Pretreatment with bone marrow–derived mesenchymal stromal cell–conditioned media confers pulmonary ischemic tolerance

Billanna Hwang, MPH,[a,b] W. Conrad Liles, MD, PhD,[b,c] Rachel Waworuntu, BS,[b] and Michael S. Mulligan, MD[a,b]

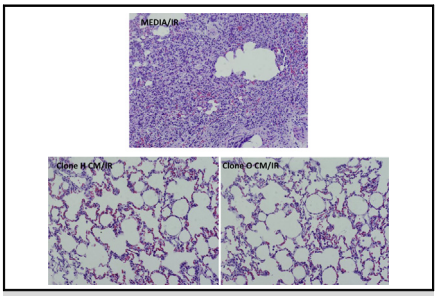
ABSTRACT

Objective: Mesenchymal stromal cell–based therapies have demonstrated efficacy in treating a variety of diseases. Despite the potential benefits, there are still significant hurdles that need to be overcome for clinical use. We describe a cell-free–based immunotherapy approach for inducing pulmonary ischemic tolerance by using mesenchymal stromal cell–conditioned media.

Methods: In our well-established lung ischemia–reperfusion model, we pretreated with mesenchymal stromal cell–conditioned media 30 minutes before injury. To determine the degree of lung injury, we assessed for changes in lung vascular permeability, proinflammatory cytokines and cellular infiltrates in bronchoalveolar lavage, and histopathology. Macrophage and T-cell subsets were assessed by immunohistochemistry.

Results: Pretreatment with mesenchymal stromal cell–conditioned media conferred protection against lung ischemia–reperfusion injury. This protection is characterized by a significant reduction in proinflammatory cytokines, a decrease in infiltrating inflammatory cells, and increases in M2-like macrophages and regulatory T cells.

Conclusions: Cell-free mesenchymal stromal cell–conditioned media therapy confers pulmonary ischemic tolerance. This therapy uses paracrine factors that provide beneficial protective effects by immunomodulating the inflammatory response in resident and infiltrating cell subsets. (J Thorac Cardiovasc Surg 2016;151:841-9)



MSC-CM confers pulmonary ischemic tolerance.

Central Message

MSC-CM–based therapy can be a substantial option for protecting or reducing the severity of lung IR injury.

Perspective

MSC-CM–based therapeutic strategy would set precedence in the field of lung IR injury.

See Editorial Commentary page 849.

Despite advances in preservation and supportive care, ischemia–reperfusion (IR) injury remains a major cause of primary graft failure after lung transplantation. Clinically significant reperfusion injury followed by major graft dysfunction develops in 15% to 30% of patients.[1-3] Patients with IR injury have a higher incidence of acute graft rejection and are more prone to early-onset obliterative bronchiolitis and chronic rejection.[1-3]

Mesenchymal stromal cells (MSCs) are a category of nonhematopoietic, stem-like cells found within the bone marrow niche and are capable of differentiating into a variety of cell types and immunomodulating cellular responses.[4-8] Preclinical and clinical studies have reported that administration of MSCs before injury is protective against a variety of diseases.[9-13] Most recently, MSCs have been shown to reduce the severity of lung injury in a variety of preclinical models of acute respiratory distress syndrome.[9-13] Despite the potential benefits, there are still significant hurdles that need to be overcome to use MSC-based therapy clinically. This includes the requirement for large cell numbers for therapeutic use, lack of MSC quality control and characterization, and cell transformation after continuous in vitro passaging. Direct administration of MSCs has been effective in clinical trials, but if the beneficial effects of conditioned media are as good as those seen with cell-based therapy, this therapeutic strategy would be simpler with fewer potential limitations.

From the [a]Department of Surgery, University of Washington School of Medicine, Seattle, Wash; [b]Center for Lung Biology, University of Washington, Seattle, Wash; and [c]Department of Medicine, University of Washington School of Medicine, Seattle, Wash.

Funded by the National Institutes of Health (Bethesda, Md) RO1-Role of TLR-4 in Lung Reperfusion Injury Grant R01HL093097. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or its subsidiary institutes and centers.

Read at the 41st Annual Meeting of The Western Thoracic Surgical Association, Whistler, British Columbia, Canada, June 24-27, 2015.

Received for publication July 17, 2015; revisions received Nov 4, 2015; accepted for publication Nov 18, 2015.

Address for reprints: Billanna Hwang, MPH, Division of Thoracic Surgery, University of Washington, Center for Lung Biology 850 Republican St, Box 358052, WA 98109 (E-mail: bhwang@uw.edu).

0022-5223/$36.00

http://dx.doi.org/10.1016/j.jtcvs.2015.11.043

Abbreviations and Acronyms

BAL = bronchoalveolar lavage
IL = interleukin
IR = ischemia–reperfusion
MSC = mesenchymal stromal cell
MSC-CM = mesenchymal stromal cell–conditioned media
PBS = phosphate-buffered saline

In the present study, we investigated a ''cell-free therapy'' approach for inducing ischemic tolerance by pretreating with mesenchymal stromal cell–conditioned media (MSC-CM). This therapeutic intervention would set precedence in this field by using the protective immunomodulatory paracrine factors of MSCs and avoiding limitations that have been reported in previous studies.

MATERIALS AND METHODS

Experimental Animals

Pathogen-free, adult, male Long-Evans rats (Harlan Sprague-Dawley, Indianapolis, Ind), weighing 275 to 300 g, were used for all in vivo experiments. Approval for all experimental protocols was granted by the University of Washington Animal Care Committee. Animals received humane care in compliance with the ''Principles of Laboratory Animal Care'' established by the National Society for Medical Research and the ''Guide for the Care and Use of Laboratory Animals'' developed by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (Publication No. 86-23, revised 1996).

