ORIGINAL ARTICLE

Combined Mesenchymal Stromal Cell Therapy and Extracorporeal Membrane Oxygenation in Acute Respiratory Distress Syndrome

A Randomized Controlled Trial in Sheep

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Abstract

Rationale: Mesenchymal stromal cell (MSC) therapy is a promising intervention for acute respiratory distress syndrome (ARDS), although trials to date have not investigated its use alongside extracorporeal membrane oxygenation (ECMO). Recent preclinical studies have suggested that combining these interventions may attenuate the efficacy of ECMO.

Objectives: To determine the safety and efficacy of MSC therapy in a model of ARDS and ECMO.

Methods: ARDS was induced in 14 sheep, after which they were established on venovenous ECMO. Subsequently, they received either endobronchial induced pluripotent stem cell–derived human MSCs (hMSCs) ($n = 7$) or cell-free carrier vehicle (vehicle control; $n = 7$). During ECMO, a low V_T ventilation strategy was employed in addition to protocolized hemodynamic support. Animals were monitored and supported for 24 hours. Lung tissue, bronchoalveolar fluid, and plasma were analyzed, in addition to continuous respiratory and hemodynamic monitoring.

Measurements and Main Results: The administration of hMSCs did not improve oxygenation (Pa_{O_2}/Fi_{O_2} mean difference = −146 mm Hg; $P = 0.076$) or pulmonary function. However, histological evidence of lung injury (lung injury score mean difference = −0.07; $P = 0.04$) and BAL IL-8 were reduced. In addition, hMSC-treated animals had a significantly lower cumulative requirement for vasopressor. Despite endobronchial administration, animals treated with hMSCs had a significant elevation in transmembrane oxygenator pressure gradients. This was accompanied by more pulmonary artery thromboses and adherent hMSCs found on explanted oxygenator fibers.

Conclusions: Endobronchial hMSC therapy in an ovine model of ARDS and ECMO can impair membrane oxygenator function and does not improve oxygenation. These data do not recommend the safe use of hMSCs during venovenous ECMO.

Keywords: ARDS; ECMO; MSC; models; animal

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At a Glance Commentary

Scientific Knowledge on the Subject: Mesenchymal stromal cell (MSC) therapy is a promising novel intervention for acute respiratory distress syndrome (ARDS). Trials to date have failed to explore the safety of cell therapy during extracorporeal membrane oxygenation (ECMO). Preclinical investigations have found that MSC therapy during ECMO attenuate the efficacy of ECMO.

What This Study Adds to the Field: This study, employing a 24-hour large animal model of ARDS and ECMO, examined the safety and efficacy of MSC therapy. We found that endobronchially administered MSCs adhere to, and impair, membrane oxygenators *in vivo*. MSCs did not improve oxygenation or other ventilatory parameters but did reduce the severity of histological evidence of lung injury. Our data also suggest that MSCs may reduce circulatory shock associated with ARDS. There were no adverse effects of MSC administration on renal or liver function.

The quest for an effective pharmacological treatment for the acute respiratory distress syndrome (ARDS) has been unsuccessful. Recently, mesenchymal stromal cells (MSCs) have attracted attention as a candidate therapy for ARDS (1).

MSCs are multipotent adult stem cells found in tissues of mesodermal origin, such as bone marrow (2). Therapeutic interest in these cells has arisen because of their pleiotropic immunomodulatory abilities. During acute inflammation, MSCs appear to be immunosuppressive, influencing both innate and adaptive immune responses (3). In ARDS, their beneficial effects are believed to be mediated in a variety of ways, including secretion of antiinflammatory paracrine factors (4), restoration of epithelial and endothelial integrity (5), enhancement of alveolar fluid clearance (6), direct antimicrobial activity (7), and mitochondrial transfer (8). In preclinical models of acute lung injury, MSCs have been shown to reduce mortality (9). A phase 2 study has been conducted in patients with ARDS with no reported infusion-related adverse events (10).

To date, trials of MSCs in ARDS have excluded patients supported with extracorporeal membrane oxygenation (ECMO). The use of ECMO in acute severe respiratory failure has increased substantially in the last decade, and is now an established tool for supporting those with refractory illness (11). The use of MSCs during ECMO, while potentially attractive, raises some unique considerations. First, MSCs are large cells, with an average diameter between 10 and 30 μm (12), which, when administered therapeutically, may pose a risk to the patency of a membrane oxygenator. Second, a defining characteristic of MSCs is avid plastic adherence (13); this too may threaten membrane oxygenators, which are constructed largely from plastics. Recent *ex vivo* and small animal experimentation has confirmed these concerns (14, 15). Conversely, immunomodulation by MSCs may provide additional benefits for patients on ECMO, where the institution of extracorporeal support results in an additional inflammatory insult (16).

Given the paucity of evidence to support the safe use of MSC therapy during ECMO, we conducted a controlled trial of clinical-grade induced pluripotent stem cell (iPSC)-derived human MSCs (hMSCs) in an ovine model of ARDS, supported with venovenous ECMO (VV-ECMO). The primary objective was to assess the safety of MSC therapy and to investigate its effect on physiologic and biologic markers of pulmonary and systemic injury.

Methods

Study Design

Ethical approvals were obtained from University Animal Ethics Committees of Queensland University of Technology and the University of Queensland (QUT1600001108 and UQPCH/483/17) and authorization for *in vivo* use of hMSCs was granted by the Australian Department of Agriculture (2017/075). The study was conducted in accordance with the *Australian Code for the Care and Use of Animals for Scientific Purposes* (17) and is reported in compliance with Animal Research: Reporting of *In Vivo* Experiments guidelines (18). Detailed methods and a comprehensive description of the experiments and analyses are provided in the online supplement. A schematic of the study protocol is provided in Figure 1.

