Stem Cell Research 17 (2016) 25–31

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Human mesenchymal stem cells attenuate early damage in a ventilated pig model of acute lung injury

Yuben Moodley [a,b,c,*], Marian Sturm [a,d], Kathryn Shaw [d], Chiko Shimbori [e], Dino B.A. Tan [a,c], Martin Kolb [e], Ruth Graham [f]

[a] School of Medicine and Pharmacology, University of Western Australia, Nedlands, Western Australia, Australia
[b] Department of Respiratory Medicine, Fiona Stanley Hospital, Murdoch, Western Australia, Australia
[c] Stem Cell Unit, Institute for Respiratory Health, Nedlands, Western Australia, Australia
[d] Cell and Tissue Therapies WA, Royal Perth Hospital, Perth, Western Australia, Australia
[e] Department of Medicine, Pathology, and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada
[f] Department of Anesthesia, University of Manitoba, Winnipeg, Canada

ARTICLE INFO

Article history:
Received 9 November 2015
Received in revised form 6 May 2016
Accepted 11 May 2016
Available online 12 May 2016

Keywords:
Acute lung injury (ALI)
Mesenchymal stem cells (MSC)
Oleic acid (OA)
Nuclear factor-light chain enhancer of activated B cells (NF-κB)
Pig

ABSTRACT

Acute lung injury/acute respiratory distress syndrome (ALI/ARDS) is a major cause of global morbidity and mortality. Mesenchymal stem cells (MSC) have shown promise in treating inflammatory lung conditions. We hypothesised that human MSC (hMSC) can improve ALI/ARDS through their anti-inflammatory actions. We subjected pigs (n = 6) to intravenous oleic acid (OA) injury, ventilation and hMSC infusion, while the controls (n = 5) had intravenous OA, ventilation and an infusion vehicle control. hMSC were infused 1 h after the administration of OA. The animals were monitored for additional 4 h. Nuclear translocation of nuclear factor-light chain enhancer of activated B cells (NF-κB), a transcription factor that mediates several inflammatory pathways was reduced in hMSC treated pigs compared to controls ($p = 0.04$). There was no significant difference in lung injury, assessed by histological scoring in hMSC treated pigs versus controls ($p = 0.063$). There was no difference in neutrophil counts between hMSC-treated pigs and controls. Within 4 h, there was no difference in the levels of IL-10 and IL-8 pre- and post-treatment with hMSC. In addition, there was no difference in hemodynamics, lung mechanics or arterial blood gases between hMSC treated animals and controls. Subsequent studies are required to determine if the observed decrease in inflammatory transcription factors will translate into improvement in inflammation and in physiological parameters over the long term.

1. Introduction

Acute respiratory distress syndrome (ARDS) is characterized by refractory hypoxemia in patients with bilateral lung infiltrates. The Berlin definition of ARDS is based on the degree of hypoxemia: mild (200 mm Hg $PaO_2/FiO_2 \leq 300$ mm Hg), moderate (100 mm Hg $PaO_2/FiO_2 \leq 200$ mm Hg), and severe ($PaO_2/FiO_2 \leq 100$ mm Hg). In addition, the condition must develop within one week of a known clinical insult or new/worsening respiratory symptoms. Bilateral opacities on chest imaging that is not fully explained by effusions, lobar/lung collapse or nodules and the respiratory failure must not be fully explained by cardiac failure or fluid overload (Ranieri et al., 2012). A National Institutes of Health study estimated the incidence of ARDS to be 75/100,000 population in the USA, while in Australia the incidence is estimated at 30/100,000 admissions (Ware and Matthay, 2000).

ARDS may be caused by direct or indirect injuries to the lung including aspiration of gastric contents and sepsis (Petty and Ashbaugh, 1971). The early phase of lung injury is characterized by epithelial and endothelial cell damage leading to a compromised alveolar-capillary barrier and exudation of fluid into the alveolar space, followed by infiltration of inflammatory cells such as neutrophils. Progression from acute lung injury (ALI) to a fibro-proliferative phase observed 5–7 days after the injury may be reversible or persistent (Leaver and Evans, 2007).

