Administration of mesenchymal stem cells during ECMO results in a rapid decline in oxygenator performance

Jonathan Edward Millar,[1] Viktor von Bahr,[1,2] Maximillian V Malfertheiner,[1,3] Katrina K Ki,[1] Meredith A Redd,[4] Nicole Bartnikowski,[1] Jacky Y Suen,[1] Danny Francis McAuley,[5] John F Fraser[1]

[1]Critical Care Research Group, University of Queensland, Brisbane, Queensland, Australia
[2]Department of Physiology and Pharmacology, Section for Anesthesiology and Intensive Care Medicine, Karolinska Institutet, Stockholm, Sweden
[3]Department of Internal Medicine II, Cardiology and Pneumology, University Medical Center Regensburg, Regensburg, Germany
[4]Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia
[5]Queen's University Belfast, Wellcome-Wolfson Centre for Experimental Medicine, Belfast, UK

Correspondence to
Dr Jonathan Edward Millar, Critical Care Research Group, University of Queensland, The Prince Charles hospital, Brisbane QLD 4032, Australia ; j.millar@doctors.org.uk

Received 18 December 2017
Revised 15 March 2018
Accepted 19 March 2018
Published Online First 5 April 2018

Check for updates

To cite: Millar JE, von Bahr V, Malfertheiner MV, *et al*. *Thorax* 2019;**74**:194–196.

ABSTRACT

Mesenchymal stem cells (MSCs) have attracted attention as a potential therapy for Acute Respiratory Distress Syndrome (ARDS). At the same time, the use of extracorporeal membrane oxygenation (ECMO) has increased among patients with severe ARDS. To date, early clinical trials of MSCs in ARDS have excluded patients supported by ECMO. Here we provide evidence from an *ex-vivo* model of ECMO to suggest that the intravascular administration of MSCs during ECMO may adversely impact the function of a membrane oxygenator. The addition of clinical grade MSCs resulted in a reduction of flow through the circuit in comparison to controls (0.6 ±0.35 L min^{-1}vs 4.12 ± 0.03 L min^{-1}, at 240 minutes) and an increase in the transoygenator pressure gradient (101±9 mmHg vs 21±4 mmHg, at 240 minutes). Subsequent immunohistochemistry analysis demonstrated quantities of MSCs highly adherent to membrane oxygenator fibres. This study highlights the potential harm associated with MSC therapy during ECMO and suggests further areas of research required to advance the translation of cell therapy in this population.

INTRODUCTION

There is growing interest in the potential of mesenchymal stem cells (MSCs) as a therapy for acute respiratory distress syndrome (ARDS). Phase I studies have been reported,[1 2] while a larger phase II study has recently completed recruitment (NCT02097641). Several more are under way (NCT02611609, NCT03042143, NCT02804945). However, these trials have excluded patients supported by extracorporeal membrane oxygenation (ECMO), which is increasingly used in patients with severe ARDS.[3] While the use of MSCs during ECMO has been described in case reports,[4] the compatibility of MSCs and ECMO has not been systematically evaluated. Here, we present data to suggest that the intravascular administration of MSCs during ECMO may have consequences for oxygenator function.

METHODS

Ex-vivo ECMO was conducted based on our previously described simulated model.[5] Briefly, permanent life support circuits (Maquet, Germany) incorporating a Quadrox D Oxygenator, a RotaFlow pump and Bioline tubing were used. Circuits were primed with 500 mL 0.9% sodium chloride (Baxter, Australia), after which this was exchanged for fresh human whole blood (final volume, 420±50 mL). Circulation was commenced at 2000 revolutions per minute (RPM), using a smooth transition clamp to provide a resistance permitting a blood flow of 4 L/min. After baseline sampling, 10 mL of calcium chloride (CSL, Australia), 12 mL of 8.4% sodium bicarbonate (Baxter) and 400 units of sodium heparin (Pfizer, Australia) were added to the circuit to achieve a pH of between 7.3 and 7.5 and an activated clotting time (ACT) ≥180 s. Pressure across the oxygenator was measured using a silicone-based pressure transducer (Omega Engineering, USA). Circulating blood temperature was maintained at 37°C±0.5°C. A solution of saline, adenine, glucose and mannitol (Macopharma, Australia) was infused at a rate of 4 mL/hour to ensure pressure equilibrium within the circuit. Fresh gas flow was set at 2.5 L/min (5% CO_2, 21% O_2, 74% N_2).