Animal Model

A total of 14 healthy Border Leicester Cross ewes, aged between 1 and 3 years and weighing between 46 and 55 kg (mean = 52.6 ± 3 kg), was randomly assigned to one of two groups: endobronchial iPSC-derived hMSC treatment ($n = 7$) or endobronchial carrier vehicle only ($n = 7$).

In brief, animals were anesthetized with a combination of ketamine, midazolam, and fentanyl. Continuous neuromuscular blockade was maintained by infusion of vecuronium. In the supine position, animals were tracheostomized and ventilated using a low-V_T strategy (6 ml/kg actual body weight [ABW]). After instrumentation, acute lung injury was induced by combining an intravenous infusion of oleic acid (0.06 ml/kg, O1008; Sigma-Aldrich) with endobronchial *Escherichia coli* LPS (LPS, 100 μg, O55:B5; Sigma-Aldrich). Once a Pa_{O_2}/Fi_{O_2} ratio <100 mm Hg (positive end-expiratory pressure ≥ 10 cm H_2O) was obtained (Time 0 [T_0]), animals were established on VV-ECMO via a right-sided jugular–jugular configuration (T_1) and positioned in sternal recumbency. VV-ECMO was combined with a lower-V_T strategy (4 ml/kg ABW) for 22 hours, at which time (T_{23}) ECMO was stopped and a standardized recruitment maneuver was performed. Animals were returned to pre-ECMO ventilatory settings for 1 hour before being killed (T_{24}).

iPSC-derived hMSCs

After 1 hour of VV-ECMO (T_2), animals received a fixed dose of 3×10^8 iPSC-derived hMSCs suspended in a carrier vehicle (hMSC) or carrier vehicle alone (vehicle control). Cells were provided by Cynata Therapeutics Ltd. (CYP-001; Cynata Therapeutics Ltd.). These cells were at least 99% positive for CD-73, CD-90, and CD-105, but negative for CD-31 and CD-45. The total volume of vehicle was 60 ml (57.5% plasmalyte-A, 40% flexbumin 25%, and 2.5% DMSO). Cells were at leats 97% viable before administration. The distribution of delivery is described in Figure E1 in the online supplement.

Statistical Analysis

An *a priori* sample size calculation, based on the primary outcome of Pa_{O_2}/Fi_{O_2} ratio at 24 hours, is detailed in the online supplement. Data are expressed as mean