Many strategies have been directed at reducing lung injury and augmenting tissue repair in ALI/ARDS with limited beneficial outcomes. Low tidal volume ventilation and prone positioning have proven to be effective in reducing mortality (Slutsky and Ranieri, 2000; Guerin et al., 2013). However, a randomised control trial of "Conventional ventilatory support vs extracorporeal membrane oxygenation (ECMO) for severe adult respiratory failure (CESAR)" showed that ECMO had limited success in preventing lung injury (Ware and Matthay, 2000; Peek et al.,

* Corresponding author at: Centre for Respiratory Health, School of Medicine and Pharmacology, Fiona Stanley Hospital Unit, Harry Perkins Research Institute, Robin Warren Drive, 102-118 Murdoch Drive, Murdoch, WA 6150, Australia.
E-mail address: yuben.moodley@uwa.edu.au (Y. Moodley).

http://dx.doi.org/10.1016/j.scr.2016.05.005

2006). Given the significant mortality associated with ARDS, alternative therapies aimed at reducing lung injury are needed.

Stem cell therapy has raised the possibility of augmenting lung repair and restituting damaged lung tissue. Human bone marrow derived mesenchymal stem cells (hMSC) have improved a variety of animal models of acute lung injury and ex vivo models of human lung injury (Ortiz et al., 1999; Lee et al., 2009; Asmussen et al., 2014). Although there have been significant advances in MSC therapy including the commencement of clinical trials, there are still critical areas that need further elucidation. There is a paucity of studies testing MSC in large animal models of ALI. In addition, there is conflicting data regarding the onset of benefit of MSC. Several studies have shown beneficial physiological effects of MSC at time points of 24–48 h post-infusion (Asmussen et al., 2014; Devaney et al., 2015), whilst Rojas et al. (2014) showed beneficial effects within 2 h of MSC infusion (Rojas et al., 2014). Therefore, determining the early effects of MSC on inflammation and lung physiology in a large animal model of ALI would inform clinical studies about the timelines to expect improvement and the impact of MSC on the early pathogenesis of the disease.

There are several proposed mechanisms by which hMSC act including the release of anti-inflammatory mediators such as nitric oxide, IL-1R antagonist, angiopoietin and tumour necrosis factor-inducible gene (TSG-6) (Moodley et al., 2013). Recently, MSC were shown to release exosomes including donating mitochondria that then improve the energetics of damaged tissue thus augmenting repair (Islam et al.). Based on the pathogenesis of ALI/ARDS and the mechanisms of action of MSC, we hypothesized that MSC would improve the early inflammatory and physiological changes in this condition. In this study, we treated an oleic acid (OA) induced-ventilatory pig model of ALI with hMSC and measured physiological and inflammatory outcomes during the initial 4 h after treatment.

2. Materials and methods

2.1. Preparation of hMSC

Human MSC were isolated from donor bone marrow as previously described (Herrmann et al., 2012), and culture expanded in Dulbecco's modified eagle medium (DMEM; Gibco, Life Technologies) containing 10% fetal bovine serum (FBS; HyClone, Thermo Scientific). Bone marrow for MSC manufacturing was collected under the approval from the Royal Perth Hospital Ethics Committee for clinical therapy and the need for consent to use the cells for research was waived because the cells manufactured were surplus to requirements.

The batch of hMSC used in our study expressed the cell surface markers CD105, CD90 and CD73, but not CD45, CD34, CD11b, and the human leucocyte antigen (HLA)-DR. Cells were harvested at *P*3 and cryopreserved at 5×10^6 cells/ml in 10% dimethyl sulfoxide (DMSO; Wakchemi), 10% porcine serum (HyClone, Thermo Scientific) and 80% sodium chloride solution (0.9%) in OriGen cryogenic bags using a controlled rate freezer. Cells were then stored at −196 °C in vapour nitrogen. Cell aliquots containing 40×10^6 cells along with control bags (same vehicle but without cells) were prepared at the Cell and Tissue Therapies WA (CTTWA) facility, cryopreserved in cryobags, shipped on dry ice to the University of Manitoba and stored at −80 °C in a secure fridge.

