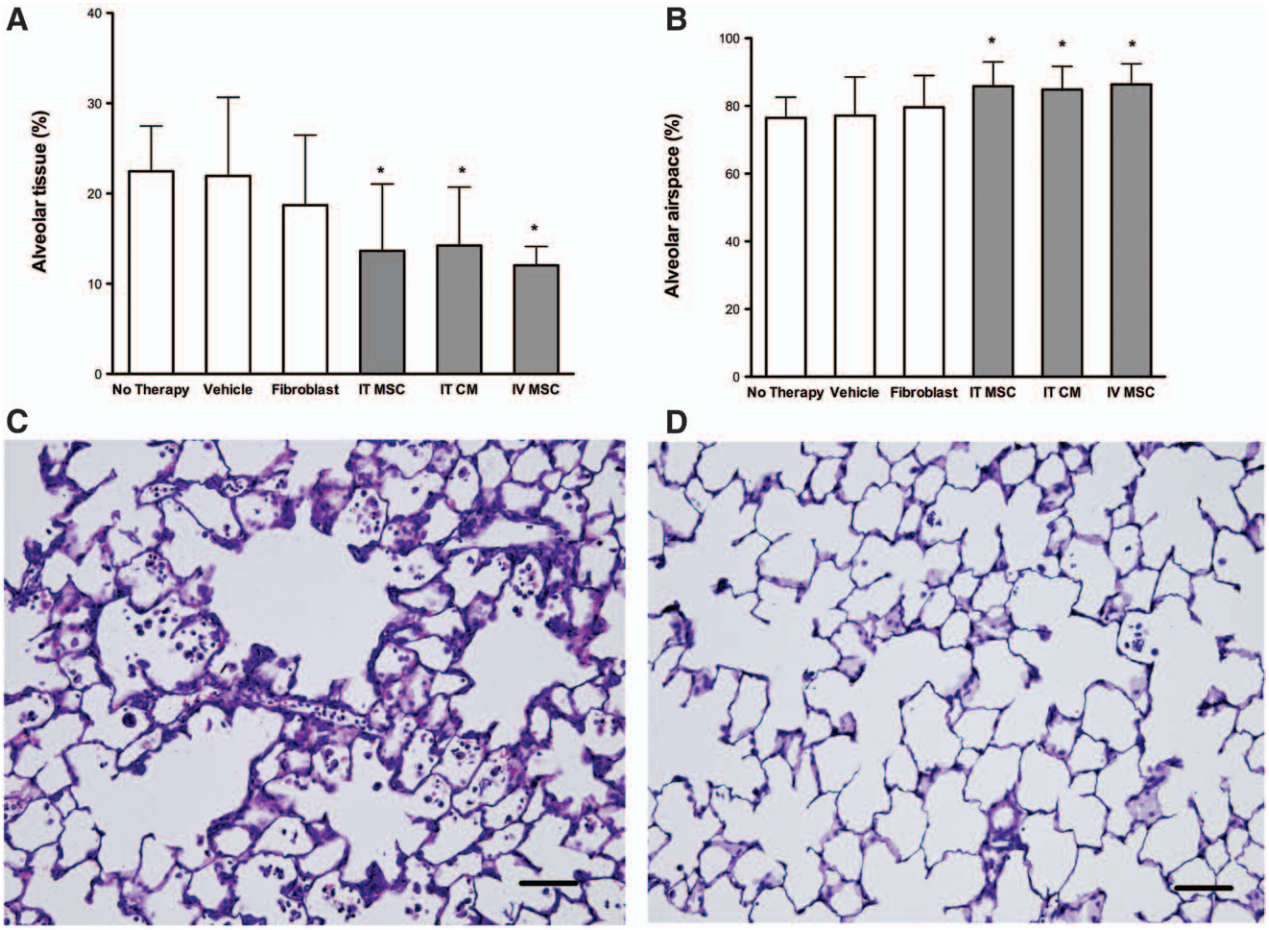

Fig. 5. MSC therapy enhances the resolution of structural lung injury after VILI. MSC therapy enhanced resolution of histologic injury as evidenced by decreased alveolar lung tissue (*A*) and increased alveolar airspace fraction (*B*). Representative photomicrographs of lung from a vehicle-treated (*C*), and IT MSC-treated (*D*) animal demonstrate greater resolution of lung injury with MSCs at 48 h (n = 8 animals per group). Scale bar is 200 μm. CM = conditioned medium; IV = intravenous; IT = intratracheal; MSC = mesenchymal stromal cell; No Therapy = no treatment given; Vehicle = treatment with vehicle alone; VILI = ventilation induced lung injury. *Significantly ($P < 0.05$) different from Vehicle, fibroblast, and No therapy groups.