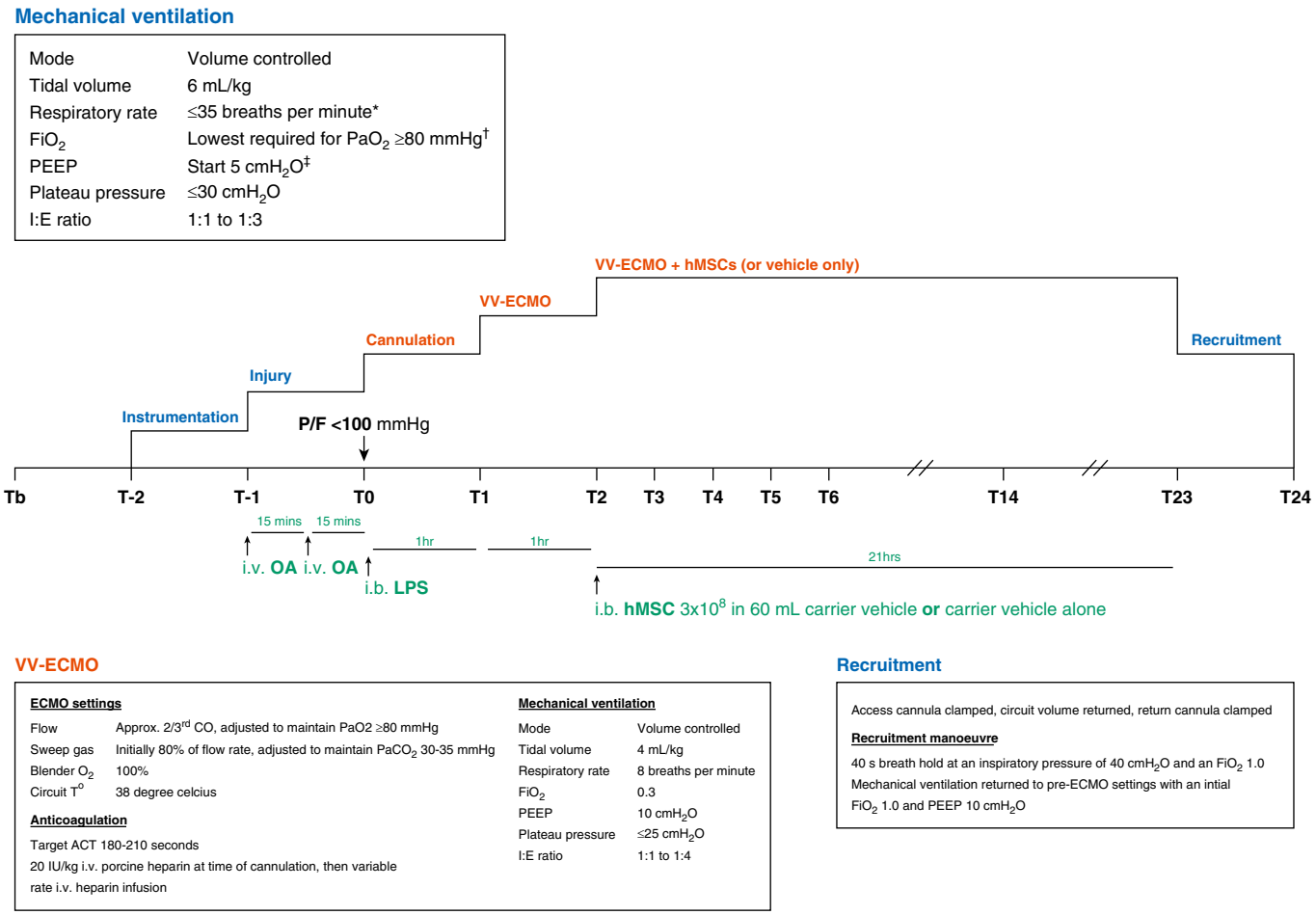

Figure 1. Study schematic. *Adjusted to maintain pH 7.30–7.45. Permissive hypercapnia was tolerated to a minimum pH of 7.15. †Pa_{O_2} = 55–80 mm Hg. If, despite an $F_{I_{O_2}}$ of 1.0, oxygenation targets were not met, positive end-expiratory pressure (PEEP) was increased, maintaining plateau pressure ⩽32 cm H_2O. ‡Total PEEP (extrinsic PEEP + intrinsic PEEP) did not exceed 20 cm H_2O. PEEP was permitted to be reduced to 5 cm H_2O to maintain plateau pressure ⩽30 cm H_2O. If, despite a PEEP of 5 cm H_2O, plateau pressure was >30 cm H_2O, V_T was reduced in 1-ml/kg steps until set at 4 ml/kg. ACT = activated clotting time; ECMO = extracorporeal membrane oxygenation; hMSC = human mesenchymal stromal cell; I:E = inspiratory: expiratory time ratio; OA = oleic acid; P/F = $Pa_{O_2}/F_{I_{O_2}}$ ratio; VV = venovenous.

(±SD) or median (interquartile range [IQR]) if nonnormally distributed. Analysis was undertaken in Graphpad Prism (v 8.1.2; GraphPad Software). Longitudinal data were analyzed by fitting a mixed model. This model uses a compound symmetry covariance matrix and is fit using restricted maximum likelihood. Where a significant interaction was observed, *post hoc* comparisons were undertaken. Correction for multiple comparisons was made using the Benjamini-Hochberg method (false discovery rate restricted to 5%). Nonlongitudinal data were compared using an unpaired *t* test or a Mann-Whitney test, as appropriate. Categorical data were compared using the chi-square test. Statistical significance was assumed if the *P* value was less than 0.05.

Results

Baseline characteristics at injury (T_0) are shown in Table 1 and Table E1. All animals completed the study protocol, and were killed at T_{24}.

Respiratory Variables

The use of ECMO facilitated a lower-V_T ventilation strategy (median [IQR], 4 [4–4] ml/kg ABW). The median (IQR) ECMO flow rate was 2.75 L/min (2.5–3.25 L/min), with a sweep gas flow of 3 L/min (2–3.5 L/min). During VV-ECMO, animals had a median (IQR) Pa_{O_2} of 109 mm Hg (94–131 mm Hg) and a Pa_{CO_2} of 32 mm Hg (30–35 mm Hg). There were no significant differences in these parameters between groups (Figure E2). Animals were adequately anticoagulated during ECMO, as measured by activated partial thromboplastin time ratios. The dose of heparin was not significantly different between groups (Figure E2).

Because VV-ECMO controls gas exchange, native lung function was assessed 1 hour after cessation of extracorporeal flow and after the performance of a standardized lung recruitment maneuver (T_{24}). As shown in Figure 2, both the $Pa_{O_2}/F_{I_{O_2}}$ ratio ($P = 0.076$) and the oxygenation index ($P = 0.153$) were numerically better in the carrier vehicle–only group; the differences were not statistically significant.

The plateau airway and driving pressures were similar between groups at T_{24} (Figure 2). Static lung compliance was

Table 1. Baseline Physiological Characteristics

Characteristics	Overall (*n* = 14)	Vehicle (*n* = 7)	hMSCs (*n* = 7)
Weight, kg	52.6 ± 3	52.4 ± 3.2	52.9 ± 2.6
Pre-ECMO V_T, ml/kg	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1
At time of injury (T_0)			
Peak airway pressure, cm H_2O	31.5 ± 4.2	31.9 ± 3.3	31.1 ± 4.9
Plateau pressure, cm H_2O	26.5 ± 3.9	26.1 ± 3.9	26.9 ± 3.8
Driving pressure, cm H_2O	16.5 ± 3.9	16.1 ± 3.9	16.9 ± 3.8
Static compliance, ml/cm H_2O	21 ± 5.0	19 ± 4.6	23 ± 4.6
Pa_{O_2}/Fi_{O_2}	59 ± 20	58 ± 23	61 ± 17
Pa_{CO_2}, mm Hg	41 (38–46)	38 (38–41)	44 (41–48)
pH	7.36 ± 0.05	7.38 ± 0.04	7.34 ± 0.05
Bicarbonate, mmol/L	23.2 ± 1.5	23.6 ± 1.4	22.9 ± 1.5
Base deficit, mmol/L	1.30 ± 1.31	0.94 ± 1.15	1.66 ± 1.37
Arterial lactate, mmol/L	1.6 ± 0.7	1.8 ± 0.6	1.4 ± 0.7
Heart rate, beats/min	102 (96–117)	108 (98–128)	98 (95–103)
Mean arterial pressure, mm Hg	103 ± 19	96 ± 16	111 ± 19
Central venous pressure, mm Hg	13 (11–13)	12 (11–13)	13 (13–14)
Mean pulmonary artery pressure, mm Hg	25 (16–29)	20 (17–27)	28 (19–29)

Definition of abbreviations: ECMO = extracorporeal membrane oxygenation; hMSCs = human mesenchymal stromal cells.

also similar, both during and after ECMO (Figure 2). The use of a protocolized recruitment maneuver did not improve compliance after cessation of extracorporeal support in either group (Figure 2).