2.2. Ventilation of pigs

After receiving approval from the University of Manitoba Animal Care Ethics Committee (submitted, Sep. 2012), a total of 12 farm bred female pigs (20–22 kg) were fasted overnight with free access to water. An intramuscular injection of ketamine, midazolam and atropine (10/0.1/0.02 mg/kg) was given for sedation. Anaesthesia was induced with isoflurane by inhalation via a nosecone. The animals were intubated with a 6.0 cuffed endotracheal tube and mechanically ventilated with a tidal volume = 10 ml/kg, inspired oxygen concentration = 50%, positive end-expiratory pressure (PEEP) = 5 cm H_2O and respiratory rate adjusted to maintain $PaCO_2$ in the normal range. Anaesthesia was maintained with 1.5 to 2 MAC Isoflurane during surgical preparation. An intravenous infusion of Ringer's Lactate (10 ml/kg/h) was established in an ear vein. Through a cut-down, a 5 ft Swan Ganz catheter was floated into the pulmonary artery via the external jugular vein to measure pulmonary artery pressure, pulmonary capillary wedge pressure, right atrial pressure and cardiac output by thermodilution in triplicate. A femoral artery cannula was placed for continuous arterial pressure measurement and blood gas sampling. A femoral venous cannula was advanced in the IVC above the diaphragm for OA administration.

Anaesthesia was then switched to a continuous infusion of propofol/ketamine/rocuronium, 10/2.5/0.5 mg/kg/h to allow for ventilation with an Esprit ® ventilator, which ensured more precise control of tidal volume, PEEP and respiratory mechanic measurements for the experimental period. Muscle relaxation was required to prevent spontaneous ventilator efforts during low tidal volume ventilation which interfere with accurate measurements of lung mechanics. The animal was allowed to stabilize for 15 min after surgical preparation, then baseline hemodynamics, arterial blood gases and lung function (peak and mean airway pressures, total respiratory system compliance, by interrupter method, and dead space) were determined.

2.3. Oleic acid induced injury

ALI was then induced with a well-established protocol (Froehlich et al., 2008). OA was infused at 0.2 ml/kg/h until static respiratory compliance decreased by at least 40% and arterial PaO_2 decreased to <100 mm Hg at an FIO_2 of 50%. Dopamine was infused at 5–10 mcg/kg/min during OA infusion to maintain arterial blood pressure > 60 mm Hg, then discontinued. When a stable injury had been achieved, (no further change in blood gases or lung compliance for 15 min) ventilation was maintained with a low tidal volume strategy (V_T = 7 ml/kg, PEEP 10, FiO_2 50%) for the duration of experiments. The animals were allowed to stabilize for 1 h and measurements repeated. The animals were then randomly assigned to a sham treatment group (n = 5) or hMSC group (n = 6).

2.4. MSC administration

The dose of MSC of 2×10^6 cells/kg administered intravenously was based on our studies in other disease cohorts (Forbes et al., 2014). In preparation for infusion, the cryobag was thawed in a water bath of sterile saline at 37 °C. An equal volume of sterile Ringer's Lactate was added to the bag to stabilize the cells. The cells (2×10^6 cells/kg) or vehicle control were infused immediately following preparation over 10 min. DMSO, used in the cryopreservation of the MSC was infused with the cells and DMSO present in the vehicle control was administered to controls in equivalent volumes to the experimental group but without the cells. Cell viability was assessed using the Vi-Cell XR cell analyser (trypan blue based determination) post cryopreservation and in our laboratory was 87% viability.

Blood gases and lung function measurements were determined immediately after infusion and then hourly for 4 h. Lung oedema fluid aspirate samples were obtained by gentle suction of a soft catheter wedged into a distal airway at baseline OA injury (1 h post OA infusion) and at 4 h of ventilation post lung injury. Samples were centrifuged at 3000 g for 10 min and the supernatant stored at −80 °C. The animals were sacrificed using Euthanyl 100 mg/kg with lung inflation maintained at 10 cm PEEP.

2.5. Histology and lung aspirate

Lung tissue samples were obtained from upper, middle and lower lobes and fixed in formalin. The lungs were then removed *en bloc*,