Clinical-grade induced pluripotent stem cells (iPSCs) derived human MSCs (Cynata Therapeutics, Australia) were obtained and stored in the vapour phase of liquid nitrogen until use. Cells were suspended in a vehicle composed of Plasmalyte-A (Baxter) (57.5%), Flexbumin 25% (Baxter) (40%) and dimethyl sulfoxide (DMSO 2.5%). iPSC-derived MSCs used in these experiments were between passages 3 and 5. Fourteen ex-vivo ECMO experiments were undertaken, divided as follows: circuits injected with 40×10^6 MSCs, circulated for 4 hours or until flow reached 0 L/min (n=4); circuits injected with 20×10^6 MSCs, circulated for 4 hours or until flow decreased by 25% from baseline (n=4); and control circuits, circulated for 4 hours (n=6). When physiological conditions were obtained (PaO_2 ≥80 mm Hg, $PaCO_2$ 30–50 mm Hg), MSCs in vehicle were thawed to room temperature (>95% viability), agitated to eliminate clumping, and then immediately administered to the circuit, after the oxygenator and before the pump head, by slow injection over 30 s.

Whole blood samples were collected from the circuit at 30 s, 15, 30, 60, 120 and 240 min. Erythrocytes were lysed, and residual cells were washed as described previously.[6] Fc receptors were blocked using Human TruStain FcX (BioLegend, USA), and MSCs were stained with mouse antihuman monoclonal antibodies—CD45-PECy7 (BioLegend),

Thorax: first published as 10.1136/thoraxjnl-2017-211439 on 5 April 2018. Downloaded from http://thorax.bmj.com/ on April 10, 2024 by guest. Protected by copyright.

Table 1 Conditions during ex-vivo ECMO

	40×10⁶ iPSC-MSCs (n=4)	20×10⁶ iPSC-MSCs* (n=4)	Control (n=6)
Time to 25% decrease in blood flow (min±SE)	68±32	99±21	–
Time to 50% decrease in blood flow (min±SE)	85±39	–	–
Blood flow at 2000 RPM (L/min±SE)			
30 s	4.05±0.02	4.04±0.02	4.03±0.05
15 min	3.79±0.35	4.01±0.04	4.03±0.05
30 min	2.83±0.98	4.12±0.07	3.98±0.06
60 min	1.94±1.14	3.95±0.20	4.12±0.03
120 min	1.39±0.92		4.09±0.05
240 min	0.60±0.35		4.12±0.03
Transoxygenator pressure gradient (mm Hg±SE)			
30 s	20±6	21±2	19±3
15 min	27±12	21±3	21±2
30 min	51±24	22±3	25±4
60 min	55±31	28±4	24±3
120 min	50±18		25±4
240 min	101±9		21±4
MSCs detectable in blood (cells/µL±SE)			
30 s	21.8±1.9		
30 min	9.4±4.2		
60 min	6.9±3.4		
120 min	4.1±3.8		
240 min	0.3±0.2		

*Circuits terminated after a 25% reduction in blood flow (3 L/min), performed to optimise conditions for microscopy.
–, Did not occur; ECMO, extracorporeal membrane oxygenation; iPSC, induced pluripotent stem cell; MSCs, mesenchymal stem cells; RPM, revolutions per minute.

CD73-PerCP, CD90-APC and CD105-FITC (Abcam, UK)—according to the manufacturers' protocol. Precision Count Beads (BioLegend) were then added to determine the fate of circulating MSCs, quantified using a two-laser FACSCanto I flow cytometer (BD Biosciences, USA).

After termination of each experiment, oxygenators were flushed with 1000 mL 0.9% NaCl, perfused with 500 mL paraformaldehyde (Merck, Germany) and rinsed again with 1000 mL 0.9% NaCl. Samples of the heat and gas exchange fibres were then retrieved.