Hemodynamic Variables

This model of acute lung injury was associated with the development of hyperdynamic shock, which worsened over time (Figure 3). The administration of hMSCs resulted in significantly lower cumulative vasopressor doses (Figure 3). At T_4, mean arterial pressure (MAP) was significantly higher in the hMSC-treated group ($P = 0.001$), even though these animals received lower doses of noradrenaline (Figure 3). By T_{14}, MAP was again similar between groups, although vasopressor requirements continued to be lower in hMSC-treated animals. In addition, there were lower arterial lactate concentrations, higher arterial base excesses, and lower mean pulmonary artery pressures from 12 hours (T_{14}) after instillation in the hMSC group (Figure 3); however, these were not statistically significant. Cumulative fluid balance at T_{24} was similar in both groups (vehicle control, 2,713 ± 970 ml vs. hMSCs, 2,992 ± 1,237 ml; $P = 0.648$).

Histopathology and Lung Injury

The blinded assessment of lung tissue was conducted by an independent expert veterinary pathologist. Sections of the right lower lobe were prepared, and a lung injury score was calculated (19). The administration of hMSCs resulted in significantly lower scores ($P = 0.04$; Figure 4), principally mediated by a reduction in neutrophil infiltration.

There were no significant differences in lung wet/dry ratio or BAL total protein concentration (Figure 4). BAL fluid inflammatory cell counts are detailed in Table E4. There were no significant differences in these counts over time. Similarly, there was no difference in lung tissue homogenate gene expression (as assessed by quantitative PCR) between groups (Figure E3).

In a *post hoc* analysis, pulmonary arterial thrombosis was noted in five hMSC-treated animals, but only one animal receiving carrier vehicle alone ($P = 0.031$).

Inflammatory Cytokines

BAL and plasma cytokine concentrations were assessed longitudinally (Figures E4 and E5). In BAL, statistically significant differences in IL-8 were observed at T_3, T_{14}, and T_{23} ($P = 0.013$, 0.016, and 0.028, respectively). In plasma, cytokine trajectories were similar between groups (Figure E5).

Hematological and Biochemical Measurements

A summary of hematological and biochemical values are provided in Tables E2 and E3. This lung injury model was associated with the development of acute kidney injury and abnormal liver function, although there were no significant differences in indices between groups. The administration of hMSCs resulted in a significantly lower lymphocyte count at T_{24} ($P = 0.047$; Figure E5).

Cell–ECMO Membrane Interaction and Cell Fate

The administration of hMSCs was associated with a significant increase in the transoxygenator pressure gradient, becoming apparent 4 hours after cell delivery (Figure 5). By T_{23}, the mean pressure gradient in the hMSC group reached 64 (±37) mm Hg versus 17 (±9) mm Hg in the vehicle-only group. The instillation of carrier vehicle alone was associated with a reduction in the ECMO pump speed to flow ratio over time, a finding not observed in the hMSC group (Figure 5). During the study, there were no instances of pump or oxygenator failure requiring a component exchange. Likewise, there was no evidence of clotting on the oxygenator surface by visual examination in either group.

Membrane oxygenators from animals treated with hMSCs were isolated and preserved at the termination of ECMO. Subsequent deconstruction and staining of the fiber bundles ($n = 7$) revealed adherent cells exhibiting surface markers consistent with those of hMSCs (Figure E6). Similar cell populations were not apparent in vehicle-only controls ($n = 3$).