Isolation and Characterization of Bone Marrow Mesenchymal Stromal Cells

Clonal rat MSC lines were generated by Beverly Torok-Storb at Fred Hutchison Cancer Research Center. Bone marrow from adult rats was obtained, and buffy coats were plated on plastic adherent tissue culture dishes at 1 to 2 $\times$ 10^6 cells/mL. Primary adherent cells were exposed to LXSN-16 E6E7 retrovirus for 2 hours and 4 μg/mL polybrene (BEV ref). Virus containing media was removed, and adherent cells were incubated with media containing polybrene for an additional 5 hours.

All clones expressed typical MSC markers: CD105, CD73, CD90, CD166, and CD146. Bone marrow MSC did not express hematopoietic markers CD45, CD14, and CD34. Mesenchymal stem cell Adipogenic and Osteogenic differentiation kits were used to assess the differentiation potential of all MSC clones (Trevigen, Gaithersburg, Md).

Conditioned Media Infusions

Seven clones were investigated in this study. All MSC lines differ in morphology, function, surface phenotype, and gene expression. Media used to grow these cells included Roswell Park Memorial Institute, 5% Pen/Strep, and 10% fetal bovine serum.

Different clonal rat MSC lines were expanded between passages 6 and 12, and conditioned media were collected for in vivo and in vitro experiments. Conditioned media were centrifuged at 1200 rpm for 10 minutes at 4°C to remove any cells and debris. A final volume of 200 μL was infused intratracheally in experimental rodents. Endotoxin studies on MSC-CM were performed using a Pierce LAL chromogenic endotoxin quantitation kit (Thermoscientific, Carlsbad, Calif).

Ischemia–Reperfusion Model

Pathogen-free Long-Evans rats (weight, 250-275 g) were used for all experiments. Rats were anesthetized with 2.5% isoflurane, and a 14-gauge angiocatheter was inserted into the trachea through a midline neck incision. Rats were connected to a CWE ventilator (CWE Inc, Ardmore, Pa), and settings were maintained at an inspired oxygen content of 60% with a positive end-expiratory pressure of 2 cm H2O and respiratory rate of 80 breaths/min.

A left thoracotomy was performed, and the left lung was mobilized atraumatically. Heparin (50 units) was administered through the penile vein. After 5 minutes of circulation, a noncrushing clamp was placed across the left lung hilum. The clamp was removed after 90 minutes, and the left lung was reventilated and reperfused for 4 hours. A midline laparotomy and sternotomy were performed, and animals were euthanized by aortic transection. The heart–lung block was excised, and the pulmonary circulation was flushed with 20 mL of phosphate-buffered saline (PBS).

Mesenchymal Stromal Cell–Conditioned Media Treatment Protocol and Experimental Groups

Ten cohorts were studied. Negative control animals did not undergo pretreatment or surgical manipulation. Animals in the positive control groups received media only before IR. The remaining 7 cohorts received MSC-CM from different MSC clones. Conditioned media were infused intratracheally 30 minutes before undergoing IR.

Lung Permeability Index

Animals received Evans blue dye at a dose of 20 mg/kg of body weight intravenously 30 minutes before the end of the reperfusion. After the reperfusion, a midline abdominal incision was performed and the abdominal aorta and vein were severed. The left ventricle was vented with a small incision at the apex of the heart. The mitral apparatus was dilated with the left atria using a 14-gauge cannula passing through the mitral valve and into the left atrium to allow free flow of effluent blood from the lung. The pulmonary vasculature was flushed by injecting 10 mL of PBS with a 20-gauge cannula from the pulmonary artery. The left lung was excised and snap-frozen in liquid nitrogen. The frozen lung was homogenized in 2 mL of PBS, diluted with 4 mL of formamide, and incubated at 60°C for 24 hours. The homogenate was centrifuged at 8000 rpm for 5 minutes at room temperature. The supernatants were collected and measured by spectrophotometry at 620 nm.

Histology

Lungs from all experimental groups were biopsied and fixed in 4% paraformaldehyde. After fixation, lungs were embedded into paraffin, cut into 5-μm sections, and stained with hematoxylin–eosin.

Immunohistochemistry

Immunohistochemistry and histology were performed by the University of Washington Histopathology Shared Resource. Five-micron sections were cut, deparaffinized, and rehydrated in Dako Wash Buffer (Dako, Carpinteria, Calif). Slides were antigen retrieved in a Black and Decker (Baltimore, Md) steamer for 20 minutes in preheated Trilogy buffer (Cell Marque, Hot Springs, Ariz) and cooled for 20 minutes. Slides were rinsed 3 times in wash buffer, and all subsequent staining steps were performed at room temperature using the Dako Autostainer. Endogenous peroxide activity was blocked using 3% H_2O_2 for 8 minutes followed by protein blocking. Slides were blocked in 15% goat serum and 5% canine serum in tris-buffered saline containing 1% bovine serum albumin for 10 minutes. Antibodies were used at 10 μg/mL and incubated for 60 minutes and were detected using biotinylated goat anti-rat (112-065-167, Jackson ImmunoResearch Laboratories Inc, West Grove, Pa) at 1:200 for 30 minutes followed by Vector Elite ABC (Vector Laboratories, Burlingame, Calif).