For immunohistochemistry analysis, fibre sections were blocked for 2 hours in phosphate buffered saline (PBS) containing 2% heat inactivated sheep seerum (HISS) (Sigma, Australia) and 0.5% Triton X-100 (Sigma). Samples were incubated overnight at 4°C with primary antibodies: mouse Ab to CD105-FITC (Abcam, 1:10), or mouse Ab to CD90-PE (Abcam, 1:50), and rabbit antibody to β1 tubulin-488 conjugated (Abcam, 1:50). The fibres were washed with PBS followed by 1-hour incubation with secondary antibodies to boost the fluorescent signal followed by additional PBS washes. Images were acquired with a wide-field Nikon deconvolution (TiE) microscope, and confocal z-stacks were acquired with Zeiss LSM710 Airyscan.

RESULTS

In all circuits to which MSCs were administered, blood flow through the ECMO oxygenator decreased by at least 25% within 4 hours, with a corresponding increase in the transoxygenator pressure gradient (table 1). When 40×10⁶ MSCs were administered, flow was reduced to <1.5 L/min in all circuits by 4 hours, and in one case within 30 min. ACT was maintained ≥180 s throughout all experiments. Microscopy of deconstructed oxygenators demonstrated the widespread adherence of MSCs to plastic fibres (figure 1).

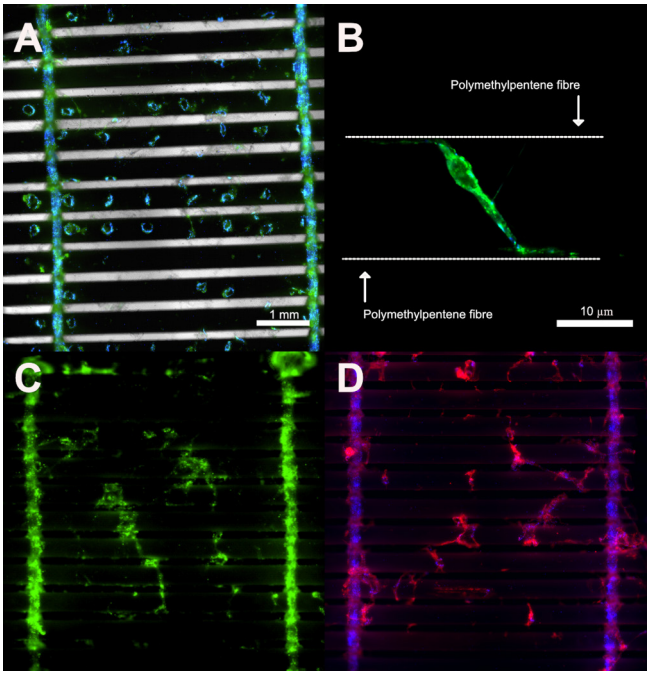

Figure 1 ECMO oxygenator fibres after the administration of iPSC-derived human MSCs. (A) Wide-field microscopy of CD105-stained MSCs (green) with a bright field overlay of a section of PU heat exchange fibres. MSCs can be seen adherent between adjacent fibre strands. (B) A confocal image of an MSC bridging two PU fibres. (A,B) DAPI-stained nuclei (blue). (C) A sample of PMP gas exchange fibres taken from the core of the oxygenator bundle and stained for CD105. (D) A similar section of PMP gas exchange taken from the periphery of the oxygenator and stained for CD90 with DAPI-stained nuclei. DAPI, 4',6-diamidino-2-phenylindole; ECMO, extracorporeal membrane oxygenation; iPSC, induced pluripotent stem cell; MSC, mesenchymal stem cell; PMP, polymethylpentene; PU, polyurethane.

DISCUSSION

This is the first study to directly address the feasibility of MSC therapy during ECMO. Our data suggest that intravascular administration of MSCs during ECMO may have important consequences for oxygenator function, as well as for their efficacy as a therapy for severe ARDS in this setting. This may have occurred due to the characteristic plastic adhesiveness of MSCs. A limitation of our study is that we did not assess gas exchange across the oxygenator.

MSC use during ECMO has been described previously but has either been administered before the commencement of ECMO,[7] by intratracheal administration[8] or during a pause in flow.[4] These methods of administration may not always be possible in severely ill patients with ARDS who are reliant on continuous high-flow ECMO for oxygenation.