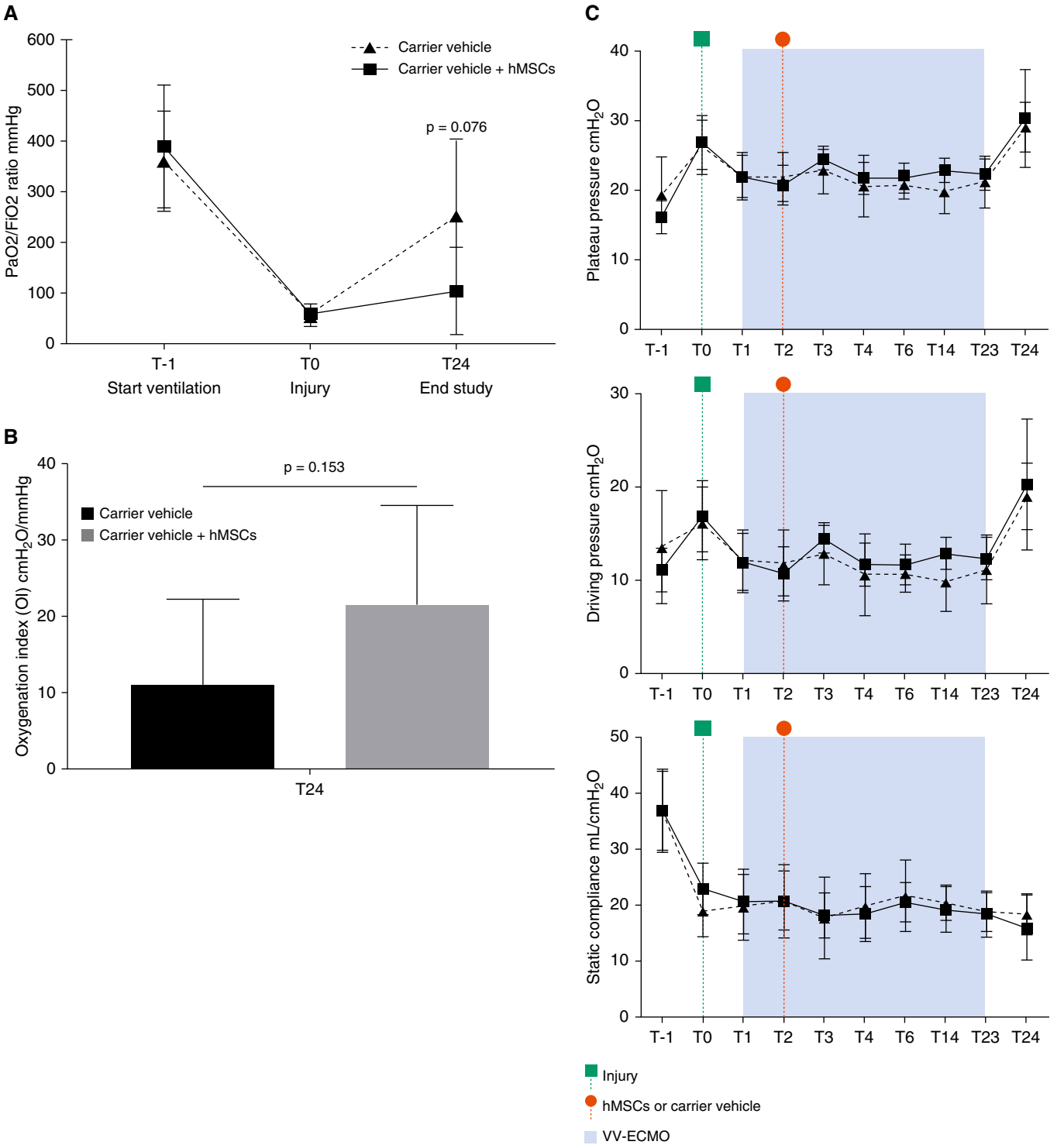

Figure 2. Oxygenation and respiratory parameters. (*A*) $Pa_{O_2}/F_{I_{O_2}}$ ratio. (*B*) Oxygenation index. (*C*) Airway pressures and lung compliance. Data are presented as mean (±95% confidence interval [CI]). Where error bars intersect the *x*-axis, the 95% CI includes zero. hMSC = human mesenchymal stromal cell; VV-ECMO = venovenous extracorporeal membrane oxygenation.

Discussion

We performed a trial of clinical-grade iPSC-derived hMSCs, given endobronchially, for acute lung injury in sheep during VV-ECMO. The main findings of this study can be summarized as follows: *1*) with regard to the primary outcome, hMSCs did not improve oxygenation at 24 hours; *2*) hMSCs did not improve pulmonary mechanics, but did improve the severity of histological lung injury and reduced the