and intratracheal MSC therapies were equally effective in restoring lung function, despite markedly different lung accumulation profiles, demonstrates that their precise disposition within the animal was of lesser importance, and supports a paracrine mechanism of action. These findings are supported by previous data demonstrating that MSCs act in large part *via* the secretion of paracrine mediators.[10,32] MSC administration modestly increased alveolar keratinocyte growth factor concentrations, although it was only significantly increased after intratracheal administration. Keratinocyte growth factor improves alveolar epithelial wound repair, is secreted in excess by MSCs,[11] and has been implicated in the mechanism by which MSCs enhance pulmonary epithelial wound repair.[11] Intriguingly, Islam et al.[33] recently demonstrated that MSCs release mitochondria-containing microvesicles, which can restore lung epithelial function after injury, providing another potential mechanism by which the MSC "secretome" may restore lung function. The finding that nonstem cells, *i.e.*, fibroblasts, did not have any therapeutic effect, suggests that the reparative effects of MSCs are a function of the stem/stromal cell properties of MSCs.

Intratracheal Delivery Route as Effective as Intravenous Route

In this study, intratracheal MSCs therapy was as effective as intravenous MSCs in restoring physiologic lung function and facilitating recovery of structural integrity after severe VILI. Intratracheal MSC therapy improved measures of alveolar epithelial and endothelial barrier function, including wet:dry ratios and BAL protein concentrations to a similar extent to that seen with intravenous MSC therapy. Both intravenous and intratracheal MSC therapy decreased alveolar concentrations of the key proinflammatory cytokines tumor necrosis factor-α and interleukin-6. Of interest,

intravenous MSC therapy enhanced alveolar interleukin-10 concentrations, a finding not seen in animals that received intratracheal MSCs or MSC medium. This finding, which has been previously reported,[20] suggests that MSCs enhance interleukin-10 secretion *via* an interaction with one or more cell types encountered in the circulation. Intravenous MSCs have been demonstrated to enhance macrophage interleukin-10 secretion in the setting of systemic sepsis.[28] In our study, the increased alveolar interleukin-10 concentrations in animals that received intravenous MSCs are not explained by alterations in alveolar macrophage proportion or absolute numbers. The significance of MSC-induced interleukin-10 secretion in this repair model is unclear, given the fact that both intravenous and intratracheal MSCs were equally efficacious in repairing the injured lung after VILI.

This study suggests that the intratracheal route is a viable alternative to the intravenous route for MSC delivery to promote repair in the lung. These results extend previous findings demonstrating that intratracheal MSC therapy is effective in attenuating the injury phase of ALI.[8–10] The potential advantages of the intrapulmonary route of delivery include the ability to deliver larger numbers of cells directly to the injury zone, their ease of administration in the clinical setting *via* the tracheal tube, and the potential for reduced systemic effects. In contrast, intravenous MSC delivery is not without its risks. Although intravenous MSCs can home to injured organs[34] including the lung,[35] these MSCs are also trapped in the vasculature of the lung,[34] potentially leading to pulmonary capillary plugging, reduced pulmonary vascular compliance, pulmonary hypertension, and right ventricular failure. Intracoronary MSC administration after myocardial infarction caused microvascular plugging, which reduced coronary blood flow, underlining the importance of these concerns.[36] The development of pulmonary hypertension may be particularly deleterious in patients with ALI/ARDS.[37] Consequently, intratracheal MSC delivery may be a useful therapeutic approach in ALI/ARDS patients.

Limitations

A number of limitations deserve consideration. First, our studies were conducted in a preclinical rodent model and caution is required in considering clinical relevance. Second, baseline data are not available on these animals. However, we have characterized the effect of high lung stretch in detail in this model in a previous publication.[20] Last, we did not examine the effects of MSCs in protectively ventilated or unventilated animals, as the effects on uninjured animals would be expected to be limited.

Conclusions

In conclusion, intratracheal MSCs enhance lung repair after VILI to a similar extent to that seen with intravenous administered MSCs. The mechanism of action of the intratracheal MSCs seems to be due to MSC secretion of paracrine factors. Intratracheal MSC therapy seems to have considerable promise for the treatment of patients suffering from VILI and ARDS.

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