At a circuit concentration of 48–95×10³ MSCs/mL, our study may have underestimated the effect of MSCs on oxygenator performance. Previous studies in ARDS have used up to 10×10⁶ MSCs/kg,[1] which assuming an average blood volume of 70 mL/kg[9] and equal distribution would result in a higher

Thorax: first published as 10.1136/thoraxjnl-2017-211439 on 5 April 2018. Downloaded from http://thorax.bmj.com/ on April 10, 2024 by guest. Protected by copyright.

circulating cell concentration (approximately 143×10^3 MSCs/mL). This must be weighed against the possibility that indirect intravascular administration, peripherally or after the membrane oxygenator, may reduce the number of MSCs reaching the oxygenator surface, most likely as a result of entrapment in the pulmonary circulation. These data also support further investigation of MSC therapy during extracorporeal carbon dioxide removal or cardiopulmonary bypass, both of which share functionally similar membrane gas exchange devices with ECMO.[10]

Alternative routes of administration, such as intrabronchial, may have advantages in the setting of ECMO, when prolonged bronchoscopy is possible. This should be evaluated in any future study. In light of these results, further investigations using MSCs in ECMO should explore safety considerations in an intact animal model before progression to clinical trials.

Correction notice This article has been corrected since it was published Online First. Errors with in Table 1 was corrected.

Contributors JEM, DFM and JFF conceived and designed the study. VvB and MVM designed and modified the ex-vivo ECMO model. JEM, VvB and MVM conducted the ex-vivo experiments. KKK, MAR, NB and JYS analysed the generated samples. All authors have participated in the drafting and critical revision of the manuscript.

Funding This study was supported by the Intensive Care Society (UK) New Investigator Award and by the National Health and Medical Research Council (NHMRC), Australia (APP1079421). VvB is funded by Gålöstiftelsen Foundation, Olle Enqvist Byggmästare Foundation, Karolinska Institutet Fernström Foundation, and the Royal Swedish Academy of Sciences, Claes Adelsköld Foundation.

Competing interests Cynata Therapeutics provided mesenchymal stem cells in kind to this study. The company was not involved in the commission, design or analysis of the study.

Ethics approval The study was approved by the Metro North Ethics Committee (HREC/16/QPCH/221).

Provenance and peer review Not commissioned; externally peer reviewed.

© Article author(s) (or their employer(s) unless otherwise stated in the text of the article) 2019. All rights reserved. No commercial use is permitted unless otherwise expressly granted.

REFERENCES

1 Wilson JG, Liu KD, Zhuo H, *et al*. Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. *Lancet Respir Med* 2015;3:24–32.
2 Zheng G, Huang L, Tong H, *et al*. Treatment of acute respiratory distress syndrome with allogeneic adipose-derived mesenchymal stem cells: a randomized, placebo-controlled pilot study. *Respir Res* 2014;15:39.
3 Sweeney RM, McAuley DF. Acute respiratory distress syndrome. *Lancet* 2016;388:2416–30.
4 Simonson OE, Mougiakakos D, Heldring N, *et al*. In Vivo Effects of Mesenchymal Stromal Cells in Two Patients With Severe Acute Respiratory Distress Syndrome. *Stem Cells Transl Med* 2015;4:1199–213.
5 Shekar K, Fung YL, Diab S, *et al*. Development of simulated and ovine models of extracorporeal life support to improve understanding of circuit-host interactions. *Crit Care Resusc* 2012;14:105–11.
6 Ki KK, Faddy HM, Flower RL, *et al*. Platelet concentrates modulate myeloid dendritic cell immune responses. *Platelets* 2017:1–10.
7 Kocyildirim E, Cárdenes N, Ting A, *et al*. The Use of GMP-Produced bone marrow-derived stem cells in combination with Extracorporeal Membrane Oxygenation in ARDS: an Animal Model. *Asaio J* 2017;63:324–32.
8 Jungebluth P, Holzgraefe B, Lim ML, *et al*. Autologous peripheral blood mononuclear cells as treatment in refractory acute respiratory distress syndrome. *Respiration* 2015;90:481–92.
9 Feldschuh J, Enson Y. Prediction of the normal blood volume. Relation of blood volume to body habitus. *Circulation* 1977;56:605–12.
10 Strueber M. Artificial lungs: are we there yet? *Thorac Surg Clin* 2015;25:107–13.

Thorax: first published as 10.1136/thoraxjnl-2017-211439 on 5 April 2018. Downloaded from http://thorax.bmj.com/ on April 10, 2024 by guest. Protected by copyright.