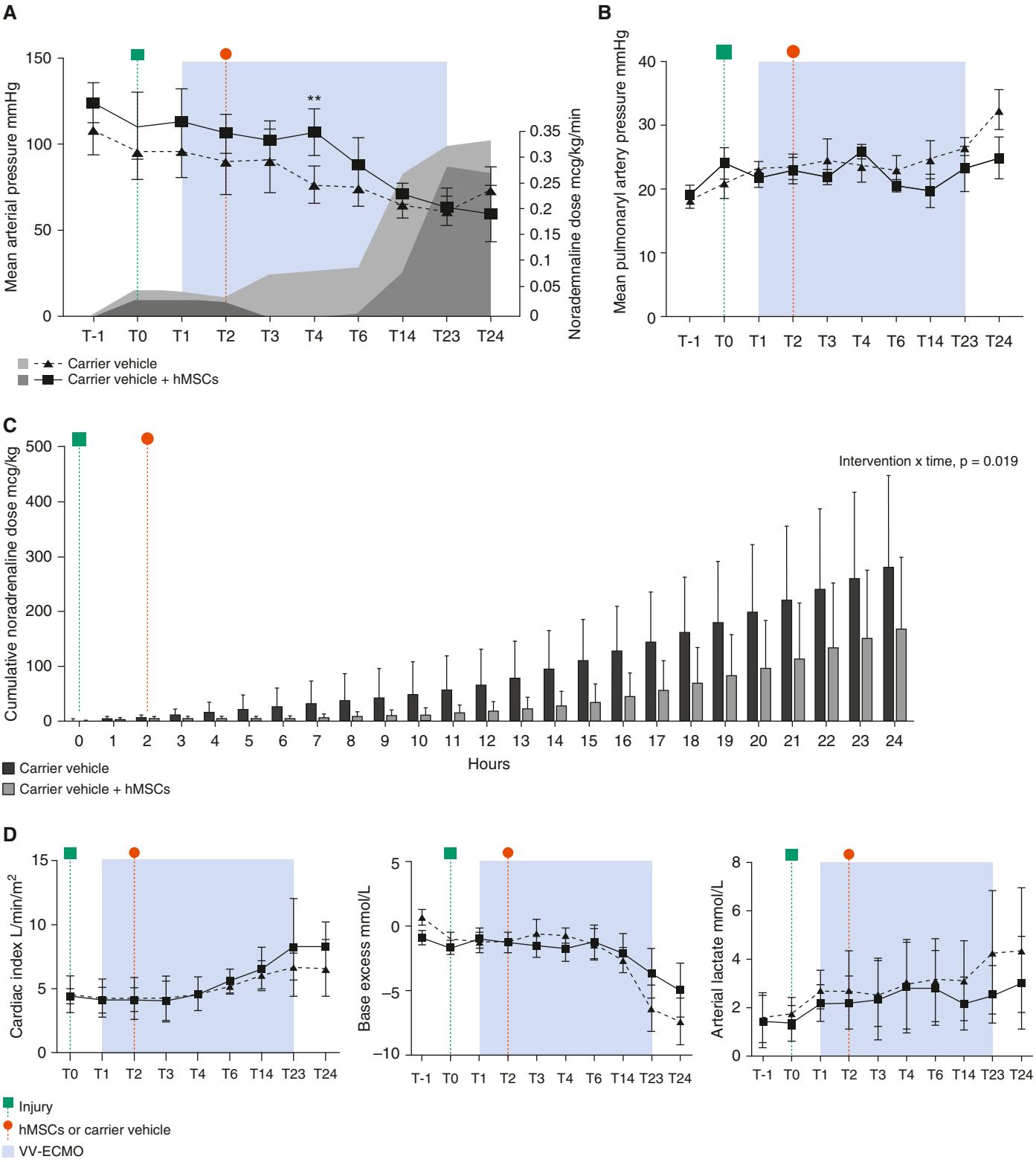

Figure 3. Hemodynamic variables. (*A*) Mean arterial pressure and vasopressor dose (mean). (*B*) Mean pulmonary artery pressure. (*C*) Cumulative vasopressor dose. (*D*) Cardiac index, base deficit, and arterial lactate. Data are presented as mean (±95% confidence interval [CI]). Where error bars intersect the *x*-axis, the 95% CI includes zero. $^{**}P < 0.01$. hMSC = human mesenchymal stromal cell; VV-ECMO = venovenous extracorporeal membrane oxygenation.

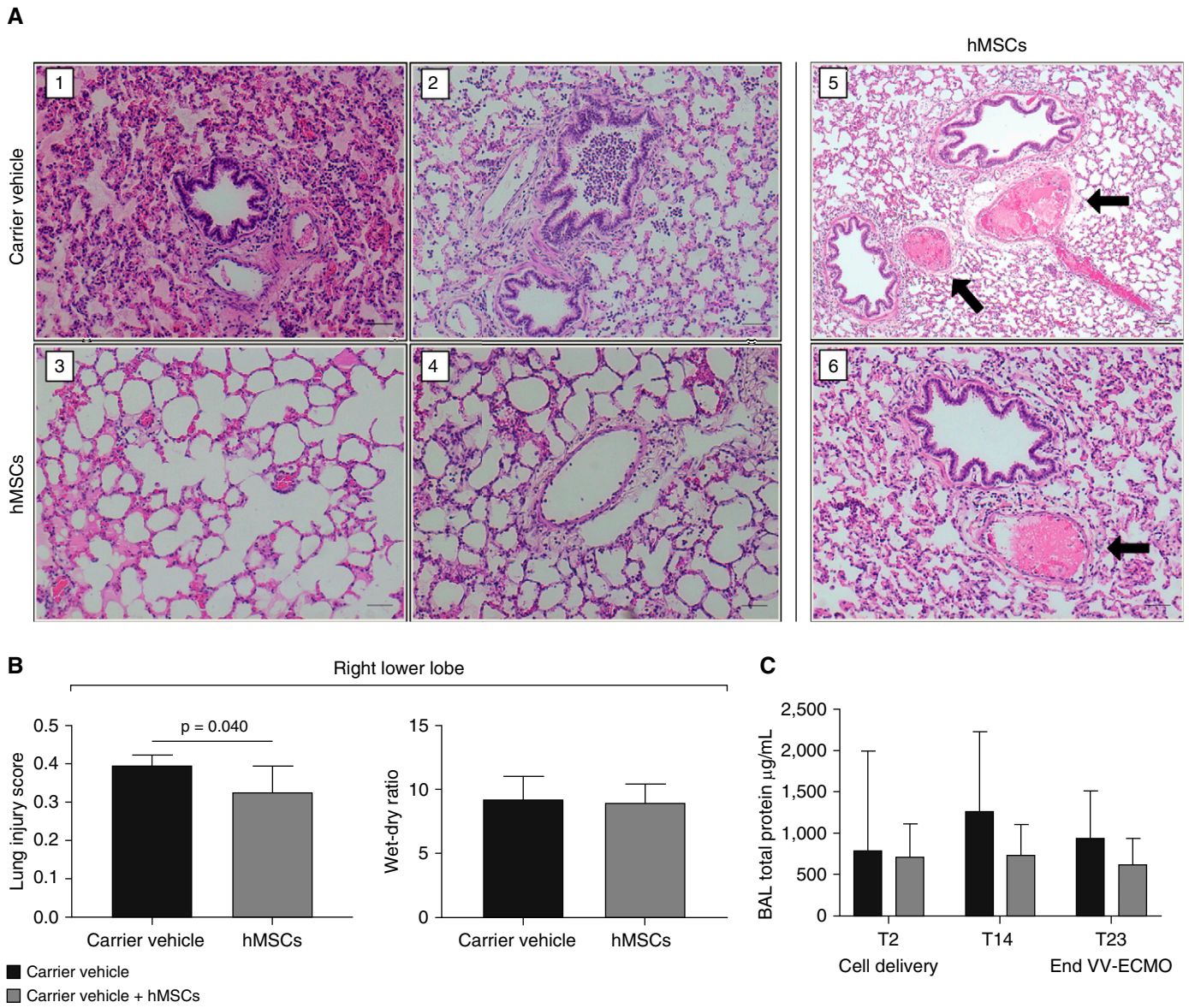

Figure 4. Histopathology and lung injury. (*A*) Representative images of lung parenchyma. All animals showed evidence of diffuse alveolar damage; however, the frequency and degree of injury differed between groups. Panel 1: extensive alveolar edema with interstitial leukocyte infiltration. Panel 2: marked leukocyte infiltration within alveolar spaces and larger airways. Panel 3: some loss of alveolar structure with edema; however, a reduction in interstitial and alveolar leukocytes. Panel 4: preservation of alveolar architecture with few leukocytes in the alveolar spaces. Panels 5 and 6: representative images of pulmonary arterial and arteriolar emboli (black arrows) in animals receiving human mesenchymal stromal cells (hMSCs). Panels 1–4: scale bars, 100 μm. Panels 5 and 6: scale bars, 200 μm. (*B*) Composite lung injury score, lung wet/dry ratio (right lower lobe). (*C*) BAL total protein concentration. Data are presented as mean (±95% confidence interval). VV-ECMO = venovenous extracorporeal membrane oxygenation.

concentration of BAL IL-8; *3*) in spite of endobronchial administration, hMSCs adhered to and impacted the function of a commercial membrane oxygenator *in vivo*, with an increase in the transmembrane oxygenator pressure gradient, and more pulmonary arterial thromboses were noted in hMSC-treated lungs; and *4*) hMSCs reduced the depth and severity of shock.

This study was conducted in a large animal model of ARDS and ECMO, which replicates several important clinical features (20). The "double-hit" injury applied in this study resulted in acute severe hypoxemic respiratory failure consistent with modern criteria for the use of VV-ECMO (11). To support the severe acute respiratory failure, we employed a commercial ECMO device that is in widespread clinical use. In addition, our protocolized intensive care was consistent with clinical best-practice standards (21). A common criticism of preclinical trials of MSCs has been the use of heterogeneous, non–clinical grade cell products (1); however, we tested a commercial hMSC product that is under investigation in clinical trials.

MSCs have been administered to patients with respiratory failure on ECMO

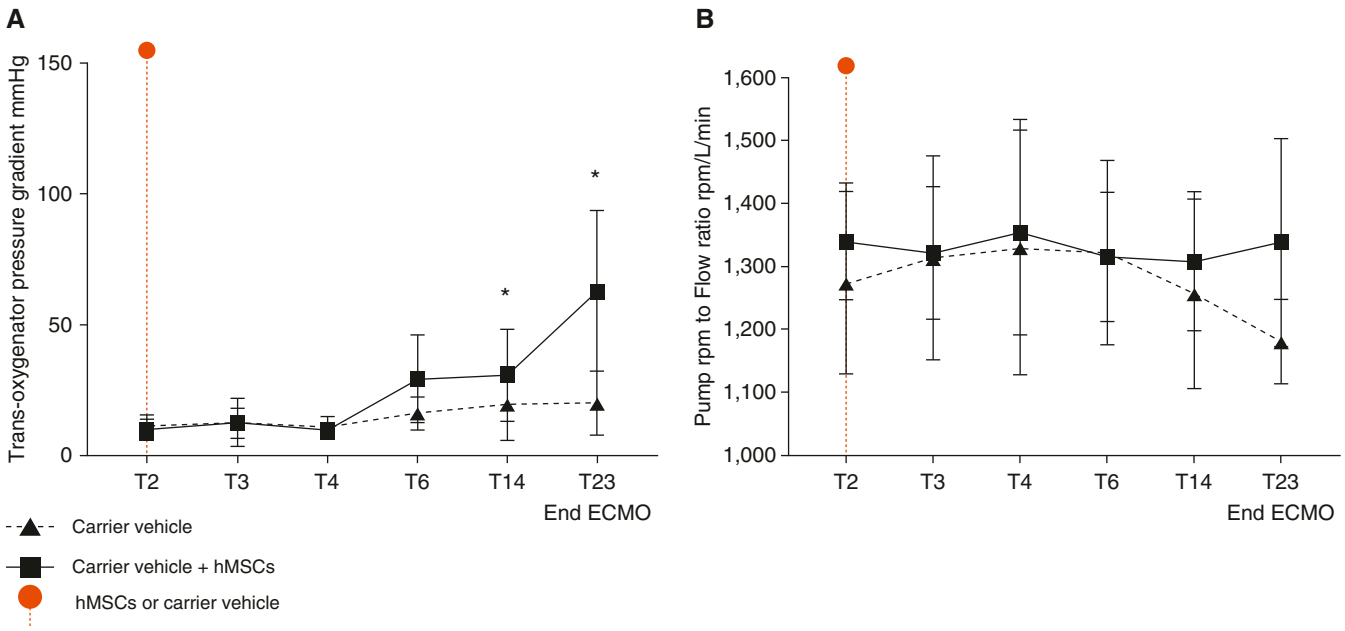

Figure 5. Cell–extracorporeal membrane oxygenation (ECMO) interaction. (*A*) Transmembrane oxygenator pressure gradient. (*B*) Pump revolutions per minute/flow ratio. Data are presented as mean (±95% confidence interval [CI]). Where error bars intersect the *x*-axis, the 95% CI includes zero. *$P = < 0.05$. hMSC = human mesenchymal stromal cell.

(22, 23). These reports, which include only three patients, did not describe infusion-related adverse events, and failed to fully characterize the interaction between MSCs and the extracorporeal device. Kocyildirim and colleagues (24) have conducted the only other preclinical study involving both MSCs and ECMO for respiratory failure. Their ovine-based 6-hour pilot study did not report on the impact of MSC administration on the performance of ECMO.

hMSCs and Pulmonary Function

In this study, the administration of hMSCs failed to improve oxygenation at T_{24}. Furthermore, animals receiving hMSCs had a trend for worse oxygenation index values. In a phase 2a study of 60 patients with ARDS, the intravenous administration of hMSCs did not significantly improve Pa_{O_2}/FI_{O_2} ratio, although there was a signal toward improvement in oxygenation index in a *post hoc* analysis (10). Preclinical studies of MSCs in ARDS have reported improvements in oxygenation, although few have produced lung injury as severe (9). In a recent systematic review of preclinical models combining ARDS and ECMO, only four achieved Pa_{O_2}/FI_{O_2} values <100 mm Hg (20). The degree of lung injury in this model may explain why oxygenation is impaired in the treated group, despite improvements in inflammation and lung injury. Emerging research has highlighted the procoagulant effects of transplanted MSCs. These appear to be primarily mediated by MSC expression of tissue factor (25), but also by the secretion of procoagulant microvesicles (26) and by direct enhancement of platelet deposition (27). In preclinical experiments MSCs have been associated with the development of pulmonary emboli *in vivo* (28). In this study, despite the use of heparin, almost all hMSC animals ($n = 6$) had histological evidence of pulmonary arterial thrombosis postmortem. The presence of exogenous hMSCs within the disordered pulmonary vasculature may have contributed to impairments in oxygenation, tempered by the fact that there was no increase in mean pulmonary artery pressure in treated animals.

Animals receiving hMSCs had improved composite histologic lung injury scores postmortem. The components of the score most influenced by hMSCs were neutrophil numbers in the alveolar and interstitial space. Multiple studies of MSCs in preclinical models of ARDS have demonstrated their ability to reduce neutrophil infiltration (29) and neutrophil extracellular trap formation (30). In this study, BAL neutrophil counts did not differ between groups. This may reflect the technical challenges of obtaining and assessing BAL cell counts. In a recent porcine model of ARDS and MSC therapy, a reduction in neutrophil infiltration was correlated with a reduction in BAL IL-8 concentrations (31), a finding confirmed in this study.

hMSCs and the Systemic Inflammatory Response

Multiple preclinical models (32) and recent clinical trials (33–36) have examined the use of MSCs in the treatment of septic shock. Animals receiving hMSCs required less vasopressor support throughout the experiment to achieve an equivalent or higher MAP. A similar, early, but nonsustained reduction in vasopressor requirement has previously been described in a large-animal model of septic shock treated with MCSs (37). hMSCs did not alter plasma concentrations of proinflammatory cytokines over extended time periods in this study, a finding that has previously been identified in other preclinical (38) and clinical studies (33). A recent phase I dose-escalation study of MSCs in patients with septic shock demonstrated that the maximum effect of cell therapy on plasma cytokine levels occurred at 4 hours after administration and declined with time (35). In this study,

that time period coincides with the maximum separation in vasopressor dose, MAP, and levels of IL-1β and IL-6 between groups. This may indicate that repeat dosing of MSCs will be required for optimal therapeutic efficacy.

hMSCs and ECMO

The risk posed by MSCs to membrane oxygenators has been postulated for some time (1), but has only recently been shown to have an experimental basis. Our group has previously reported the ability of hMSCs to tightly adhere to the membrane fibers of a commercial oxygenator. This may have been the result of the known plastic avidity of MSCs (13). Recently, Cho and colleagues (15) reported the loss of systemically administered MSCs in an *ex vivo* model of venoarterial ECMO.

Given the emerging signal that systemically administered MSCs may interact with membrane oxygenators, we decided to test endobronchial instillation in this study. Cardenes and colleagues (39) have used 18F-fluorodeoxyglucose labeling to track the fate of both systemically and endobronchially administered MSCs in an ovine model of ARDS. Although systemically administered cells have a wide biodistribution in the first 5 hours, endobronchially administered cells were retained at the site of instillation. There are key differences in our approach, including the means of inducing lung injury and its severity.

Limitations

This study has some limitations. First, although our model of injury replicates several relevant features, including severe respiratory and hemodynamic failure, clinical ARDS is usually caused by infection and develops over several days, often in patients with other comorbidities (40). Second, MSCs are known to exhibit different functional responses dependent on the contemporary milieu, which, in some circumstances, may be detrimental (41). This study may have modeled only one phase of acute lung injury, and so hMSCs may have exerted an effect that may differ in other phases. Third, although our model extended 21 hours after cell or vehicle delivery, this may have been too short a period to observe some beneficial effects of the intervention. For example, it may be that the favorable effect of hMSCs on histological injury may have translated to improvements in oxygenation over a longer time period. Conversely, an extension of the study period in the face of rising transoxygenator pressure gradients in the hMSC group may have ultimately led to circuit failures. Fourth, the use of a lung recruitment maneuver and the assessment of native lung function off ECMO may have had several adverse effects, and we cannot be certain that these effects did not differ between groups. This approach was taken due to the challenge of assessing native lung function during ECMO, particularly where a lower V_T ventilatory strategy has been adopted. The study protocol was designed before the publication of the ART (Alveolar Recruitment for ARDS Trial) randomized controlled trial (42). Fifth, the addition of an uninjured control group may have provided further insights into the distribution of hMSCs during VV-ECMO. Finally, the dose and method of delivery of hMSCs remain a matter of conjecture. Based on the findings of our previous work (14), we chose not to investigate intravenous administration. Likewise, based on clinical trial experience, we opted to administer a single, fixed dose of hMSCs. It is possible that varying the dose and/or route of administration of hMSCs may alter their efficacy and safety profile during ECMO.

Conclusions

In a 24-hour ovine model of ARDS and VV-ECMO, we found that hMSC therapy was associated with impairment of the membrane oxygenator. The use of cell therapy did not result in improvements in oxygenation, the primary outcome of this study, but was associated with a reduction in histological evidence of lung injury and inflammation in the lung. Given these data, we cannot currently recommend the administration of hMSCs during ECMO. ■

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

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